PCCP

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/pccp

PCCP

COMMUNICATION

Proton-Electron Sequential Transfers Mechanism: A Theoretical Evidence about Its Biological Relevance

L. Muñoz-Rugeles and J. R. Alvarez-Idaboy*[a]

Received 00th January 20xx,
Accepted 00th January 20xx

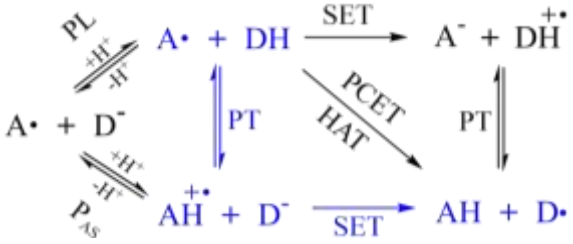
DOI: 10.1039/x0xx00000x

www.rsc.org/

Density functional theory calculations, using SMD continuum model, indicate that hydrogen transfer from totally protonated uric to a tryptophanyl radical in proteins corresponds to a sequential mechanism. Modeling in methyl butanoate indicates that this mechanism is more important in a hydrophobic medium than in water.

Recent studies have shown that damage caused by radicals to tryptophan and tyrosine residues in proteins can be efficiently repaired by uric (H_3Ur) and ascorbic acids (H_2Asc) at physiological pH.[1,2] However, neither the repair reaction mechanism, nor the participating species, have been identified. Even though no experimental or theoretical evidence is available, the repair activity has usually been assumed to be performed by the main fractions of uric and ascorbic acids i.e. the urate (H_2Ur^-) and ascorbate ($HAsc^-$) anions.[3] Reaction between the damaged protein radical (A·) and the repairer antioxidant (DH), (in this case, the tryptophanyl radical, TrpN·, and the H_3Ur acid, respectively) could, in principle, occur either in one reaction step or in multiple steps. The favored mechanism would depend on the intrinsic and environmental features of the reactants. The possible reaction mechanisms are shown in Scheme 1. As can be seen there are several possibilities, in blue the one we are studying in the present work.

In PCET reactions, one proton and one electron are transferred as separated particles between different sets of orbitals, and the process is associated with significant molecular charge redistribution.[4–6] The contributions to the electron density of the HOMO and SOMO orbitals come essentially from atomic orbitals involved in the single electron transfer (SET), rather than from the ones involved in the proton transfer (PT). For example, in the phenoxyl radical-phenol classical PCET model (PhO-PhOH), the electron density on the acceptor and donor oxygen atoms in the transition state involves 2p-π atomic orbitals which are nearly perpendicular to the reaction coordinate, (Figure S1 in supporting information). Then, PT occurs between the oxygen σ-orbitals while SET occurs via the channel provided by oxygen-oxygen π-orbitals interaction present in the HOMO orbital.[4,5]



Scheme 1. Repair reactions mechanisms.

If one proton and one electron are transferred in separated steps, the reaction mechanism is sequential. Sequential proton-loss electron transfer (SPLET) is an important reaction mechanism for phenolic compounds with free radicals.[7] In an SPLET reaction, one proton is first lost by DH in the proton loss step (PL), thus generating the D^- anion, which is a better electron donor than DH. The second step is a SET reaction and the products are the D· radical and the A^- anion. However, if the electron affinity of A· is small, it could capture a proton from the solvent before the SET step occurs (protonation assisted by solvent, P_{AS}) and form the $AH^{+\cdot}$ radical cation, which is a better electron acceptor than A·. This variant of SPLET is denoted as $SP_{AS}ET$.

Another possibility for hydrogen transfer sequential reactions is that both PT and SET occur directly between reactants. If the SET step between A· and DH occurs before the PT step, the

[a] Departamento de Física y Química Teórica, Facultad de Química, Universidad Nacional Autónoma de México, México DF 044510, México. E-mail: jidaboy@unam.mx

† Electronic supplementary information (ESI) available: Details on computational methodology. Fig. 1. Transition states structures and their HOMO and SOMO orbitals for the HAT and PCET models. Fig. S2. Hirshfeld atomic charges and spin density variation as a function of the reaction coordinate for the HAT and PCET models. Fig. S3. The intrinsic reaction coordinates for PEST transitions states. Fig. S4. Distribution diagrams at pH=7.4 for the ascorbic a uric acids. Table S1. Single electron transfer thermodynamics data calculated from Marcus theory. Cartesian coordinates of main stationary points. See DOI: 10.1039/x0xx00000x

mechanism could be called electron-proton sequential transfer (EPST). On the contrary, if the SET step between A· and DH occurs after the PT step, the mechanism would be called proton-electron sequential transfer (PEST). In either PEST or EPST, the electronic density of the HOMO and SOMO orbitals remains almost invariant through the PT step.

