Dalton Transactions

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Cite this: DOI: 10.1039/x0xx00000x

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Direct NMR detection of the unstable "red product" from reaction between nitroprusside and 2-mercaptosuccinic acid†

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Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

The reaction between nitroprusside (NP, $[Fe^{II}(CN)_5NO]^{2-}$) and organic thiolates (RS^-) in aqueous solution has long been known to produce an unstable red intermediate thus often being referred to as the "red product" (RP) in the literature. While RP has always been formulated as $[Fe^{II}(CN)_5N(O)SR]^{3-}$, it is rather difficult to study it in aqueous solution because it is not only unstable but also exhibits rapid ligand exchange. All previous studies of RP have relied on UV-vis, IR, kinetics measurement, and analysis of decomposed products. Herein we report the first comprehensive multinuclear (^1H, ^{13}C, ^{15}N, ^{17}O) NMR characterization of the RP produced from reaction between NP and 2-mercaptosuccinic acid (MSA). The NMR chemical shifts obtained for RP are compared with those from the free ligand (S-nitrosothiol, RS-N=O) prepared *in situ* by reaction of MSA with $NaNO_2$. We also showed that useful thermodynamic and kinetic properties of RP formation can be readily obtained from ^1H NMR studies.

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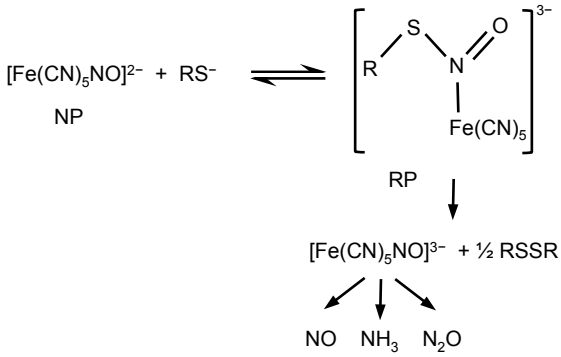
Introduction

S-nitrosothiols (RSNOs) have been intensely investigated in the past 20 years, because of their important roles in protein post-translational modification, NO-mediated bioactivity, and potential therapeutic applications.[1-10] The general mechanism of RSNO bioregulatory action however remains unknown. Recently, the reactivity of RSNOs towards H_2S under physiological conditions has attracted considerable attention[11-15] and also generated controversies.[16-20] This new reaction pathway adds further complication for possible "cross talks" between the two major gaseous signaling molecules, NO and H_2S. While transition metal catalyzed RSNO formation/decomposition has long been considered to be a possible pathway for RSNO bioregulatory function in biological systems, the field of coordination chemistry of RSNOs remains largely unexplored. For example, it is well known that metal ions such as Hg^{2+} and Cu^+ can catalyze the decomposition of RSNOs.[1,21] But the detailed mechanism has not yet been firmly established. Recently, Kozhukh and Lippard[22] showed that Zn^{2+} can also catalyze RSNO decomposition to release gaseous NO and N_2O. In this case, however, RSSR was not found among the decomposed products. In general, well characterized transition metal coordination complexes containing RSNO ligands are extremely rare.[23,24] Among them the so-called "red products" (RPs), which are produced from reaction between nitroprusside (NP, $[Fe^{II}(CN)_5NO]^{2-}$) and organic thiolates (RS^-) in aqueous solution, are the most extensively studied.[25-35] It is commonly accepted that the RP is $[Fe^{II}(CN)_5N(O)SR]^{3-}$, where RSNO is coordinated to the Fe(II) center in the κ^1-N binding mode. However, RPs are generally unstable, decomposing rapidly to produce RSSR and $[Fe^{II}(CN)_5NO]^{3-}$. The latter can further undergo complex decomposition to produce NO, N_2O and NH_3 among other species, depending strongly on experimental conditions, as illustrated in Scheme 1. Because of this extraordinary instability, no crystal structure has ever been reported for any RP. The closet analogs to RPs are a series of Ir(III)-N(O)RS complexes reported by Doctorovich and co-workers[36,37] In addition to their poor stabilities, RPs often exhibit rapid ligand exchange as indicated in Scheme 1, making them rather difficult to characterize by NMR. As a result, all previous studies of RPs have relied on UV-vis, IR, kinetics measurement, and chemical analysis of decomposed products. While RPs themselves are diamagnetic, many decomposed products are

