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ARTICLE

Thermodynamics of Various F420 Coenzyme Models as Sources of Electrons, Hydride Ions, Hydrogen Atoms and Protons in Acetonitrile

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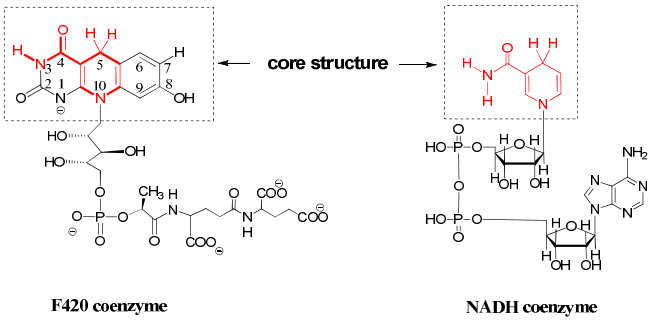
Abstract: 32 F420 coenzyme models with the alkylation of the three different N atoms (N_1, N_3 and N_{10}) in the core structure (XFH^-) were designed and synthesized and the thermodynamic driving forces (defined in terms of the molar enthalpy changes or the standard redox potentials in this work) of the 32 XFH^- releasing hydride ions, hydrogen atoms and electrons, the thermodynamic driving forces of the 32 $XFH^{\bullet}$ releasing protons and hydrogen atoms and the thermodynamic driving forces of $XF^{-\bullet}$ releasing electrons in acetonitrile were determined using titration calorimetry and electrochemical methods. Effects of methyl group at N_1, N_3 and N_{10} and a negative charge on N_1 and N_{10} atoms on the six thermodynamic driving forces of the F420 coenzyme models and their related reaction intermediates were examined, the results show that seating arrangements of the methyl group and the negative charge have remarkably different effect on the thermodynamic properties of the F420 coenzyme models and their related reaction intermediates. Effects of the substituents at C_7 and C_8 on the six the thermodynamic driving forces of the F420 coenzyme models and their related reaction intermediates were also examined, the results show that the substituents at C_7 and C_8 have good Hammett linear free energy relationships with the six thermodynamic parameters. Meanwhile, a reasonable diagnosis of possible reactions of the members between F420 family and NADH family in vivo were given according to a thermodynamic analysis platform constructed using the elementary step thermodynamic parameter of F420 coenzyme model $2FH^-$ and NADH model MNAH releasing hydride ions in acetonitrile. The information disclosed in this work could not only supply a gap of the chemical thermodynamics of F420 coenzyme models as one class of very important organic source of electrons, hydride ions, hydrogen atoms and protons, but also strongly promote the fast development of the chemistry and applications of F420 coenzyme.

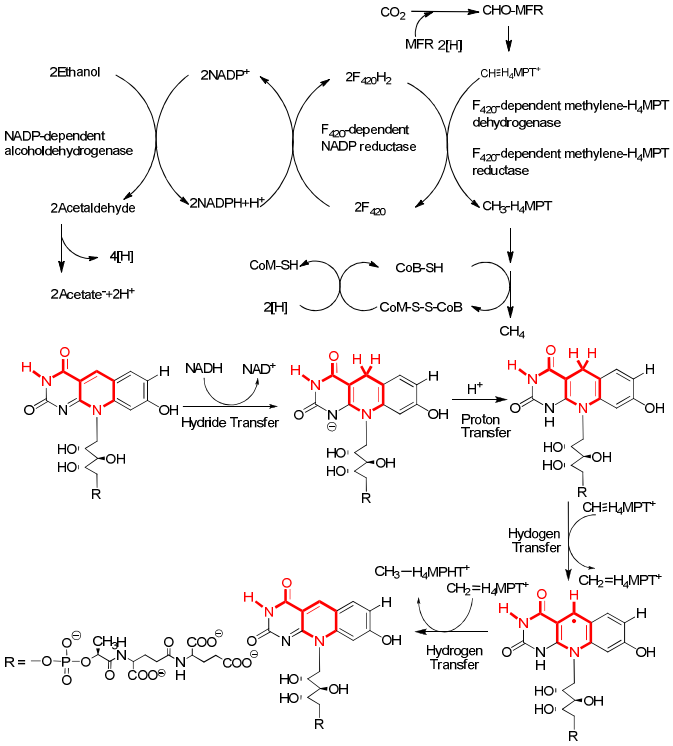
Introduction

F420 coenzyme or 8-hydroxy-5-deazaflavin (Scheme 1) is a coenzyme involved in redox reactions in methanogens, in many Actinobacteria, and sporadically in other bacterial lineages.[1] It is a flavin derivative. The coenzyme is a substrate for coenzyme F420 hydrogenase,[2] 5,10-methylenetetrahydro-methanopterin reductase and methylenetetrahydromethano-pterin dehydrogenase.[3,4] A particularly rich natural source of F420 is Mycobacterium smegmatis, in which several dozen enzymes use F420 instead of the related cofactor FMN used by homologous enzymes in most other species.[5] Although F420 coenzyme contains a deazaflavin moiety, it is biochemically analogous to the nicotinamide coenzymes (Scheme 1).



Scheme 1. Comparison of the core structure between F420 coenzyme and NADH coenzyme.

This coenzyme is essential for energy metabolism in methanogenic Archaea, which involves the conversion of CO_2, several other one-carbon compounds, and acetate to CH_4 likely by hydride transfer from the reduced F420 (Scheme 2).[6-9] In Streptomyces, F420 coenzyme is involved in the biosynthesis of tetracycline, lincomycin, and other natural products.[10-14] In some cyanobacteria, a precursor of F420 coenzyme is a cofactor in DNA photolyases for DNA repair.[15,16] In Mycobacterium tuberculosis, an F420-dependent glucose-6-phosphate dehydrogenase is required for the reductive activation of a series of nitroimidazo-oxazine compounds for their anti-tubercular effects.[17-19]

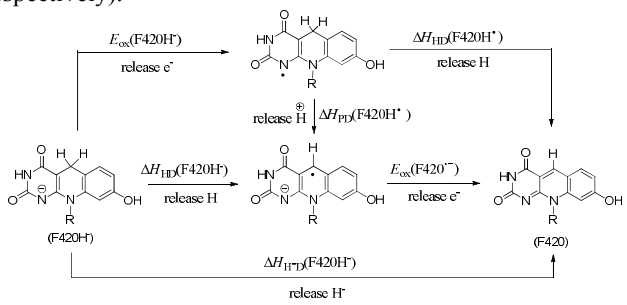


Scheme 2. An example of the biological functions of F420 in vivo.

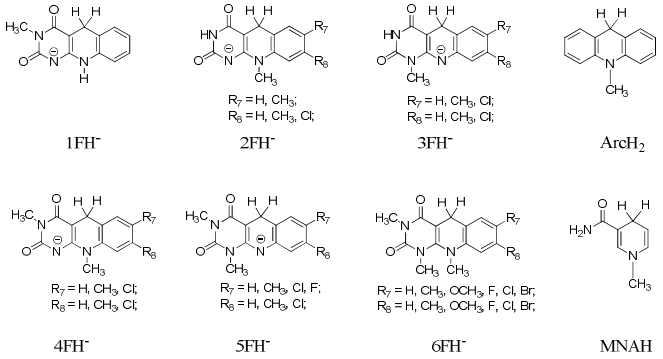
Since F420 coenzyme extensively takes part in the conversion and biosynthesis of many important living active molecules and the role of the F420 coenzyme essentially is to donate or accept hydride ions, hydrogen atoms and electrons, the oxidation mechanism of the reduced F420 coenzyme in vivo has attracted much attention of many chemists and biochemists.[20] In order to clarify the oxidation mechanism of the reduced F420 coenzyme in vivo and make the better artificial alternatives to the coenzyme, the various analogous compounds with substituents at various positions on the core structure of the F420 coenzyme molecule have been designed and synthesized and a lot of very important chemical mimic studies on the oxidation reactions of the reduced F420 coenzyme in vivo have been reported.[17-20] Although the oxidation of the reduced F420 has received extensive investigations, the real oxidation mechanism of the reduced F420 coenzyme by donating hydride anions in vivo is not clear still. The main reason is that one hydride ion is consisted of two electrons and on proton, which means that the mechanism of the hydride transfer from the reduced F420, in fact, is quite complex (Scheme 3). From Scheme 3, it is clear that a detailed thermodynamic analysis of each elementary step for the hydride transfer from the reduced F420 coenzyme and its models must be performed to completely clarify the oxidation mechanism of the reduced F420 coenzyme in vivo. In this work, 32 model compounds of F420 coenzyme with substituents at various positions on the core structure of F420 coenzyme shown in Scheme 4 were designed and synthesized, and the thermodynamic driving forces of the six elementary steps for the hydride transfer in acetonitrile were determined by using efficient experimental method. Since the center core structure of F420 coenzyme is biochemically analogous to that of the nicotinamide coenzyme, N-methyl-1,4-dihydronicotinamide (MNAH) as a typical model compound of the nicotinamide coenzyme was also examined for comparison.

Since the uracil structure is a common structure of many bioactive compounds, it is clear that the effect of the uracil moiety in F420 coenzyme on the thermodynamic driving forces of F420 coenzyme to release hydride ions, hydrogen atoms and electrons is also valuable chemical information to understand the function of the uracil structure in biologically active molecules. In this work, N-methyl-9,10-didihydroacridine ($AcrH_2$) was also chosen as another artificial model of F420 coenzyme for comparison (Scheme 4).