In this work, we have studied the repairing reactions of TrpN· by H_3Ur and H_2Asc acids using density functional theory. Results indicate that, at pH 7.4, these reactions occur via a PEST mechanism for H_3Ur, and via an $SP_{AS}ET$ for H_2Asc. Moreover, in a nonpolar medium (i.e. low dielectric constant), PEST is shown to be the only important repair mechanism.

Geometry optimizations and frequency calculations have been carried out using the M05-2X functional[8] and the 6-31+G(d,p) basis set, in conjunction with the SMD continuum model,[9] using water and methyl butanoate as solvents. Thermodynamic corrections at 298.15 K were included in the calculation of relative energies. The rate constants (k) were calculated using conventional transition state theory (TST)[10–12] and 1 M standard state. For the single electron transfer reactions (SET) $\Delta G^{\ddagger}$ was calculated using the Marcus theory[13,14]. The orbitals were obtained for the optimized TS structures using restricted open-shell M05-2X with 6-31+G(d,p) basis set.[5] Quantitative measures of the selected lone pair-π and π - π interactions in these orbitals were calculated using the overlap expressions from Mulliken.[5,15] The data for the analysis of the atomic charges of the H-donor, H-acceptor, and transferring H atoms, as a function of the reaction coordinate,[6] were obtained using the points on the ground state reaction path (generated from intrinsic reaction coordinate calculations, IRC) and the Hirshfeld partition scheme. More detailed information about the methodology used in the calculations can be found in the supporting information.

The reaction free energies (ΔG_{rx}), relative to isolated reactants, for a SET from H_3Ur and H_2Asc to TrpN· are found to be large and positive: 34.55 and 41.28 kcal/mol respectively. It implies that these completely protonated acids are poor electron donors, and that the first step of EPST does not occur. Moreover, SPLET does not occur either, because TrpN· does not accept electrons even from good electron donors such as H_2Ur^- and $HAsc^-$. The corresponding ΔG_{rx} values are 17.86 and 13.05 kcal/mol, respectively. Therefore, TrpN· is clearly a poor electron acceptor. Thermodynamic data calculated from Marcus theory are included in Table S1, in supporting information.

Table 1. Reaction free energies (ΔG_{rx}, kcal/mol), activation free energies ($\Delta G^{\ddagger}$, kcal/mol) and rate constants (k, $M^{-1}s^{-1}$) for reaction steps of a PEST mechanism with uric (H_3Ur) and (H_2Asc) ascorbic acids in water.

Reaction step	Repairer	ΔG_{rx}	$\Delta G^{\ddagger}$	k [b]
PT	H_3Ur	1.50 [a]	2.11	$7.39x10^9$
	H_2Asc	-0.68 [a]	0.00	$6.66x10^9$
SET	H_2Ur^-	-6.18	0.97	$7.43x10^9$
	$HAsc^-$	-10.98	0.15	$7.42x10^9$

[a] These free energies of reaction have been calculated using experimental values of pk_a. [b] The k values have not been corrected for molar fractions of reagents at pH 7.4.