Scheme 1 Formation of RP and its subsequent decomposition in aqueous solution.



paramagnetic and thus can be studied by EPR. Clearly, it is highly desirable to have a more direct means of probing the formation and transformation of unstable RPs. Some time ago, Stasicka and co-workers[33] reported that the RP generated from reaction between NP and 2-mercaptosuccinic acid (MSA) is relatively stable. We decided to utilize the stability of this particular RP to obtain its multinuclear (^{17}O, ^{15}N, ^{13}C, ^{1}H) NMR signatures. The present work was also motivated by our recent finding that the red-violet and blue transient intermediates in Gmelin reaction (between nitroprusside and hydrogen sulfide) are in fact $[Fe(CN)_5N(O)S]^{4-}$ and $[Fe(CN)_5N(O)SS]^{4-}$, respectively.[38] The thionitro ligand in the former complex, $[SNO]^-$, is the deprotonated form of HSNO, which can be considered to be the smallest S-nitrosothiol. Thus, $[Fe(CN)_5N(O)S]^{4-}$ may be seen as a special type of RP. Indeed, aqueous solution of $[Fe(CN)_5N(O)S]^{4-}$ exhibits nearly the same red coloration (λ_{max} = 530 nm) as do all RPs. Another objective of this work is to continue our effort to explore the use of ^{17}O NMR as a new technique for probing highly reactive and unstable intermediates/products.[39] The advantage of ^{17}O NMR over the more conventional ^{15}N NMR in studying NO related compounds is that the very short ^{17}O spin-lattice relaxation time allows very rapid data acquisition, thus permitting detection of very short-lived species as we demonstrated recently.[38]

Results and discussion

Figure 1 shows the UV-vis and ^{1}H NMR spectra of the reaction solution containing NP and MSA in a 1:1 molar ratio. The formation of the RP is evident from the brilliant red color of the solution (λ_{max} = 526 nm). Under the conditions employed in this study, this RP has a half-life ($t_{½}$) of about 2 hrs at pH 11. Interestingly, the ^{1}H NMR spectrum of the reaction solution displays two sets of peaks suggesting that RP and free MSA are at equilibrium. This observation also suggests that, for MSA, the reversible process of the RP formation is slow on the NMR timescale (*vide infra*). Because of this rather slow ligand exchange process, it is possible to fully characterize RP for the first time by multinuclear (^{1}H, ^{17}O, ^{15}N, ^{13}C) NMR. As seen from Figure 2, the ^{17}O NMR signal obtained for the RP, $[Fe^{II}(CN)_5N(^{17}O)SR]^{3-}$, appears at 1036 ppm. This ^{17}O NMR signal is quite broad with a full width at the half-height (FWHH) of ca. 4.2 kHz. This is because the relatively large size of the RP induces a very rapid ^{17}O nuclear quadrupole relaxation. The ^{15}N NMR signal for the RP is at 607 ppm. In both cases, weaker signals from NP were also observed, in agreement with the conclusion drawn from the ^{1}H NMR data shown in Figure 1 that both RP and free ligands are present. To compare these NMR data with those from a free RSNO ligand, we reacted MSA with $NaNO_2$ in a 1:0.7 molar ratio and immediately recorded NMR spectra. As shown as insets in Figures 2a and 2b, the ^{17}O and ^{15}N NMR signals from a free RSNO ligand appear at 1200 and 761 ppm, respectively. It is immediately clear that, upon coordination to the Fe(II) center, both ^{17}O and ^{15}N chemical shifts of the RSNO ligand change significantly. Considering that the $\delta(^{17}O)/\delta(^{15}N)$ ratio is typically 1.8 for nitroso compounds,[42] the observed nearly equal ^{15}N and ^{17}O coordination shifts (ca. 160 ppm) are consistent with the κ^1-N binding mode as shown in Scheme 1. Similar ^{15}N coordination shifts were also observed for C-nitroso metal compelxes in the κ^1-N mode of binding.[43,44] It is interesting to note that, for the κ^1-O mode of binding, while the ^{15}N coordination shifts (200 ppm) are similar to those found for the κ^1-N complexes, significantly larger ^{17}O coordination shifts (600 ppm) were detected.[42] This illustrates the uniqueness of ^{17}O NMR to differentiate between the two distinct modes of metal binding. Table 1 summarizes the ^{15}N and ^{17}O NMR data reported for related compounds in the literature. A parallelism between ^{15}N and ^{17}O chemical shifts is clearly seen for the RSNO-related compounds. However, it is important to point out that it took only 4 min to acquire the ^{17}O NMR data shown in Figure 2a, whereas the corresponding ^{15}N NMR spectra shown in Figure 2b were recorded in 50 min. This drastically short experimental time in ^{17}O NMR experiments is due to the rapid quadrupolar relaxation, which makes it possible to detect very short-lived species.[38]