In addition, since the most biochemical processes with F420 coenzymes as hydride and electron sources in living body all take place in the polar organic regions constructed with enzyme proteins rather than with pure water, the chemical information of F420 coenzyme as hydride and electron sources in the polar organic regions constructed with enzyme proteins should be more important and valuable than that in the pure aqueous solution. In order to derive the characteristic chemical information of F420 coenzyme as hydride and electron sources in the polar organic regions constructed with enzyme proteins, in this work, acetonitrile was chosen as the solvent to imitate the polar organic regions constructed with enzyme proteins, because the polarity of acetonitrile (ε = 37.5) is quite close to that of peptide bond in proteins (ε = 37.0, 37.8 and 38.3 for $HCONMe_2$, $MeCONMe_2$ and N,N-dimethylbenzamide, respectively).[21]



Scheme 3. Possible pathways of the reduced F420 coenzyme to release hydride ions.

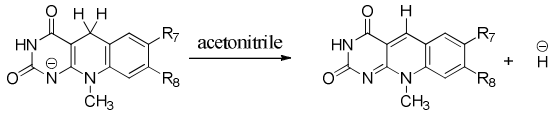


Scheme 4. Structures of the model compounds of the reduced F420 and NADH coenzymes examined in this work.

Results

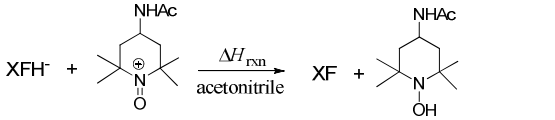
The thermodynamic driving force of the reduced F420 coenzyme models (XFH^-) (Scheme 4) to release hydride ions in this work is defined as the molar enthalpy change of the reduced F420 coenzyme models (XFH^-) to release hydride ions to form the corresponding the oxidized F420 coenzyme models (XF) in acetonitrile at 298 K (eqs 1 and 2), $\Delta H_{H^-D}(XFH^-)$, which can be used to directly scale the hydride-donating ability of the reduced F420 coenzyme models in acetonitrile. Since the free hydride anion in acetonitrile is not available, it is difficult to directly determine the thermodynamic driving force of the reduced F420 coenzyme models in acetonitrile using conventional experimental methods. However, the thermodynamic driving force of the reduced F420 coenzyme models (XFH^-) to release hydride ions in acetonitrile, $\Delta H_{H^-D}(XFH^-)$, can be obtained from the molar reaction enthalpy change of XFH^- with a strong hydride acceptor, $TEMPO^+$ (eqs 3 and 4), because Figure 1 gave a strong evidence that the reaction of XFH^- with $TEMPO^+$ in acetonitrile takes place by hydride transfer. In eq 4, ΔH_{rxn} is the molar enthalpy change of the reaction in acetonitrile (eq 3), which can be determined using titration calorimetry (Figure 2);[22] $\Delta H_{H^-A}(TEMPO^+)$ is the molar enthalpy change of $TEMPO^+$ to accept hydride ions in acetonitrile, i.e., the hydride affinity of $TEMPO^+$ in acetonitrile, which has been determined previously in our group (-105.6 kcal/mol).[23-24] The molar enthalpy changes of the reactions of XFH^- with $TEMPO^+$ in acetonitrile (eq 3) are listed in Table 1. The thermodynamic driving forces of the reduced F420 coenzyme models (XFH^-) to release hydride ions in acetonitrile are summarized in Table 2.

The thermodynamic driving forces of the reduced F420 coenzyme models (XFH^-) to release hydrogen atoms as well as the thermodynamic driving forces of $XFH^\bullet$ to release hydrogen atoms and protons in this work are also defined as the molar enthalpy changes of the corresponding chemical processes. In order to obtain the molar enthalpy change values of XFH^- to release hydrogen atoms, as well as the molar enthalpy change values of $XFH^\bullet$ to release hydrogen atoms and protons in acetonitrile, three thermodynamic cycles were constructed according to the chemical process of XFH^- to release hydride ions to form the corresponding XF in acetonitrile (Scheme 5). From the three thermodynamic cycles, three eqs 5-7 can be derived according to Hess's law.[25] In eqs 5-7, $\Delta H_{H^-D}(XFH^-)$ and $\Delta H_{HD}(XFH^-)$ are the molar enthalpy changes of XFH^- to release hydride ions and hydrogen atoms in acetonitrile, respectively; $\Delta H_{HD}(XFH^\bullet)$ and $\Delta H_{PD}(XFH^\bullet)$ are the molar enthalpy changes of $XFH^\bullet$ to release hydrogen atoms and protons in acetonitrile, respectively; $E^o_{ox}(XFH^-)$, $E^o_{red}(XF)$, $E^o(H^{0/-})$ and $E^o(H^{+/0})$ are the standard redox potentials of XFH^-, XF, H^+ and H^- in acetonitrile, respectively. It's obvious that $\Delta H_{HD}(XFH^-)$, $\Delta H_{HD}(XFH^\bullet)$ and $\Delta H_{PD}(XFH^\bullet)$ can be obtained as long as the $\Delta H_{H^-D}(XFH^-)$, $E^o_{ox}(XFH^-)$, $E^o_{red}(XF)$, $E^o(H^{0/-})$ and $E^o(H^{+/0})$ are available. In fact, $\Delta H_{H^-D}(XFH^-)$ are available from the above work in Table 2; $E^o(H^{0/-})$ and $E^o(H^{+/0})$ can be achieved from literature;[26] $E^o_{ox}(XFH^-)$ and $E^o_{red}(XF)$ can be obtained from direct experimental measurements (Figure 3). The detailed values of $\Delta H_{H^-D}(XFH^-)$, $\Delta H_{HD}(XFH^-)$, $\Delta H_{HD}(XFH^\bullet)$ and $\Delta H_{PD}(XFH^\bullet)$ together with the standard oxidation potentials of XFH^-, $E^o_{ox}(XFH^-)$, and the standard reduction potentials of XF, $E^o_{red}(XF)$, in acetonitrile are all summarized in Table 2.



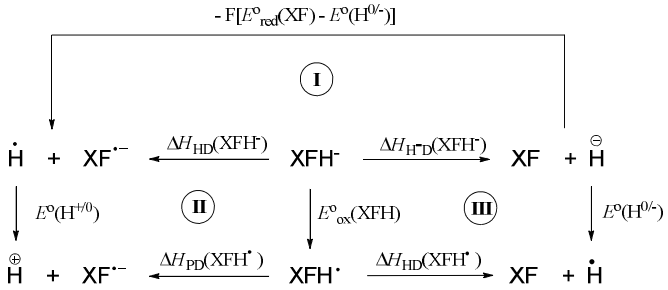
XFH^- XF (1)

$$\Delta H_{H^-D}(XFH^-) = H_f(XF) + H_f(H^-) - H_f(XFH^-) \quad (2)$$



$TEMPO^+$ TEMPOH (3)

$$\Delta H_{H^-D}(XFH^-) = \Delta H_{rxn} - \Delta H_{H^-A}(TEMPO^+) \quad (4)$$



Scheme 5. Related Thermodynamic Cycles for the Constructions of eqs 5-7.

$$\Delta H_{HD}(XFH^-) = \Delta H_{H^-D}(XFH^-) - F[E_{red}(XF) - E^o(H^{0/-})] \quad (5)$$
$$\Delta H_{PD}(XFH^\bullet) = \Delta H_{HD}(XFH^-) - F[E_{ox}(XFH^-) - E^o(H^{+/0})] \quad (6)$$
$$\Delta H_{HD}(XFH^\bullet) = \Delta H_{H^-D}(XFH^-) - F[E_{ox}(XFH^-) - E^o(H^{0/-})] \quad (7)$$

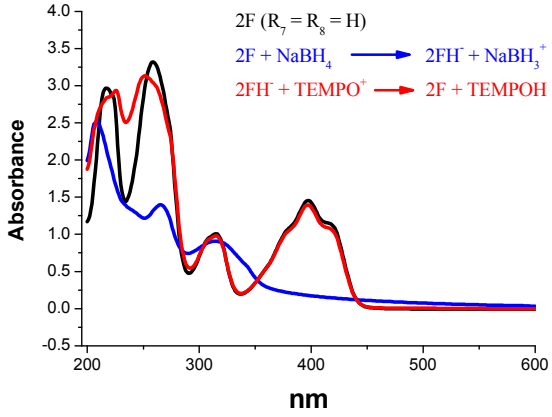


Figure 1. UV-vis spectra: the spectrum of pure 2F ($R_7 = R_8 =$ H) (7.0 mg) in 100 mL acetonitrile (black line), after adding $NaBH_4$ (1.5 mg, ~1.3 eq) in situ (blue line) and then by adding $TEMPO^+$ (14.0 mg, ~1.4 eq) (red line), the absorption of 2F around 400 nm appeared again.

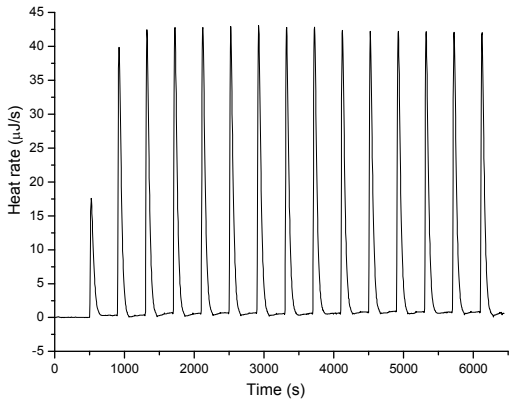


Figure 2. Isothermal titration calorimetry (ITC) for the reaction heat of $6FH^-$ (R_7 = R_8 = H) with $TEMPO^+$ in acetonitrile at 25 °C. Titration was conducted by adding 15 μL of $TEMPO^+$ (2.9 mM) every 400s into the acetonitrile containing the $6FH^-$ (ca. 8.0 mM), which was obtained in situ from the reaction of the corresponding 6F with $NaBH_4$.