The pk_a values for the tryptophanyl radical cation ($Trp^{\cdot+}$) and for the H_3Ur and the H_2Asc acids are close to each other (4.7, 5.8 and 4.2, respectively). Therefore, a PT from the protonated acids to TrpN· can be expected to occur, thus supporting the existence of a PEST repair mechanism. The ΔG_{rx} values for PT from the acids to TrpN·, calculated using experimental values of pk_a, are 1.50 and -0.68 kcal/mol for H_3Ur and H_2Asc, respectively, (Table 1). Even though the proton transfer between TrpN· and H_3Ur is endergonic, relative to the isolated reagents, Marcus theory predicts that SET occurs rapidly if the charge separation increases in both systems during the PT reaction.[16] In this case, when the TrpN· radical is protonated to form the $Trp^{\cdot+}$ radical cation, its electron affinity increases and the ΔG_{rx} values for SET from H_2Ur^- and $HAsc^-$ are -6.18 and -10.98 kcal/mol, respectively, (Table 1). The theoretical ΔG_{rx} values for PT from the acids to TrpN· are 1.37 kcal/mol for H_3Ur and 1.29 kcal/mol for H_2Asc. The theoretical value for H_3Ur acid is in excellent agreement with the experimental value while it is acceptable for H_2Acs acid.

The activation free energy ($\Delta G^{\ddagger}$) for PT between TrpN· and H_3Ur is 2.11 kcal/mol; for TrpN· and H_2Asc it occurs without a barrier. Therefore the PT reaction rate (k_{PT}) is diffusion controlled for both systems, i.e. $k_{PT} \sim 10^9\ M^{-1}\ s^{-1}$, (Table 1). However, k_{PT} values, corrected for molar fractions of reagents at pH 7.4, are $1.77x10^8$ and $2.09x10^6\ M^{-1}s^{-1}$ for repair reactions with H_3Ur and H_2Asc, respectively. Information about the calculation of corrected rate constants can be found in the supporting information. The $\Delta G^{\ddagger}$ values for SET between a $Trp^{\cdot+}$ radical cation and the H_2Ur^- and $HAsc^-$ monoanions are 0.97 and 1.47 kcal/mol, respectively, considering the reaction from isolated reactants to isolates products. Therefore, after PT step, SET rate constants (k_{SET}), are diffusion controlled for both systems (Table 1).

The transition states of the studied repair reactions and their HOMO and SOMO electronic densities are shown in Figure 1. The SOMO density is completely localized over the TrpN· radical, while the HOMO density involves only the repairers. According to the above discussion, this clearly indicates that PT and SET occur in separated steps, as expected for a PEST mechanism of hydrogen transfer. The contribution percentage from repairer-TrpN· pairs to HOMO and SOMO orbitals of the transition states are 100%-0% and 0%-100, respectively, for both systems. For the classical HAT and PCET reactions mentioned above, the contribution percentage to SOMO and HOMO are 50%-50%, (Figure S1, supporting information).

In order to evaluate the competition between PEST and $SP_{AS}ET$ in the reaction between the $Trp^{\cdot+}$ radical cation and the anionic acids, the rate constants for the PT step in PEST should be compared with the SET rate constants (table 1), since in the $SP_{AS}ET$ mechanism P_{AS} is considered a diffusion controlled step. $SP_{AS}ET$ rate constants at pH 7.4 are $1.39x10^7$ and $1.43x10^7\ M^{-1}s^{-1}$ for repair reactions with H_2Ur^- and $HAsc^-$, respectively, (supporting information). These results indicate that, for H_3Ur acid, the PEST mechanism is more important than $SP_{AS}ET$: $k_{PEST} > k_{SPASET}$. On the other hand, for H_2Asc, $SP_{AS}ET$ is more important than PEST, and the latter mechanism is not competitive in water, i.e. $k_{SPASET} > k_{PEST}$. It is extremely important to note that there is no competition between PEST and $SP_{AS}ET$ in a lipid

medium because the only species present in lipid medium are the completely protonated fractions, H_3Ur and H_2Asc.

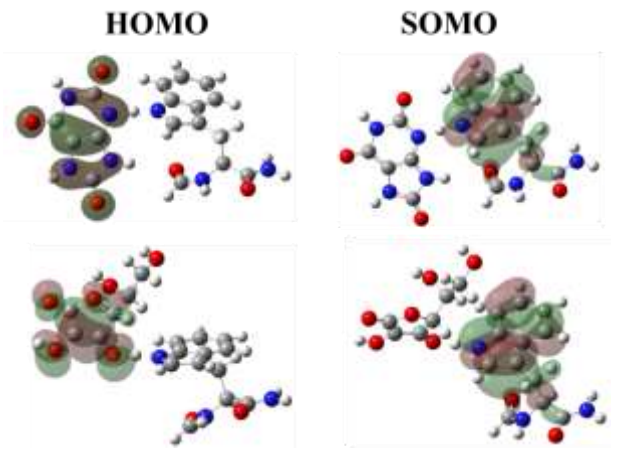


Fig. 1 Transition states and their HOMO and SOMO orbitals for repair reactions of tryptophanyl radical with the uric acid, H_3Ur-TrpN· (top) and ascorbic acid, H_2Asc-TrpN· (bottom).