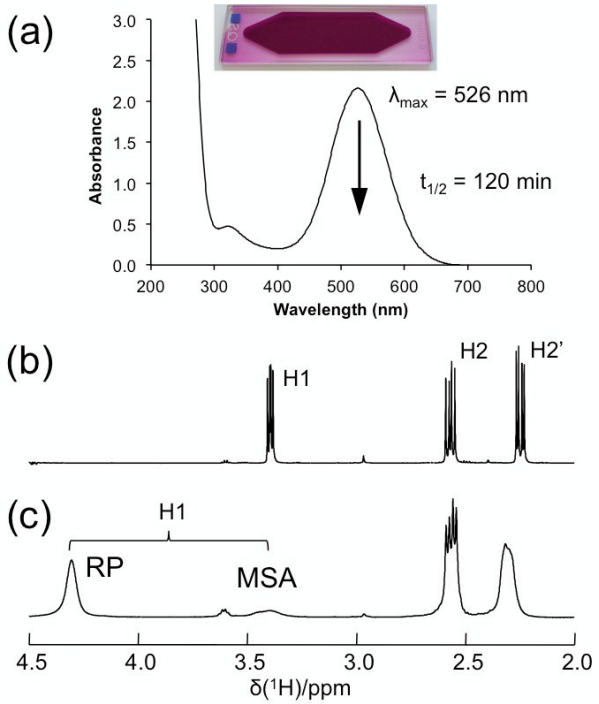


Fig. 1 (a) UV-vis spectrum (0.1 mm pathlength) of the RP prepared by reacting 50 mM MSA with 50 mM NP in aqueous solution (pH 11, 50 mM sodium carbonate buffer, 0.1 M NaCl, 0.5 mM EDTA and 12 mM KCN). The brilliant red color of the RP is shown in the inset. (b) ^{1}H NMR spectrum of 10 mM MSA in aqueous solution at pH 11. (c) ^{1}H NMR spectrum of the same reaction solution as in (a).

As this particular RP is relatively stable, we were also able to record a ^{13}C NMR spectrum at the natural abundance; see Figure 2c. The most interesting observation in the ^{13}C NMR spectrum is that the equatorial and axial CN groups appear at 167.6 and 162.8 ppm, respectively. These are drastically different from the corresponding signals found in NP, 134.5 (CN_{eq}) and 132.3 (CN_{ax}) ppm. Recently, we reported[37] that the experimental ^{13}C