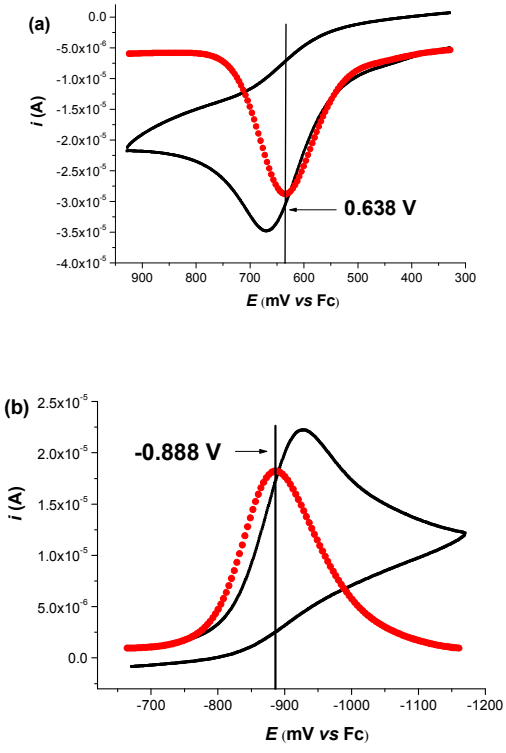


Figure 3. (a) Cyclic voltammetry (CV) and Osteryoung Square Wave Voltammetry (OSWV) of **$6FH^-$** (R_7 = R_8 = H), and (b) CV and OSWV of **6F** (R_7 = R_8 = H) in deaerated acetonitrile containing 0.1 M *n*-Bu_4NPF_6 as supporting electrolyte. The full black line: CV graph (sweep rate = 0.1 V/s), the dashed red line: OSWV graph.

Discussion

Redox Potential Scale of XFH^- and XF in Acetonitrile.

As chemical reactions with the F420 coenzyme models as hydride donors often involves electron transfer, the standard redox potentials of F420 coenzyme models should be a very important thermodynamic parameter to evaluate the electron-donating ability of the reduced forms and the electron-gaining ability of the oxidized forms. From column 8 in Table 2 it is clear that the standard oxidation potential of XFH^- in acetonitrile is 0.316V for $1FH^-$, and ranges from 0.188 to 0.316V for $2FH^-$, from 0.114 to 0.200V for $3FH^-$, from 0.192 to 0.222V for $4FH^-$, from 0.144 to 0.210V for $5FH^-$ and from 0.590 to 0.694V for $6FH^-$, respectively. Since the standard oxidation potentials of XFH^- except $6FH^-$ in acetonitrile are not large positive values, generally smaller than 0.3 V (vs Fc), the reduced F420 models, XFH^-, except $6FH^-$ should be all good electron-donors. In order to visually compare the relative electron-donating ability of XFH^- and examine the effects of charge and methyl group at the different N-positions on it, a number axis of the standard oxidation potentials of XFH^- with R_7 = R_8 = H, MNAH and $AcrH_2$ in acetonitrile is made as shown in Scheme 6.

From Scheme 6, it is clear that the standard oxidation potentials of XFH^-, MMAH and $AcrH_2$ in acetonitrile increase in the order of MNAH < $3FH^-$ < $5FH^-$ < $4FH^-$ < $2FH^-$ < $1FH^-$ < $AcrH_2$ < $6FH^-$, which means that the electron-donating abilities of the eight electron donors in acetonitrile is MNAH > $3FH^-$ > $5FH^-$ > $4FH^-$ > $2FH^-$ > $1FH^-$ > $AcrH_2$ > $6FH^-$. If the standard oxidation potentials of $1FH^-$-$5FH^-$ and that of $6FH^-$ are compared, it is found that the standard oxidation potentials of $1FH^-$-$5FH^-$ is more negative than that of $6FH^-$ by more than 0.322 V, which means that the electron-donating abilities of $1FH^-$-$5FH^-$ are stronger than that of $6FH^-$ by more than 7.4 kcal/mol. Since each of $1FH^-$-$5FH^-$ carries one negative charge, but $6FH^-$ does not, it is conceived that the negative charge can remarkably enlarge the electron-donating abilities of XFH^-. If the standard oxidation potentials of $3FH^-$ (0.143 V) and $5FH^-$ (0.166V) and those of $1FH^-$ (0.316 V), $2FH^-$ (0.226 V) and $4FH^-$ (0.202 V) are compared, it is found that the electron-donating effect of the negative charge at N_{10} position ($3FH^-$ and $5FH^-$) is greater than that at N_1 position ($1FH^-$, $2FH^-$ and $4FH^-$). The reason is that the negative charge at N_1 can be withdrawn by carbonyl oxygen atom at ortho-position. If the standard oxidation potentials of $1FH^-$ (0.316 V), $2FH^-$ (0.226 V) and $4FH^-$ (0.202 V) are examined, it is found that the electron-donating ability of $2FH^-$ is greater than that of $1FH^-$, but smaller than that of $4FH^-$, which means that for the F420 models with the negative charge at N_1 position, methyl group is an electron-donating group and the electron-donating ability of the methyl group at N_{10} is greater than that at N_3. However, if the standard oxidation potentials of $3FH^-$ and $5FH^-$ are compared, it is found that the electron-donating ability of $3FH^-$ is greater than that of $5FH^-$, which means that for the F420 models with the negative charge at N_{10} position, methyl group is an electron-withdrawing group rather than an electron-donating group.

In addition, from Scheme 6, it is also clear that if the oxidation potentials of $2FH^-$ (0.226 V) and MNAH (0.136 V) are compared, the electron-donating ability of MNAH is slightly greater than that of $2FH^-$. Since the structure of $2FH^-$ resembles that of the natural reduced F420 coenzyme and the structure of MNAH resembles that of the natural NADH coenzyme, it is conceivable that the electron-donating ability of the reduced F420 coenzyme should be close to or slightly

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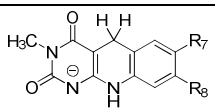
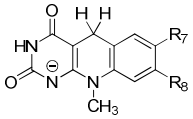
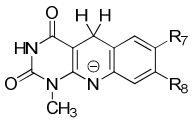
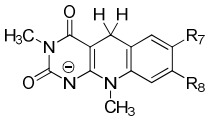
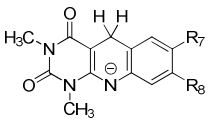
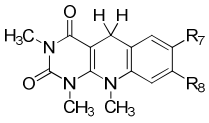
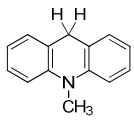
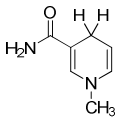
Table 1: The Molar Reaction Enthalpy Changes of XFH^- with $TEMPO^+$ in Acetonitrile at 25 °C (kcal/mol), the Oxidation Potential of XFH^- and reduction Potential of XF in Acetonitrile at 25 °C (V vs $Fc^{+/0}$)

				$E_{ox}(XFH^-)$[b]		$E_{red}(XF)$	
XFH^-	R_7	R_8	ΔH_{rxn}[a]	CV	OSWV	CV	OSWV
	H	H	-67.2	0.338	0.316	-1.703	-1.666
($1FH^-$)							
	H	H	-55.2	0.257	0.226	-1.593	-1.560
	CH_3	H	-53.6	0.235	0.205	-1.626	-1.598
	Cl	H	-63.4	0.347	0.316	-1.358	-1.326
	H	CH_3	-51.2	0.219	0.188	-1.662	-1.632
($2FH^-$)							
	H	H	-72.9	0.161	0.143	-1.858	-1.832
	CH_3	H	-74.1	0.166	0.135	-1.870	-1.843
	Cl	H	-66.5	0.232	0.200	-1.759	-1.72
	H	CH_3	-75.9	0.147	0.114	-1.922	-1.882
($3FH^-$)	H	Cl	-68.9	0.219	0.186	-1.781	-1.748
	H	H	-51.9	0.236	0.202	-1.607	-1.574
	CH_3	H	-50.6	0.231	0.198	-1.629	-1.590
	Cl	H	-61.5	0.258	0.222	-1.492	-1.456
	H	CH_3	-47.6	0.228	0.192	-1.666	-1.634
($4FH^-$)	H	Cl	-58.9	0.246	0.214	-1.499	-1.472
	H	H	-68.4	0.200	0.166	-1.947	-1.914
	CH_3	H	-69.9	0.183	0.157	-1.957	-1.938
	Cl	H	-61.4	0.241	0.210	-1.806	-1.774
	F	H	-62.3	0.231	0.201	-1.836	-1.810
($5FH^-$)	H	CH_3	-72.3	0.182	0.144	-2.004	-1.974
	H	Cl	-63.8	0.221	0.192	-1.831	-1.808
	H	H	-28.5	0.667	0.638	-0.920	-0.888
	OCH_3	H	-28.2	0.677	0.649	-0.898	-0.864
	CH_3	H	-29.4	0.657	0.620	-0.918	-0.898
	Cl	H	-25.6	0.734	0.694	-0.808	-0.786
	F	H	-25.8	0.700	0.672	-0.841	-0.808
($6FH^-$)	Br	H	-26.0	0.731	0.694	-0.833	-0.802
	H	OCH_3	-31.0	0.625	0.590	-0.991	-0.954
	H	CH_3	-29.2	0.636	0.608	-0.960	-0.924
	H	Cl	-26.0	0.737	0.696	-0.859	-0.812
	H	F	-27.8	0.702	0.652	-0.923	-0.870
	H	Br	-27.2	0.718	0.686	-0.839	-0.808
			-24.5	0.488	0.460	-0.817	-0.787
($AcrH_2$)							

-44.9	0.164	0.136	-1.501	-1.470



(MNAH)

[a] ΔH_{rxn} obtained by titration calorimetry in dry acetonitrile and expressed in kcal/mol were average values of at least two independent runs, each of which was an average value of 14 consecutive titrations, except the first. The reproducible is ±0.5 kcal/mol. [b] Measured by CV and OSWV methods in acetonitrile at 25 °C, the unit is volts vs. Fc^+/Fc^0 and reproducible to 5 mV or better.