Based on the above discussion we propose a proton-electron sequential transfer mechanism, PEST, for the repair reaction in water and lipid medium of TrpN· radical with the H_3Ur and H_2Asc acids. The reactions are shown in Scheme 2. The final products are the repaired Trp and the H_2Ur· and HAsc· radicals. To our knowledge, this is the first example of a PEST reaction.

$$\text{Step 1} \quad \text{TrpN}\cdot + \text{H}_3\text{Ur} / \text{H}_2\text{Asc} \overset{\text{PT}}{\rightleftharpoons} \text{TrpNH}^{+\cdot} + \text{H}_2\text{Ur}^- / \text{HAsc}^-$$

$$\text{Step 2} \quad \text{TrpNH}^{+\cdot} + \text{H}_2\text{Ur}^- / \text{HAsc}^- \xrightarrow{\text{SET}} \text{TrpNH} + \text{H}_2\text{Ur}\cdot / \text{HAsc}\cdot$$

Scheme 2. Steps for PEST repair mechanism.

An analysis of atomic charges and spin densities as a function of the reaction coordinate for the H-donor, the H-acceptor and the transfered H atom have been used to differentiate between PCET and HAT.[6] Thus, in this work these descriptors have been used successfully to unambiguously demonstrate that calculated transition states correspond to PT transitions states and that the repair reactions occur through a PEST mechanism (figure 2).

In the HAT model, the Hirshfeld atomic charges and spin densities of the transferred hydrogen atom are 0.02-0.04 and 0.038-0.062, respectively, (Figure S2, supporting information). For the PCET and PEST mechanisms, the proton transfer and electron transfer occur as separated particles, then the atomic charge and spin density on the transferred hydrogen must be equal to that of the proton for both mechanisms. In the PCET model, the Hirshfeld atomic charges and spin densities on the transferred hydrogen are 0.10-0.11 and 0.002-0.005, respectively, (Figure S2). In comparison, the corresponding values for the transferring hydrogen in the PEST systems are 0.11-0.12 and 0.004-0.007 for the reaction of TrpN· with H_3Ur, and 0.11-0.12 and 0.004-0.005 for the reaction of TrpN· with H_2Asc, figure 2. Therefore, the proton is transferred in the first step of the PEST mechanism, i.e. the charges on the transferred hydrogen in the PCET model correspond to the charge of a proton, and consequently the charges in the PEST model correspond to charges for a proton too. In the HAT mechanism the atomic charges are approximately one order of magnitude smaller, while spin densities are approximately one order of magnitude larger compared to PCET and PEST mechanisms.

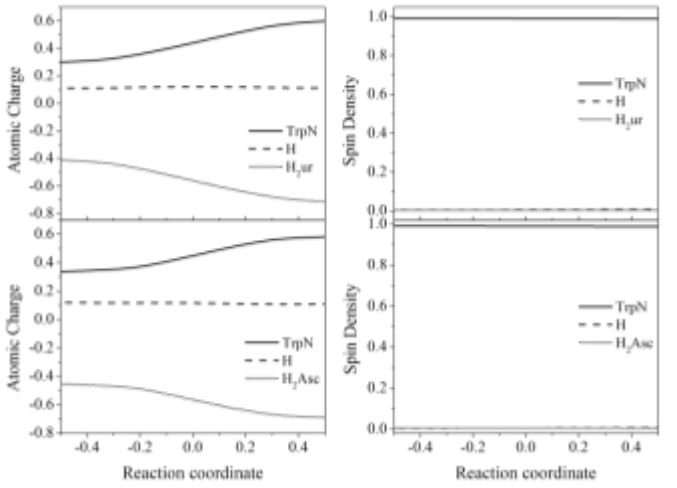


Fig. 2 Hirshfeld atomic charges and spin density variation as a function of the reaction coordinate for the hydrogen atom transfer reaction for the proton-electron sequential transfer reaction (PEST) of tryptophanyl radical with uric acid, TrpN-H_3Ur (top), and ascorbic acid, TrpN-H_2Asc (bottom).