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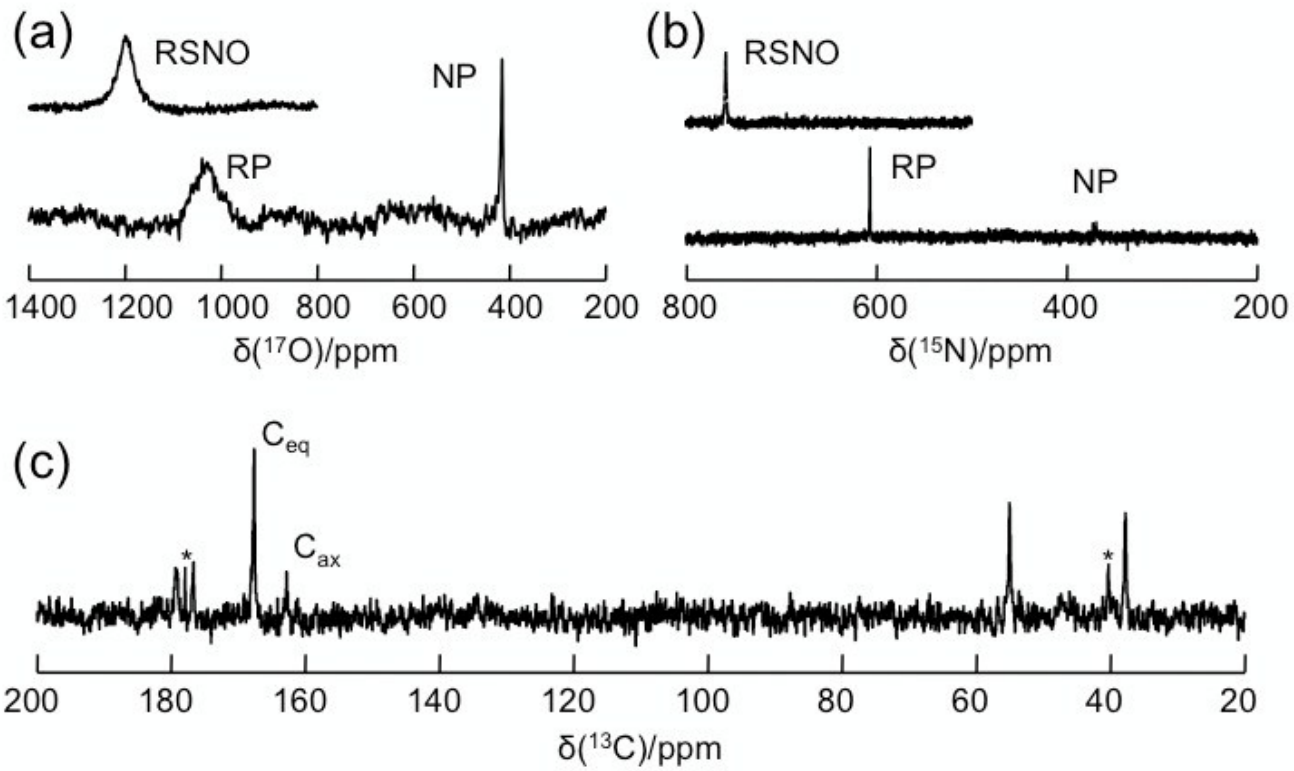

Fig. 2 (a) ^{17}O, (b) ^{15}N, and (c) ^{13}C NMR spectra of the RP prepared by reacting 100 mM MSA with 100 mM NP in aqueous solution (pH 11, 50 mM sodium carbonate, 0.1 M NaCl, 0.5 mM EDTA and 12 mM KCN). In (a) and (b), ^{17}O- and ^{15}N-labled NPs were used respectively. The experimental time to acquire the ^{17}O (recycle delay 40 ms, 5435 transients), ^{15}N (recycle delay 1.3 s, 2300 transients), and ^{13}C (recycle delay 1.2 s, 1840 transients) NMR spectra was 4, 50, and 37 min, respectively. In the insets of (a) and (b), ^{17}O and ^{15}N NMR spectra obtained for the free RSNO ligand are shown for comparison. The free RSNO ligand was prepared *in situ* by reacting MSA with $NaNO_2$ (either 60% ^{15}N and 20% ^{17}O labeled) in a 1:0.7 molar ratio. In (c) the two signals marked by * are due to the presence of a very small amount of decomposed products: $[Fe(CN)_6]^{4-}$ and RSSR. The ^{13}C NMR signals from NP were too weak to be seen because they have longer ^{13}C spin-relaxation times than those from RP and were thus partially saturated under the current experimental condition.