Table 2. The Molar Enthalpy Changes of XFH^- to Release Hydride ions and Hydrogen Atoms, the Molar Enthalpy Changes of $XFH^\bullet$ to Release Protons and Hydrogen Atoms together with the Standard Oxidation Potential of XFH^- and the Standard Reduction Potential of XF in Acetonitrile

XFH^-	R_7	R_8	$\Delta H_{H^-D}(XFH^-)$[a]	$\Delta H_{HD}(XFH^-)$[b]	$\Delta H_{HD}(XFH^\bullet)$[b]	$\Delta H_{PD}(XFH^\bullet)$[c]	$E^o_{ox}(XFH^-)$[d]	$E^o_{red}(XF)$[e]
$1FH^-$	H	H	38.0	50.4	4.7	-9.9	0.316	-1.666
$2FH^-$	H	H	50.1	60.0	18.8	1.8	0.226	-1.560
	CH_3	H	51.6	62.5	20.9	4.7	0.205	-1.598
	Cl	H	41.8	46.4	8.5	-14.0	0.316	-1.326
	H	CH_3	54.0	65.7	23.7	8.3	0.188	-1.632
$3FH^-$	H	H	32.3	48.5	3.0	-7.8	0.143	-1.832
	CH_3	H	31.1	47.6	1.9	-8.5	0.135	-1.843
	Cl	H	38.7	52.4	8.1	-5.2	0.200	-1.72
	H	CH_3	29.3	46.7	0.7	-8.9	0.114	-1.882
	H	Cl	36.3	50.6	6.0	-6.7	0.186	-1.748
$4FH^-$	H	H	53.3	63.6	22.7	6.0	0.202	-1.574
	CH_3	H	54.6	65.3	24.1	7.7	0.198	-1.590
	Cl	H	43.7	51.3	12.6	-6.8	0.222	-1.456
	H	CH_3	57.6	69.2	27.1	11.8	0.192	-1.634
	H	Cl	46.3	54.2	15.3	-3.7	0.214	-1.472
$5FH^-$	H	H	36.8	54.9	6.9	-1.9	0.166	-1.914
	CH_3	H	35.3	54.0	5.6	-2.7	0.157	-1.938
	Cl	H	43.8	58.7	13.0	0.9	0.210	-1.774
	F	H	42.9	58.6	12.2	1.0	0.201	-1.810
	H	CH_3	32.9	52.4	3.6	-3.9	0.144	-1.974
	H	Cl	41.4	57.1	11.0	-0.3	0.192	-1.808
$6FH^-$	H	H	76.7	71.2	36.0	3.5	0.638	-0.888
	OCH_3	H	77.0	70.9	36.0	2.9	0.649	-0.864
	CH_3	H	75.8	70.5	35.5	3.2	0.620	-0.898
	Cl	H	79.8	71.9	37.8	2.9	0.694	-0.786
	F	H	79.4	72.0	37.9	3.5	0.672	-0.808
	Br	H	79.2	71.6	37.1	2.6	0.694	-0.802
	H	OCH_3	74.2	70.2	34.6	3.6	0.590	-0.954
	H	CH_3	76.0	71.3	36.0	4.3	0.608	-0.924
	H	Cl	79.2	71.9	37.1	2.9	0.696	-0.812
	H	F	77.4	71.5	36.4	3.4	0.652	-0.870
	H	Br	78.0	70.6	36.2	1.8	0.686	-0.808
$AcrH_2$			81.1	73.0	44.2	9.2	0.460	-0.787
MNAH			60.7	68.4	31.3	12.0	0.136	-1.470

[a] $\Delta H_{H^-D}(XFH^-)$ values of XFH^- were estimated from eq 4, taking $\Delta H_{H^-A}(TEMPO^+)$ = -105.6 kcal/mol. [b] $\Delta H_{HD}(XFH^-)$, $\Delta H_{HD}(XFH^\bullet)$ and $\Delta H_{PD}(XFH^\bullet)$ were estimated from eqs 5～7, respectively, taking $E^o(H^{+/0})$ = -2.307 (V *vs* $Fc^{+/0}$), $E^o(H^{0/-})$ = -1.137 V (V *vs* $Fc^{+/0}$) (Fc = ferrocene), and choosing the redox potentials of XFH^- and XF measured by OSWV method (Table 1) as $E^o_{ox}(XFH^-)$ and $E^o_{red}(XF)$, since the values from OSWV were identified to be closer to the corresponding standard redox potentials than the values from CV.[27] [c] The values derived by OSWV were chosen as the standard redox potentials of XFH^- and XF.

greater than that of NADH coenzyme in vivo, which means that the reduced F420 coenzyme like NADH coenzyme can provide electrons in the conversion and biosynthesis of some important living active molecules in vivo. If the oxidation potentials of $6FH^-$ (0.638 V) and $AcrH_2$ (0.460 V) are compared, it is found that the electron-donating ability of $6FH^-$ is remarkably smaller than that of $AcrH_2$, which means that the uracil group is an electron-withdrawing group with respect to the benzene group.

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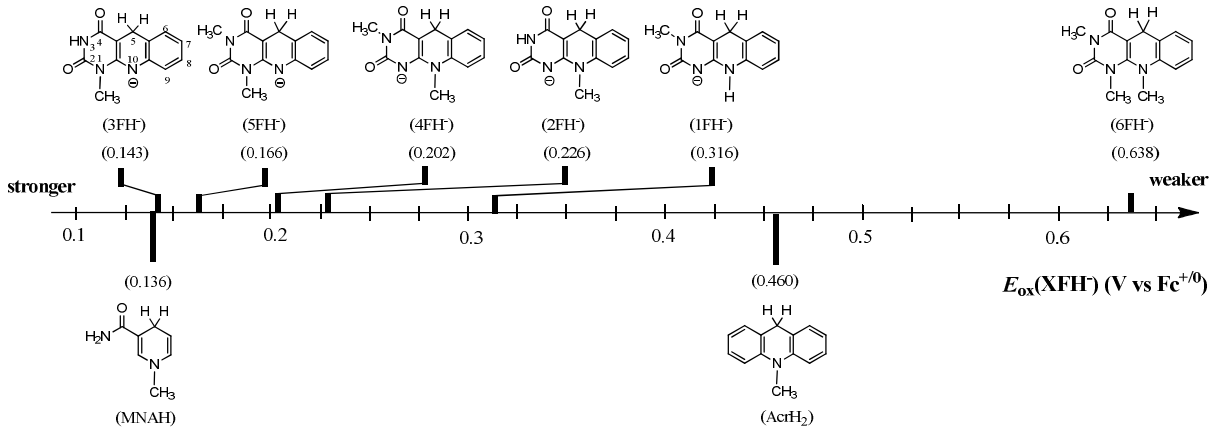
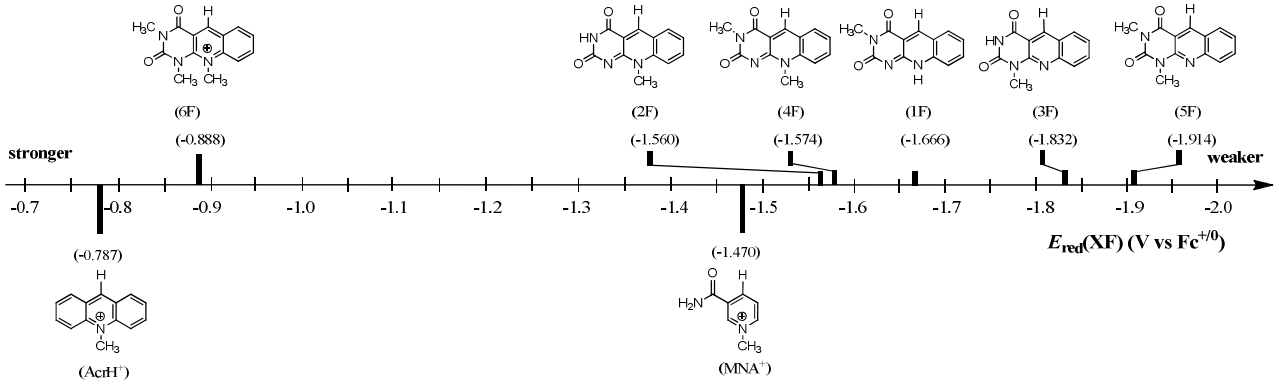
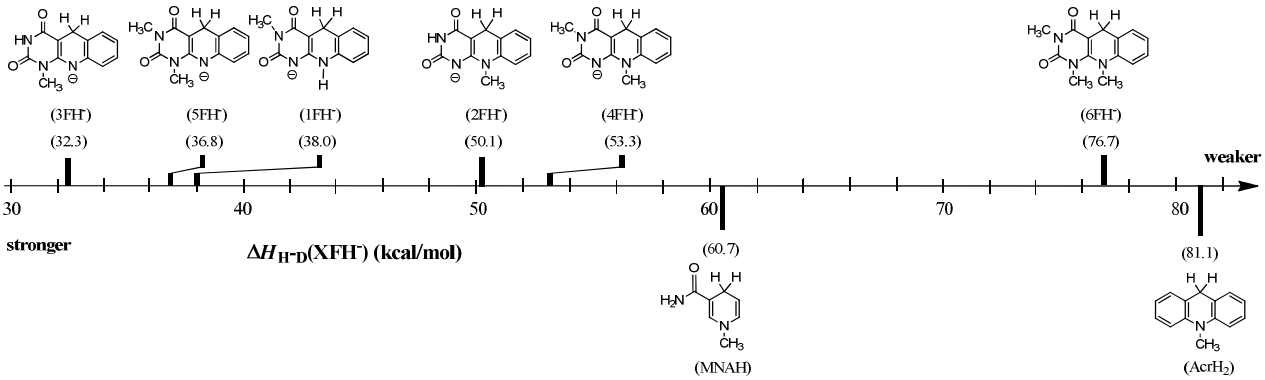


Scheme 6. Electron-Donating Ability Order of XFH^- ($R_7 = R_8 = H$), MNAH and $AcrH_2$ in Acetonitrile.