It has been shown that in a PCET mechanism, the atomic charges and spin densities of the H-acceptor and the H-donor switch signs.[6] In a PEST mechanism, the charge on the H-acceptor becomes more positive and the charge on the H-donor becomes more negative, while the spin densities for H-acceptor and H-donor are constant. In the case of the TrpN· with the H_2Asc and H_3Ur acids reactions, the observed changes in the charges and spin densities are consistent with a PT step.

Similarly to the analysis of atomic charges and spin densities used to differentiate between PCET and HAT, the evolution of the Hirshfeld dipole moment vector has been used successfully too.[6] For the PCET model, an inversion of the dipole moment component on the reaction coordinate is observed in the course of the reaction, (Figure 3). This behavior of the dipole moment vector is also observed in the case of the $PhCH_2$-$PhCH_3$ HAT model, however, the change in the dipole moment magnitude is significantly larger for the PCET reaction, i.e. the range variation is from 0.15 to -0.15 for HAT and it varies from 2.38 to -2.38 for PCET, (Figure 3). The switching of the signs indicates a change in the electronic charge distribution for the HAT and PCET mechanisms because the proton is transferred simultaneously with the electron. On the other hand, in a PEST mechanism, the dipole moment increases and the switching of the signs does not occur, i.e. the charge separation increases (figure 3).

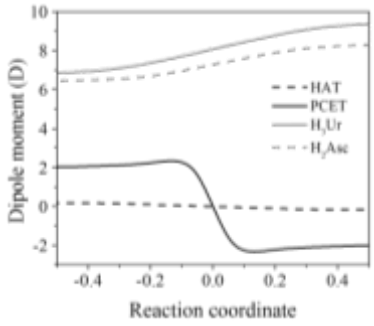


Fig. 3 Evolution of the Hirshfeld dipole moment vectors for the hydrogen atom transfer reaction (HAT) between benzyl radical and toluene, $PhCH_2$-$PhCH_3$; the proton couple electron transfer (PCET) between phenoxyl radical and phenol, PhO-PhOH; and the proton-electron sequential transfer (PEST) of tryptophanyl radical with H_3Ur and H_2Asc. The dipolar moment vectors have been projected over the vector joining the donor and acceptor atoms.

In order to assess the solvent effects present in a PEST mechanism, we analyzed the energy profile of the repair reaction with H_3Ur in methyl butanoate, and compared it with the energy profile in water, (Figure 4). The potential energy surfaces (PES), in terms of free energy (ΔG) and enthalpy (ΔH) changes, were calculated considering the formation of product complexes for the PT and ET steps of the PEST mechanisms (denoted as PTC and ETC, respectively). The PTC are metastable species, and ETC corresponds to the final product complexes, i.e. the repaired Trp and a $H_2Ur\cdot$ radical. The isolated reactants and products are indicated as R and P, respectively.

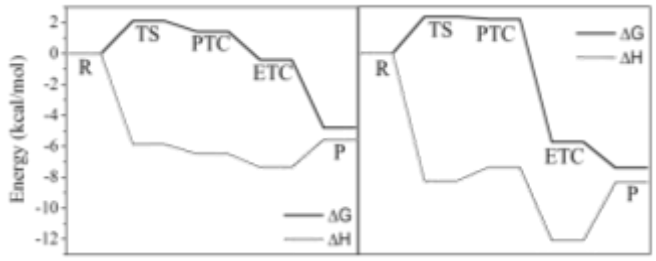


Fig. 4 Potential energy surfaces in free energy and enthalpy, ΔG and ΔH, respectively, for reaction of tryptophanyl radical and uric acid in water (left) and methyl butanoate (right). Reactants, transition state, proton transfer complex, electron transfer complex and products are identified like R, TS, PTC, ETC and P, respectively.