Table 1 Comparison of ^{15}N and ^{17}O chemical shifts (δ in ppm) and vibrational frequencies (ν in cm^{-1}) between RP and other related RSNO compounds.

Compound	$\delta(^{15}N)$	$\delta(^{17}O)$	ν_{NO}	ν_{NS}	Ref.
$[Fe(CN)_5N(O)SR]^{3-}$ (R = -CH(COO$^-$)(CH$_2$)-COO$^-$)	607	1035	1390	758	This work
$[Fe(CN)_5N(O)SEt]^{3-}$	—	—	1380	—	35
R-S-N=O (R = -CH(COO$^-$)(CH$_2$)-COO$^-$)	761	1200	1505	—	This work
R-S-N=O (R = a variety of groups)	765-830[a]	—	—	—	40
$[Fe(CN)_5N(O)S]^{4-}$	700	1028	1254	805	38
$[Fe(CN)_5N(O)SS]^{4-}$	632	938	1358	—	38
$[Fe(CN)_5N(O)H]^{3-}$	640	1099	1352	—	39
$[Fe(CN)_5(NO)]^{2-}$ (NP)	373	419	1935	—	39
$[IrCl_4(MeCN)N(O)SR]^-$ (R = -CH$_2$Ph)	—	—	1431	778	36
$[IrCl_5(NO)]^-$	—	—	2008	—	41

[a]The listed values are obtained by adding 39 ppm to those reported in ref. 39. This is because the ^{15}N chemical shifts in ref. 39 were referenced by setting the ^{15}N NMR signal of $Na^{15}NO_2$ to 570 ppm, but in our work the same signal was determined to be at 609 ppm.

signal from the CN_{eq} groups in $[Fe(CN)_5N(O)S]^{4-}$ is 174.7 ppm and this signal is predicted by quantum chemical computation to shift to 159.3 ppm, if the RSNO ligand is neutral, i.e., $[Fe(CN)_5N(O)SH]^{3-}$. We then hypothesized that the CN_{eq} signal should be a sensitive probe to the protonation state of the HSNO ligand. Indeed, our observation that the RP exhibits $\delta(^{13}C_{eq})$ = 167.6 ppm confirms this hypothesis. A full list of ^{13}C chemical shifts are provided in the ESI. The small signal at 177 ppm (marked with * in Figure 2c) is due to the presence of a trace amount of $[Fe(CN)_6]^{4-}$ from RP decomposition. Over a period of about 3.5 hrs, all the ^{13}C NMR signals from RP disappear, producing a set of new signals attributable to RSSR; see ESI.

After having obtained a complete set of multinuclear NMR data for the RP, we decided to further characterize this particular stable RP with FTIR. Figure 3 shows the FTIR spectra obtained for both free RSNO ligand and RP. The relevant IR data from the literature are also given in Table 1. The ν_{NO} stretching for the free RNSO ligand was observed at 1505 cm^{-1}, and it is shifted to a lower wavenumber in the RP, 1390 cm^{-1}. This is consistent with that reported by Schwane and Ashby.[34] A similar trend was also noted by Doctorovich and co-workers[36,37] in the Ir-RSNO complexes. As seen from Figure 3b, we were also able to detect a small peak attributable to ν_{NS} at 758 cm^{-1}. This appears to be the only reported ν_{NS} for RPs. However, we should point out that similar ν_{NS} values were reported for Ir-RSNO complexes.[36,37] It is also interesting to note that the ν_{NO} stretch of RP is higher than that found in $[Fe(CN)_5N(O)S]^{4-}$, but the ν_{NS} stretch is lower. This observation further confirms the deprotonated state of the HSNO ligand in $[Fe(CN)_5N(O)S]^{4-}$. It is also clear from Figure 3b that both NP and RP are present in solution, which is in agreement with the NMR data discussed earlier. In RP, two ν_{CN} signals were observed, 2084 and 2054 cm^{-1}, both being considerably shifted to lower wavenumbers than that found in NP, 2145 cm^{-1}. This was also noted by Stasicka and co-workers.[33]