Scheme 7. Electron-Gaining Ability Order of XF ($R_7 = R_8 = H$), MNA^+ and $AcrH^+$ in Acetonitrile.



Scheme 8. Hydride-Donating Ability Order of XFH^- ($R_7 = R_8 = H$), MNAH and $AcrH_2$ in Acetonitrile.

Concerning the electron-gaining abilities of the oxidized F420 coenzyme models, XF, as one-electron oxidants, we can find from column nine in Table 2 that the reduction potential of XF is -1.666V for 1F and ranges from -1.632 to -1.326V for 2F, from -1.882 to -1.720V for 3F, from -1.634 to -1.456V for 4F, from -1.974 to -1.774V for 5F and from -0.954 to -0.786V for 6F, respectively. Since the reduction potentials of XF except 6F are all much negative values, the electron-gaining abilities of the oxidized F420 coenzyme

models, XF, except 6F should be all quite weak. In view of the structures of 1F-5F more similar to that of F420 coenzyme than that of 6F, it is conceivable that the electron-gaining ability of the oxidized F420 coenzyme in vivo should be quite weak also, which means that the conversion of F420 coenzyme from the oxidized form to the reduced form in vivo is quite difficult by initial electron transfer.

For Scheme 7, if the reduction potentials of 1F (-1.666 V) and 4F (-1.574 V), 1F (-1.666 V) and 5F (-1.914 V) as well as 2F (-1.560 V) and 4F (-1.574 V) are compared, respectively, it is clear that the result suggests that in the oxidized F420 coenzyme models, XF, methyl group is an electron-withdrawing group at N_{10} position, but an electron-donating group at N_1 and N_3 positions. If the reduction potentials of 1F (-1.666 V) and 3F (-1.832 V) are compared, it is found that the electron-donating ability of methyl group at N_1 atom is remarkably greater than that at N_3 atom.

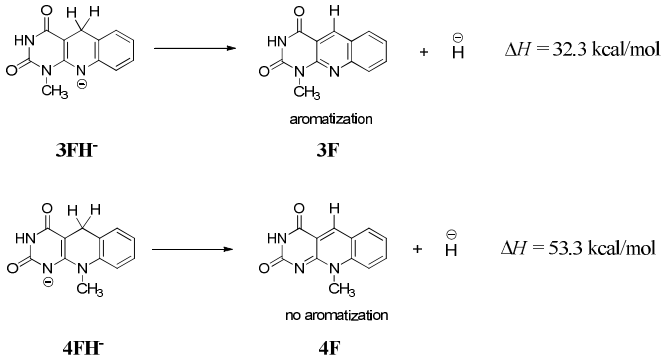
In addition, from Scheme 7, it is also found that the reduction potential of 2F (-1.560 V) is slightly more negative than that of MNA^+ (-1.470 V), which means that the electron-gaining ability of 2F is slightly smaller than that of MNA^+. Since the structure of 2F is like that of F420 coenzyme and the structure of MNA^+ resembles that of NAD^+, it is conceived that the electron-gaining ability of the oxidized F420 coenzyme in vivo should be weaker than that of NAD^+. If the reduction potentials of 6F (-0.888 V) and $AcrH^+$ (-0.787 V) are compared, it is also found that the electron-obtaining ability of 6F is smaller than that of $AcrH^+$, which means that that the uracil group is an electron-donating group rather than an electron-withdrawing group relative to benzene group, which is just reverse to the case that the reduced F420 coenzyme models, XFH^-, release electrons.

Thermodynamic Driving Force Scale of XFH^- to Release Hydride Ions in Acetonitrile.

As the thermodynamic driving force scale of XFH^- to release hydride ions in acetonitrile is described in this work using the molar enthalpy changes of XFH^- to release hydride ions in acetonitrile, the thermodynamic driving forces of XFH^- to release hydride ions in acetonitrile are examined according to the corresponding molar enthalpy changes of XFH^- to release hydride ions in acetonitrile.

From column 4 in Table 2, we can see that the molar enthalpy changes of the reduced F420 coenzyme models to release hydride ions in acetonitrile is 38.0 kcal/mol for $1FH^-$, and range from 41.8 to 54.0 kcal/mol for $2FH^-$, from 29.3 to 36.3 kcal/mol for $3FH^-$, from 43.7 to 57.6 kcal/mol for $4FH^-$, from 32.9 to 43.8 kcal/mol for $5FH^-$, and from 74.2 to 79.8 kcal/mol for 6FH, respectively. Since the molar enthalpy changes of the reduced F420 coenzyme models, XFH^- except $6FH^-$ to release hydride ions in acetonitrile are all quite small positive values, the reduced F420 coenzyme models, XFH^-, except $6FH^-$ should all be strong hydride-donors. In view of the structures of $1FH^-$-$5FH^-$ much more close to the core structure of the natural reduced F420 coenzyme than that of $6FH^-$, it is conceivable that the natural reduced F420 coenzyme should be very strong hydride donors as $1FH^-$-$5FH^-$.

From Scheme 8, it is clear that when the molar enthalpy changes of $1FH^-$ (38.0 kcal/mol) and $4FH^-$ (53.3 kcal/mol) to release hydride ions in acetonitrile are compared, it is found that hydride-donating ability of $1FH^-$ is greater than that of $4FH^-$ by 15.3 kcal/mol, meaning that methyl group at N_{10} position is a hydride-withdrawing group. When the molar enthalpy changes of $3FH^-$ (32.3 kcal/mol) and $5FH^-$ (36.8 kcal/mol) to release hydride ions in acetonitrile are compared, it is found that hydride-donating ability of $3FH^-$ is greater than that of $5FH^-$ by 4.5 kcal/mol, meaning that methyl group at N_3 is a hydride-withdrawing group. When the molar enthalpy changes of $1FH^-$ (38.0 kcal/mol) and $2FH^-$ (50.1 kcal/mol) to release hydride ions in acetonitrile are compared, it is found that hydride-donating ability of $1FH^-$ is greater than that of $2FH^-$ by 12.1 kcal/mol, meaning that the hydride-donating ability of methyl group at N_{10} position is larger than that at N_3 position by 12.1 kcal/mol. When the molar enthalpy changes of $3FH^-$ (32.3 kcal/mol) and $4FH^-$ (53.3 kcal/mol) to release hydride ions in acetonitrile are compared, it is found that the hydride-donating ability of $4FH^-$ is greater than that of $3FH^-$ by 21.0 kcal/mol. The main reason is that 3F has aromaticity, but 4F does not.



In addition, from Scheme 8, it is also clear that if the molar enthalpy changes of MNAH (60.7 kcal/mol) and $2FH^-$ (50.1 kcal/mol) to release hydride ions in acetonitrile are compared, the hydride-donating ability of $2FH^-$ is greater than that of MNAH by 10.6 kcal/mol, which means that the hydride-donating ability of the reduced F420 coenzyme could be greater than that of NADH coenzyme by about 10 kcal/mol in vivo, because the structures of $2FH^-$ and MNAH are quite similar to those of F420 and NADH, respectively. If the molar enthalpy changes of $6FH^-$ (76.7 kcal/mol) and $AcrH_2$ (81.1 kcal/mol) to release hydride ions in acetonitrile are compared, it is also clear that the hydride-donating ability of $6FH^-$ is remarkably greater than that of $AcrH_2$, which means that the uracil group is an hydride-donating group with respect to the benzene group.

Thermodynamic Driving Force Scale of XFH^- to Release Hydrogen Atoms in Acetonitrile.

From column 5 in Table 2, it is found that the molar enthalpy change scale of XFH^- to release hydrogen atoms in acetonitrile is 50.4 kcal/mol for $1FH^-$, and ranges from 46.4 to 65.7 kcal/mol for $2FH^-$, from 46.7 to 52.4 kcal/mol for $3FH^-$, from 51.3 to 69.2 kcal/mol for $4FH^-$, from 52.4 to 58.7 kcal/mol for $5FH^-$, and from 70.2 to 72.0 kcal/mol for $6FH^-$, respectively. Since the molar enthalpy changes of the reduced F420 coenzyme models, XFH^- to release hydrogen atoms in acetonitrile are all quite small, all much smaller than that of the reduced vitamin C with one negative charge (65.4 kcal/mol),[28,29] a well-known good hydrogen atom donor, the reduced F420 coenzyme models with one negative charge, XFH^-, should all be strong hydrogen atom donors, which means the reduced F420 coenzyme with one negative charge should be a good antioxidant in vivo.