For methyl butanoate $\Delta G^{\ddagger}$ is 2.37 kcal/mol, which is 0.26 kcal/mol higher compared to $\Delta G^{\ddagger}$ for the corresponding reaction in water. Therefore, the PT rate constant remains diffusion-controlled in lipid medium. Furthermore, the formation of a SET complex is more exergonic in methyl buta-noate compared to the one observed in the reaction in water. Thus the total process is more irreversible in methyl butanoate than in water. Both systems have similar behavior in terms of ΔG and ΔH. After the activation barrier is overcome, the ΔG values drop in the direction of the isolated products and the total process of repairing is essentially irreversible. On the other hand, the formation of the transition state is significantly favored by the ΔH factor, while the formation of PT complexes in water is favored by both entropy (ΔS) and ΔH, and the formation in methyl butanoate is favored by ΔS. The formation of SET complexes is favored by ΔS and ΔH in both solvents. Finally, the dissociation of the SET complex to form the final products is disfavored by ΔH, because it involves the breaking of a hydrogen bond. The similar behavior in both solvents shows that the PEST mechanism is slightly dependent on the solvent and therefore almost any solvent model may be appropriate. Consequently is safe to assume that no explicit solvent molecules are needed

Conclusions

These results represent a step towards a deeper under-standing of the protective and reparative repairing activity of antioxidant compounds that are present in high concentrations in living organisms. Moreover, due to the absence of mechanisms of enzymatic repair for damage caused by free radicals to Trp residues, the mechanism studied here becomes even more important. To date, the antioxidant activity of H_3Ur and H_2Asc acid has been ascribed solely to the anions obtained in the first deprotonation at physiological pH. However, our results indicate that the repair activity of the completely protonated fractions is not negligible, and it must be considered in the interpretation of experimental studies. Modeling in methyl butanoate indicates that the PEST mechanism is more important in a hydrophobic medium than in water, since the only fractions present in lipid medium are the completely protonated acids and, therefore, PEST is likely the only reaction mechanism.

To the best of our knowledge this is the first case of a reaction that has been shown to occur via Proton-Electron Sequential Transfer Mechanism.

References

1 A. S. Domazou, W. H. Koppenol and J. M. Gebicki, *Free Radic. Biol. Med.*, 2009, **46**, 1049–1057.
2 A. S. Domazou, H. Zhu and W. H. Koppenol, *Free Radic. Biol. Med.*, 2012, **52**, 1929–1936.
3 A. S. Domazou, J. M. Gebicki, T. Nauser and W. H. Koppenol, *Isr. J. Chem.*, 2014, **54**, 254–264.
4 J. M. Mayer, D. A. Hrovat, J. L. Thomas and W. T. Borden, *J. Am. Chem. Soc*, 2002, **124**, 11142–11147.
5 G. A. Dilabio and E. R. Johnson, *J. Am. Chem. Soc*, 2007, **129**, 6199–6203.
6 A. Sirjoosingh and S. Hammes-Schiffer, *J. Phys. Chem. A*, 2011, **115**, 2367–2377.
7 G. Litwinienko and K. U. Ingold, *Acc. Chem. Res.*, 2007, **40**, 222–230.
8 Y. Zhao, N. E. Schultz and D. G. Truhlar, *J. Chem. Theory Comput.*, 2006, **2**, 364–382.
9 A. V Marenich, C. J. Cramer and D. G. Truhlar, *J. Phys. Chem. B*, 2009, **113**, 6378–96.
10 H. Eyring, *J. Chem. Phys.*, 1935, **3**, 107.
11 M. G. Evans and M. Polanyi, *Trans. Faraday Soc.*, 1935, **31**, 875.
12 D. G. Truhlar, W. L. Hase and J. T. Hynes, *J. Phys. Chem.*, 1983, **87**, 2664–2682.
13 R. Marcus, *Rev. Mod. Phys.*, 1993, **65**, 599–610.
14 R. A. Marcus, *Pure Appl. Chem.*, 1997, **69**, 13–30.
15 R. S. Mulliken, *J. Chem. Phys.*, 1955, **23**, 1833.
16 A. Galano and J. R. Alvarez-Idaboy, *J. Comput. Chem.*, 2013, **34**, 2430–45.