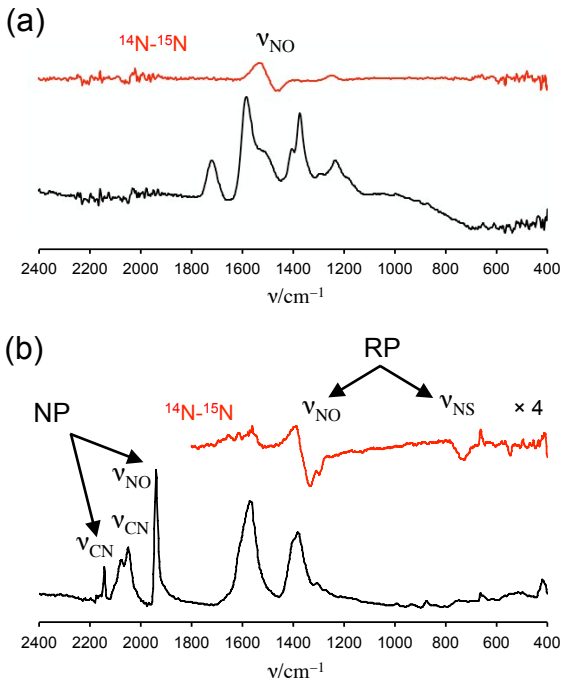

Fig. 3 ATR-FTIR spectra of (a) free RSNO ligand prepared *in situ* from mixing MSA with $NaNO_2$ (1:0.7 molar ratio) and (b) RP prepared under the same conditions as described in Fig.2b.

As mentioned earlier, the observation of both RP and MSA signals in the ^1H NMR spectra suggests that the ligand exchange is slow on the NMR timescale. This provides us with a rare opportunity to actually determine the thermodynamic and kinetic properties of the reaction between NP and MSA. To this end, we recorded ^1H NMR spectra of the NP/MSA reaction