From Scheme 9, it is clear that if the molar enthalpy changes of $3FH^-$ (48.5 kcal/mol) and $4FH^-$ (63.6 kcal/mol) to release hydrogen atoms in acetonitrile are compared, it is found that the molar enthalpy change of $4FH^-$ is greater than that of $3FH^-$ to release hydrogen atoms by 15.1 kcal/mol, but the

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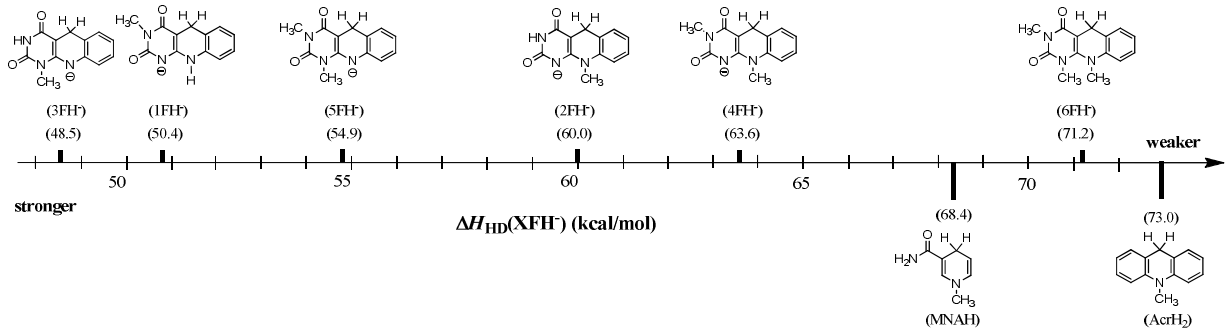
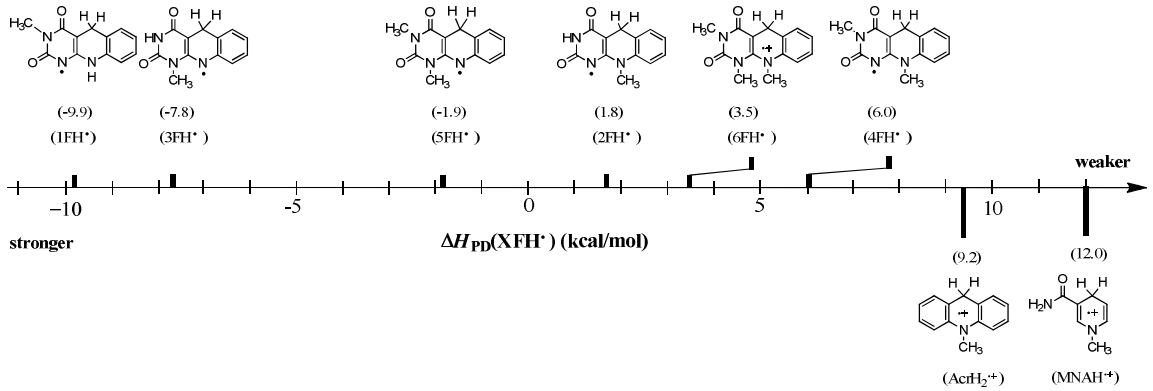
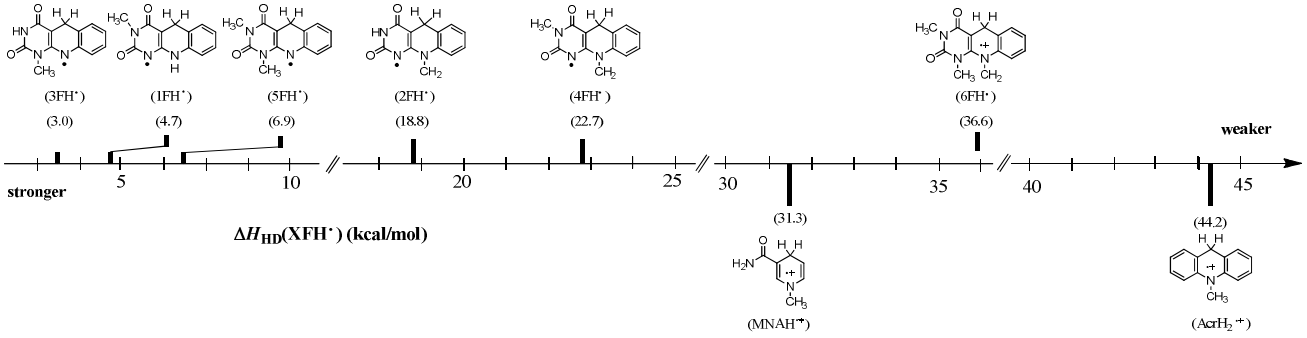


Scheme 9. Hydrogen Atom-Donating Ability Order of XFH^- ($R_7 = R_8 = H$), MNAH and $AcrH_2$ in Acetonitrile.



Scheme 10. Proton-Donating Ability Order of $XFH^•$ ($R_7 = R_8 = H$), $MNAH^{•+}$ and $AcrH_2^{•+}$ in Acetonitrile.



Scheme 11. Hydrogen Atom-Donating Ability Order of $XFH^•$ ($R_7 = R_8 = H$), $MNAH^{•+}$ and $AcrH2^{•+}$ in Acetonitrile.

difference is smaller than that of them to release hydride ions in acetonitrile (21.1 kcal/mol). The reason is that 3F has aromaticity, but $3F^{-•}$ has not. If the molar enthalpy changes of $1FH^-$ (50.4 kcal/mol) and $4FH^-$ (63.6 kcal/mol) to release hydrogen atoms in acetonitrile are compared, it is clear that the hydrogen-donating ability of $1FH^-$ is larger than that of $4FH^-$ by 13.2 kcal/mol, meaning that methylation of N_{10} atom can greatly weaken the ability of $4FH^-$ to release hydrogen atoms, the main reason could be that methyl presence is not conducive to the dihydropyridine ring planarization when one hydrogen atom at C_5 position is released.

In addition, if the molar enthalpy changes of MNAH (68.4 kcal/mol) and $2FH^-$ (60.0 kcal/mol) to release hydrogen atoms in acetonitrile are compared (see Scheme 9), it is clear that the hydrogen atom-donating ability of $2FH^-$ is greater than that of MNAH by 8.4 kcal/mol, meaning that the hydrogen-donating ability of the reduced F420 coenzyme with one negative charge could be

greater than that of NADH coenzyme by about 8 kcal/mol in vivo, because the structures of $2FH^-$ and NADH are quite similar to those of F420 and NADH, respectively. If the molar enthalpy changes of $6FH^-$ (71.2 kcal/mol) and $AcrH_2$ (73.0 kcal/mol) to release hydrogen atoms in acetonitrile are compared, it is also clear that the hydrogen-atom-donating ability of $6FH^-$ is slightly greater than that of $AcrH_2$, which means that the uracil group is a weaker hydrogen-atom-donating group than benzene group.

Thermodynamic Driving Force Scales of $XFH^\bullet$ to Release Protons and Hydrogen Atoms in Acetonitrile.

As well known, the reductions of F420 coenzyme in vivo often involve electron transfer as the initial step, which indicates that incipient radical cation intermediates can be formed and the proton transfer or hydrogen-atom transfer should be the following step. Therefore, the enthalpy changes of $XFH^\bullet$ to release protons and hydrogen atoms should be very important parameters for people to examine the reduction mechanism of F420 coenzyme and the chemical properties of the related reduction intermediates in vivo.

From the columns 6 and 7 in Table 2, we found that the enthalpy change or the enthalpy change scale of $XFH^\bullet$ to release protons is -9.9 kcal/mol for $1FH^\bullet$ and ranges from -14.0 to 8.3 kcal/mol for $2FH^\bullet$, from -8.9 to -5.2 kcal/mol for $3FH^\bullet$, from -6.8 to 11.8 kcal/mol for $4FH^\bullet$, from -3.9 to 1.0 kcal/mol for $5FH^\bullet$, from 1.8 to 5.5 kcal/mol for $6FH^\bullet$, respectively. The enthalpy change or the enthalpy change scale of $XFH^\bullet$ to release hydrogen atoms is 4.7 kcal/mol for $1FH^\bullet$ and ranges from 8.5 to 23.7 kcal/mol for $2FH^\bullet$, from 0.7 to 8.1 kcal/mol for $3FH^\bullet$, from 12.6 to 27.1 kcal/mol for $4FH^\bullet$, from 3.6 to 13.0 kcal/mol for $5FH^\bullet$, from 34.6 to 38.5 kcal/mol for $6FH^\bullet$, respectively. If the enthalpy changes of **$XFH^\bullet$** to release protons and the corresponding enthalpy changes of **$XFH^\bullet$** to release hydrogen atoms are compared, it is found that the proton-donating ability of **$XFH^\bullet$** is larger than the corresponding hydrogen-donating ability, which means that if the hydride transfer from XFH^- is initiated by electron transfer, the possibility of proton transfer in the second reaction step should be much larger than that of the hydrogen atom transfer in the second reaction step. That is, the e-H^+-e sequence hydride transfer should be most likely among the various possible multi-step mechanisms for the hydride transfer from the reduced F420 coenzyme, if the hydride transfer were initiated by single-electron transfer. Since the molar enthalpy changes of the most **$1FH^\bullet$-$5FH^\bullet$** to release protons are negative values, these compounds are unstable in thermodynamics and can release protons spontaneously.

$2FH^-$

$$\Delta H_{H\text{-}D}(2FH^-) = -21.613\ \Sigma(\sigma_p + \sigma_m) + 50.2 \quad (8)$$
$$\Delta H_{HD}(2FH^-) = -34.644\ \Sigma(\sigma_p + \sigma_m) + 59.9 \quad (9)$$
$$\Delta H_{PD}(2FH^\bullet) = -40.105\ \Sigma(\sigma_p + \sigma_m) + 1.7 \quad (10)$$
$$\Delta H_{HD}(2FH^\bullet) = -27.032\ \Sigma(\sigma_p + \sigma_m) + 19.0 \quad (11)$$
$$E_{ox}(2FH^-) = 0.232\ \Sigma(\sigma_p + \sigma_m) + 0.225 \quad (12)$$
$$E_{red}(2F) = 0.563\ \Sigma(\sigma_p + \sigma_m) - 1.550 \quad (13)$$

$3FH^-$

$$\Delta H_{H\text{-}D}(3FH^-) = 16.175\ \Sigma(\sigma_p + \sigma_m) + 32.1 \quad (14)$$
$$\Delta H_{HD}(3FH^-) = 9.652\ \Sigma(\sigma_p + \sigma_m) + 48.3 \quad (15)$$
$$\Delta H_{PD}(3FH^\bullet) = 6.135\ \Sigma(\sigma_p + \sigma_m) - 7.9 \quad (16)$$
$$\Delta H_{HD}(3FH^\bullet) = 12.726\ \Sigma(\sigma_p + \sigma_m) + 2.8 \quad (17)$$
$$E_{ox}(3FH^-) = 0.151\ \Sigma(\sigma_p + \sigma_m) + 0.142 \quad (18)$$
$$E_{red}(3F) = 0.285\ \Sigma(\sigma_p + \sigma_m) - 1.830 \quad (19)$$

$4FH^-$

$$\Delta H_{H\text{-}D}(4FH^-) = -24.547\ \Sigma(\sigma_p + \sigma_m) + 53.2 \quad (20)$$
$$\Delta H_{HD}(4FH^-) = -31.944\ \Sigma(\sigma_p + \sigma_m) + 63.5 \quad (21)$$
$$\Delta H_{PD}(4FH^\bullet) = -33.094\ \Sigma(\sigma_p + \sigma_m) + 5.8 \quad (22)$$
$$\Delta H_{HD}(4FH^\bullet) = -25.738\ \Sigma(\sigma_p + \sigma_m) + 22.6 \quad (23)$$
$$E_{ox}(4FH^-) = 0.051\ \Sigma(\sigma_p + \sigma_m) + 0.201 \quad (24)$$
$$E_{red}(4F) = 0.323\ \Sigma(\sigma_p + \sigma_m) - 1.573 \quad (25)$$

$5FH^-$

$$\Delta H_{H\text{-}D}(5FH^-) = 18.790\ \Sigma(\sigma_p + \sigma_m) + 36.4 \quad (26)$$
$$\Delta H_{HD}(5FH^-) = 10.799\ \Sigma(\sigma_p + \sigma_m) + 54.6 \quad (27)$$
$$\Delta H_{PD}(5FH^\bullet) = 8.341\ \Sigma(\sigma_p + \sigma_m) - 2.2 \quad (28)$$
$$\Delta H_{HD}(5FH^\bullet) = 16.302\ \Sigma(\sigma_p + \sigma_m) + 6.6 \quad (29)$$
$$E_{ox}(5FH^-) = 0.111\ \Sigma(\sigma_p + \sigma_m) + 0.164 \quad (30)$$
$$E_{red}(5F) = 0.346\ \Sigma(\sigma_p + \sigma_m) - 1.914 \quad (31)$$

$6FH^-$

$$\Delta H_{H\text{-}D}(6FH^-) = 7.698\ \Sigma(\sigma_p + \sigma_m) + 76.6 \quad (32)$$
$$\Delta H_{HD}(6FH^-) = 1.932\ \Sigma(\sigma_p + \sigma_m) + 71.0 \quad (33)$$
$$\Delta H_{PD}(6FH^\bullet) = -1.743\ \Sigma(\sigma_p + \sigma_m) + 3.3 \quad (34)$$
$$\Delta H_{HD}(6FH^\bullet) = 3.930\ \Sigma(\sigma_p + \sigma_m) + 36.0 \quad (35)$$
$$E_{ox}(6FH^-) = 0.159\ \Sigma(\sigma_p + \sigma_m) + 0.635 \quad (36)$$
$$E_{red}(6F) = 0.246\ \Sigma(\sigma_p + \sigma_m) - 0.884 \quad (37)$$

In Schemes 10 and 11, if $1FH^\bullet$ and $2FH^\bullet$ are compared, the proton-donating abilities and hydrogen atom-donating abilities of $1FH^\bullet$ are all greater than those of $2FH^\bullet$, which means that the methyl group at N_{10} can greatly weaken the abilities of $2FH^\bullet$ to donate protons and hydrogen atoms, the main reason could be that methyl presence is not conducive to the dihydropyridine ring planarization when the proton or hydrogen atom at C_5 position is released. If $3FH^\bullet$ and $5FH^\bullet$ are compared, the proton-donating abilities and hydrogen atom-donating abilities of $3FH^\bullet$ are all greater than those of $5FH^\bullet$, respectively, which means that the methyl group at N_3 can also remarkably weaken the ability of $5FH^\bullet$ to donate protons and hydrogen atoms, the reason could be that methyl presence at N_3 is not unfavorable to the adjustment in the structure of the uracil group when the proton or hydrogen atom at C_5 position is released. If $6FH^\bullet$ and $AcrH_2^{\bullet+}$ are compared, the proton-donating abilities and hydrogen atom-donating abilities of $6FH^\bullet$ are all greater than those of $AcH_2^{\bullet+}$, which means that the uracil group can promote the releasing of proton or hydrogen atom from $6FH^\bullet$.

The Effect of Substituents at R_7 and R_8 on the Thermodynamic Driving Forces of XFH^- to Release Hydride ions, Hydrogen Atoms and Electrons, Those of $XFH^\bullet$ to Release Protons and Hydrogen Atoms and That of $XF^{+\bullet}$ to Release Electrons.

From Table 2, it is clear that the molar enthalpy changes and the redox potentials of XFH^-, $XFH^\bullet$ and $XF^{+\bullet}$ are not only strongly dependent on the nature, number and position of the substituents at N atoms, but also dependent on the nature of the substituents at the at C_7 and C_8 positions. In order to elucidate the effect of the remote substituents on the enthalpy changes and the redox potentials, we do the Hammett linear relationship

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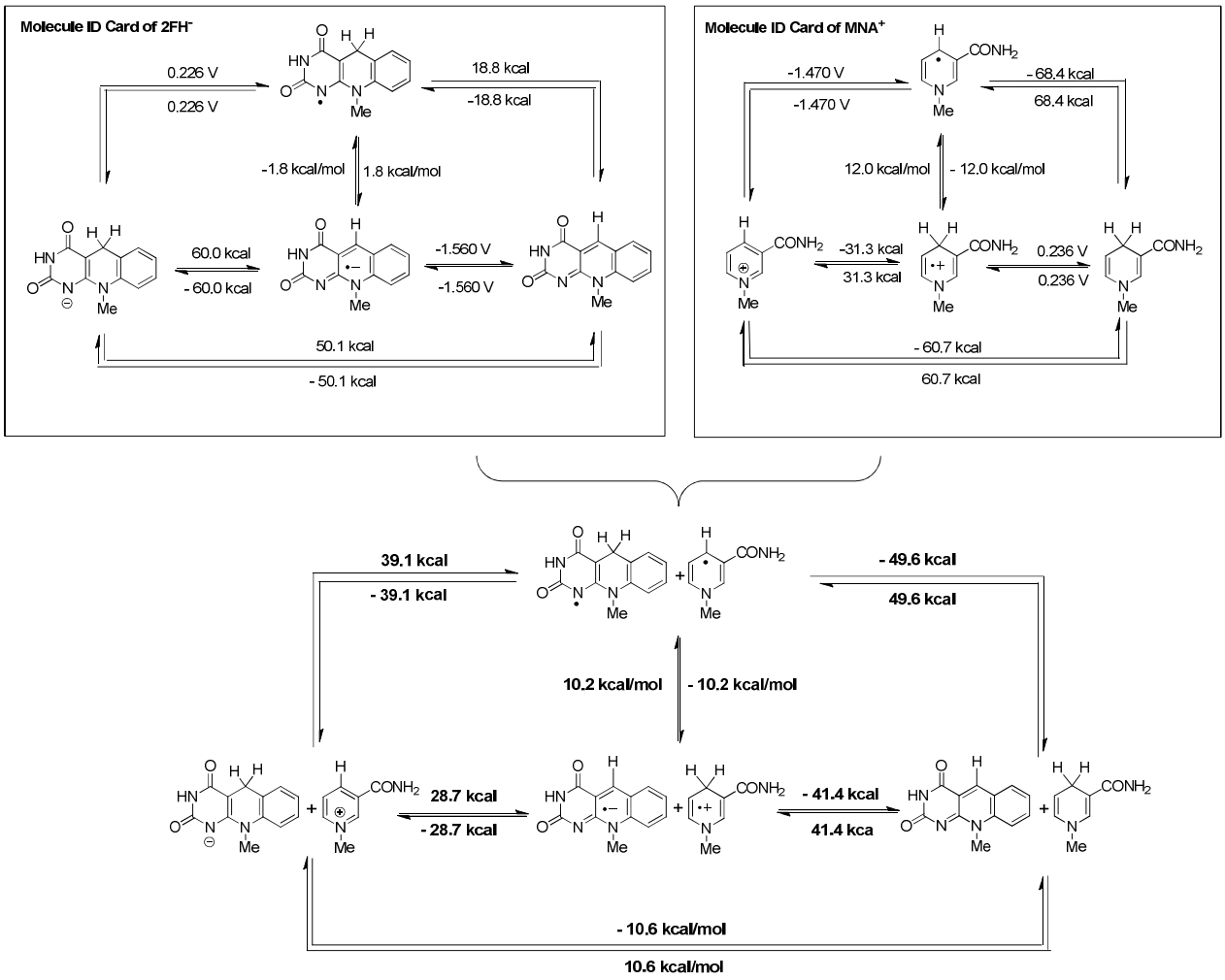

Scheme 12. Thermodynamic Analysis Platform on the Possible Reaction of the Members between $2FH^-$ Family and MNA^+ Family in Acetonitrile.