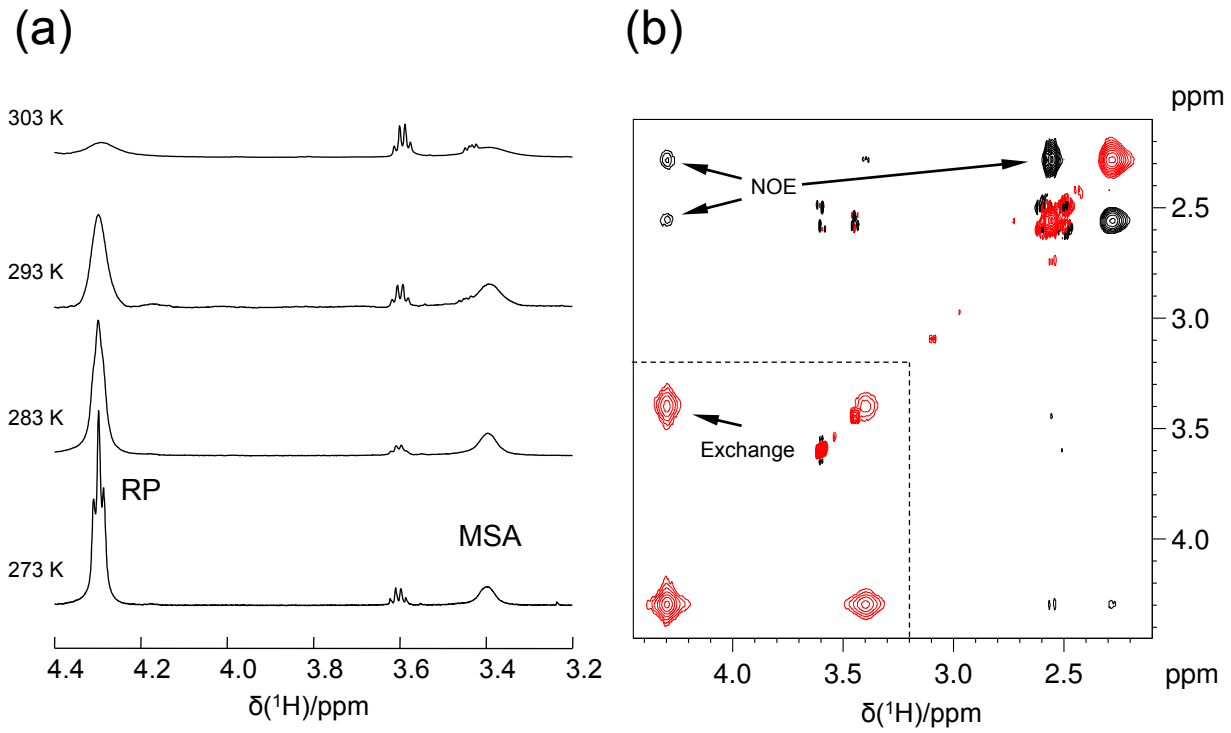

Fig. 4 (a) The H_1 region of the 1D ^{1}H NMR spectra recorded at different temperatures. (b) The entire 2D ^{1}H NOESY (EXSY) spectrum (298 K, mixing time of 2 s) of the reaction solution containing 50 mM MSA and 50 mM NP. In (b), the H_1 region is indicated with dotted lines. Note that cross peaks due to chemical exchange (red) and NOE (black) display different phases as compared with the diagonal peaks.

solution at different temperatures. As seen from Figure 4a, as the temperature of the solution decreases, both sets of signals sharpen, suggesting a slowing down of the ligand exchange, and the RP concentration increases, indicating a shift of the equilibrium towards RP production. The ligand exchange process was further confirmed by recording 2D NOESY (EXSY) spectra at different temperatures; Figure 4b. From these VT ^{1}H NMR data, we obtained the following thermodynamic and kinetic data: $\Delta H^\circ = -88.1$ kJ mol^{-1}, $\Delta S^\circ = -260.1$ J mol^{-1} K^{-1}; $\Delta H^\ddagger = 25.9$ kJ mol^{-1}, $\Delta S^\ddagger = -118.2$ J mol^{-1} K^{-1}. These are in reasonable agreement with the results from earlier temperature-jump/stop-flow kinetic measurements.[28] This is the first time that this kind of information are directly obtained for RPs from ^{1}H NMR experiments. Our results show that, as long as the ligand exchange is slow on the NMR timescale, RP formation can be readily monitored by ^{1}H NMR.

Conclusions

We have obtained a complete set of multinuclear (^{1}H, ^{13}C, ^{15}N, and ^{17}O) NMR signatures for the RP prepared from reaction between MSA and NP. We have also prepared *in situ* the corresponding RSNO by reacting RSH with $NaNO_2$. This is the first time that S-nitrosothiols in both free ligand and metal-bound states are fully characterized by multinuclear NMR. When the ligand exchange is slow on the NMR timescale, useful thermodynamic and kinetic information about the formation of RP can be readily obtained with ^{1}H NMR. This study also demonstrates the utility of ^{17}O NMR as a new technique for detecting short-lived species, which is particularly suited in the study generally unstable S-nitrosotiol compounds. One can envisgae future studies in which ^{17}O NMR can be used to monitor formation of RSNOs and to follow chemical transformation of the S-N=O group in biological systems. It is also possible to use this new approach to study chemical reactions between RSNOs and H_2S. Research along this line is underway in our laboratory.

Acknowledgements

G. W. thanks the Natural Sciences and Engineering Research Council (NSERC) of Canada for financial support.

Notes and references

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† Electronic supplementary information (ESI) available: synthetic details and additional spectroscopic data.

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TOC entry

First NMR characterization of the unstable “Red Product” produced from reaction between nitroprusside and organic thiolates.