between the sum of Hammett substituent parameters (σ_p and σ_m) and the redox potentials and the enthalpy changes (Figures S1 and S2). From the figures it is found that $\Delta H_{H\text{-}D}(XFH^-)$, $\Delta H_{HD}(XFH^-)$, $\Delta H_{PD}(XFH^\bullet)$ and $\Delta H_{HA}(XFH^\bullet)$ as well as $E_{ox}(XFH^-)$ and $E_{red}(XF)$ are all linearly dependent on the Hammett substituent parameters σ with very good correlation coefficients, which means that the Hammett linear free-energy relationships all hold in the 6 chemical and electrochemical processes. From the slopes and the intercepts of the 30 straight lines, the corresponding 30 mathematical formulas (eqs 8-37) can be easily derived. Evidently, for any substituted XFH^- at the C_7 and/or C_8 positions on the benzene ring, it is not difficult to estimate the values of $\Delta H_{H\text{-}A}(XFH^-)$, $\Delta H_{HA}(XFH^-)$, $\Delta H_{PA}(XFH^\bullet)$ and $\Delta H_{HA}(XFH^\bullet)$, as long as the corresponding Hammett substituent parameters (σ) are available, and the uncertainties of the estimations are generally less than 0.5 kcal/mol. In the same way, for any substituted XFH^- at the C_7 and/or C_8 positions on the benzene ring, the oxidation potentials of XFH^-, $E_{ox}(XFH^-)$, and the reduction potentials of XF, $E_{red}(XF)$, can also be reliably estimated if only the corresponding Hammett substituent parameters are available and the uncertainties of the estimations are generally smaller than 30 mV. Since the family of the substituent groups is very large, and the Hammett parameters of most substituents located at the para- and meta-position can be easily obtained from literature [30] it is evident that the 30 formulas (eq 8-37) should have very useful to predict the related thermodynamic parameters of the various XFH^- and their reaction intermediates.

Diagnoses of Possible Reactions of the Members between F420 Family and NADH Family.

Since F420 coenzyme and NADH coenzyme are two important bio-reductants and the hydride transfer from F420 and NADH could involve multi-step mechanism, it is conceived that F420 and NADH as well as their various reaction intermediates all could coexist in living body. In order to infer the possible reactions between F420 and NADH as well as their various reaction intermediates in vivo, a mimic thermodynamic analysis platform on the likely reactions among them was constructed according to Molecule ID Cards [31,32] of $2FH^-$ and MNAH in acetonitrile (Scheme 12). From Scheme 11, the following predictions on the reactions between the members of $2FH^-$ family and MNAH family can be made: (i) When $2FH^-$ and MNA^+ are mixed in acetonitrile, hydride is allowed from $2FH^-$ to MNA^+ to yield 2F and MNAH, but electron transfer and hydrogen atom transfer are forbidden. (ii) When neutral radical $2FH^\bullet$ and neutral radical $MNA^\bullet$ contact each other in acetonitrile, $2FH^-$ and MNA^+, $2F^{-\bullet}$ and $MNAH^{+\bullet}$ as well as 2F and MNAH could be formed by hydrogen, proton and electron transfers, respectively. (iii) When $2FH^\bullet$ and $MNA^\bullet$ met together in acetonitrile, $2FH^-$ and MNA^+ as well as 2F and MNAH could be formed by hydrogen transfer and electron transfer, respectively, but proton transfer is forbidden. (iv) When 2F and MNAH were mixed in acetonitrile, no reaction is allowed. Among the four couples of the reaction partners, the couple of 2F and MNAH is the most stable reaction partner, but the couple of $2FH^\bullet$ and $MNA^\bullet$ should be the reaction partner that has the most possible reactions. It is evident that these diagnoses should be useful for our understanding possible reactions of the members between F420 family and NADH family in vivo.

Conclusions

In this work, 32 F420 coenzyme models (XFH^-) including 6 types with the alkylation on the three different N atoms in the core structure are designed and synthesized. The thermodynamic driving forces of the 32 XFH^- to release hydride ions, hydrogen atoms and electrons in acetonitrile, the thermodynamic driving forces of $XFH^\bullet$ to release protons and hydrogen atoms in acetonitrile and the thermodynamic driving forces of $XF^{-\bullet}$ to release electrons in acetonitrile are determined. After examining the determined enthalpy changes and redox potentials as well as the effect of substituents and charge on the enthalpy changes and the redox potentials, the following conclusions can be made:

(1) The F420 coenzyme models with a negative charge ($1FH^-$-$5FH^-$) belong to strong hydride donors, the hydride-donating ability of $1FH^-$-$5FH^-$ even is greater than that of NADH model MNAH. The neutral F420 models ($6FH^-$) are weak hydride donors, but the hydride-donating ability is greater than that of NADH model $AcrH_2$.

(2) The F420 coenzyme models XFH^- all belong to weak electron donors, the electron-donating abilities of them are all smaller than that of NADH model MNAH.

(3) The F420 coenzyme models with a negative charge ($1FH^-$-$5FH^-$) are good hydrogen atom donors, the hydrogen atom-donating ability of $1FH^-$-$5FH^-$ is not only greater than that of NADH model $AcrH_2$, but also greater than that of NADH model MNAH. The neutral F420 models ($6FH^-$) belong to weak hydrogen atom donors, and the hydrogen atom-donating ability is weaker than that of NADH model MNAH, but greater than that of NADH model $AcrH_2$.

(4) The radicals or cation radicals ($XFH^\bullet$) of the F420 coenzyme models all belong to strong organic acids, the proton-donating ability of $1FH^\bullet$-$6FH^\bullet$ is not only greater than that of NADH model $AcrH_2^{+\bullet}$, but also greater than that of the NADH model $MNAH^{+\bullet}$.

(5) The radicals or cation radicals ($XFH^\bullet$) of the F420 coenzyme models are all strong hydrogen atom donors. The hydrogen-donating ability of $1FH^\bullet$-$5FH^\bullet$ is not only greater than that of $AcrH_2^{+\bullet}$, but also greater than that of $MNAH^{+\bullet}$. However, the hydrogen-donating ability of $6FH^\bullet$ is only greater than that of $AcrH_2^{+\bullet}$, but smaller than that of $MNAH^{+\bullet}$.

(6) The oxidized F420 coenzyme models (XF) are all weak electron acceptors. For 1F-5F, the electron-accepting ability is not only smaller than that of $AcrH^+$, but also smaller than that of MNA^+. But for 6F, the electron-accepting ability is only smaller than that of $AcrH^+$, but greater than that of MNA^+.

(7) Negative charge at N_1 position ($2FH^-$ and $4FH^-$) is more favorable to the electron transfer and the hydride transfer of XFH^- than that at N_{10} position ($3FH^-$ and $5FH^-$).

(8) Methyl group at N_3 position is unfavorable to the electron and hydride transfers from XFH^- with negative charge at N_{10} position ($3FH^-$ and $5FH^-$), but is favorable to the electron and hydride transfers from XFH^- with negative charge at N_1 position ($2FH^-$ and $4FH^-$).

(9) The substituents at C_7 and C_8 in XFH^-, $XFH^\bullet$ and XF have excellent Hammett linear free energy relationships to affect the enthalpy changes or redox potentials of XFH^- to release hydride ions, hydrogen atoms and electrons, the enthalpy changes of $XFH^\bullet$ to release hydrogen atoms and protons as well as the redox potentials of XF, respectively. Meanwhile, the effects of the substituents at C_7 and C_8 positions also have good additive properties.

(10) Compared to benzene group, in the reduced F420 coenzyme models, XFH^-, the uracil group is not an electron-donating group, but a hydride-donating group and a hydrogen atom-donating group. In $XFH^\bullet$, the uracil group is not only a proton-donating group, but also a hydrogen atom-donating group. In XF, the uracil group is an electron-donating group.

It is believed that the publication of this work will strongly promote the fast development of the chemistry and applications of F420 coenzyme and its various analogues.

Experimental Section

Materials. Reagent acetonitrile was purchased from Aldrich and treated by refluxing over P_2O_5 under argon before use. 1-Methyl-1,4-dihydronicotinamide (MNAH), 10-methylacridane ($AcrH_2$) and 4-acetylamino-2,2,6,6-tetramethyl-piperidine-1-oxoammonium perchlorate ($TEMPO^+$) were prepared according to the literature methods.[33,34] The typical synthetic routes of the F420 model compounds XFH^- are provided in Supporting Information.[35]

Titrated Calibration Experiments. Titrated calibration experiments were performed in acetonitrile at 298 K on a CSC isothermal titration calorimeter.[27] The reaction heats of two reactants were determined by following nine automatic injections from a 250 μL injection syringe containing 2mM of one reactant into the reaction cell (1.2 ml, containing 10mM another reactant). Injection volumes (15 μL) were delivered at 0.5 s intervals with 400s or 500s between every two injections. The reaction heat was obtained by area integration of each peak except the first one.

Measurement of Redox Potentials. The electrochemical experiments were carried out by cyclic voltammetry (CV) by using a BAS-100B electrochemical apparatus in deaerated

acetonitrile under argon atmosphere at 298 K as described previously.[36] N-Bu_4NPF_6 (0.1 M) was employed as the supporting electrolyte. A standard three- electrode cell consisting of a glassy carbon disk was used as working electrode, a platinum wire was used as counter electrode, and 0.01 M $AgNO_3$/Ag (in 0.1 M n-Bu_4NPF_6) was used as reference electrode. All sample solutions were about 1.5 mM. The ferrocenium/ferrocene redox couple ($Fc^{+/0}$) was taken as the internal standard.

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Notes and references

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Electronic Supplementary Information (ESI) available: Detailed synthetic routes and general preparation procedures of the 32 F420 coenzyme model compounds (XFH^-) as well as the plots of $E_{ox}(XFH^-)$, $E_{red}(XF)$, $\Delta H_{H^-D}(XFH^-)$, $\Delta H_{HD}(XFH^-)$, $\Delta H_{HD}(XFH^\bullet)$ and $\Delta H_{PD}(XFH^\bullet)$ against the sum of Hammett substituent parameters σ_p and σ_m.. See DOI: 10.1039/b000000x/

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