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Alkali-Metal-Ion-Assisted Hydrogen Atom Transfer in the Homocysteine Radical

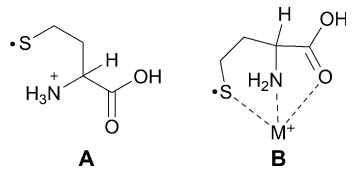
Michael Lesslie,[a] Justin Kai-Chi Lau,[b, c] John T. Lawler,[a] K. W. Michael Siu,[b, c] Jos Oomens,[d] Giel Berden,[d] Alan C. Hopkinson,*[b] and Victor Ryzhov*[a]

Abstract: Intramolecular hydrogen atom transfer (HAT) was examined in homocysteine (Hcy) thiyl radical/alkali metal ion complexes in the gas phase by combination of experimental techniques (ion-molecule reactions and infrared multiple photon dissociation spectroscopy) and theoretical calculations. The experimental results unequivocally show that metal ion complexation (as opposed to protonation) of the regiospecifically generated Hcy thiyl radical promotes its rapid isomerisation into an α-carbon radical via HAT. Theoretical calculations were employed to calculate the most probable HAT pathway and found that in alkali metal ion complexes the activation barrier is significantly lower, in full agreement with the experimental data. This is, to our knowledge, the first example of a gas-phase thiyl radical thermal rearrangement into an α-carbon species within the same amino acid residue and is consistent with the solution phase behaviour of Hcy radical.

Homocysteine (Hcy), an analogue of Cys, is biosynthesised by demethylation of methionine and is normally present in blood plasma at concentrations of 5–15 μM.[1] Severely elevated levels of Hcy (>100 μM) have been implicated as an independent risk factor for cardiovascular diseases through a condition known as hyperhomocysteinemia.[2] In addition, it has been shown that increased Hcy levels are linked to Alzheimer's disease, birth defects and osteoporosis.[3] The specific molecular mechanisms involved in these pathologies have not been fully established.[4] Studies are ongoing to elucidate relationships between Hcy and oxidative stress.[5]

One of the important chemical properties of Hcy is its ability to form a thiyl (S-based) radical, which can reversibly convert to the slightly more stable α-carbon-based radical (C_α).[6] While Hcy only differs from Cys by an additional methylene linkage, the effect of this difference on the rates to interconversion is quite profound. The intramolecular hydrogen atom transfer (HAT) resulting in a S-to-C_α radical isomerisation in an alkaline solution was approximately 10 times faster in Hcy than Cys thiyl radicals (this was also later shown to occur at physiological pH by the Strongin group).[4a,7] This difference is a direct consequence of the extra methylene linkage as it affords a 5-membered cyclic transition state during HAT in Hcy, compared to a 4-membered cyclic structure for Cys.

We were not able to reproduce the HAT in gas-phase experiments involving Hcy thiyl radical protonated at the amine (structure A).[8] This, however, was expected according to work by Schöneich et al. who found that the HAT rate decreases approximately tenfold when the charge is located on the amine next to the C_α.[9] While in solution this can be circumvented by raising the pH, gas-phase ion experiments must rely on charge/sign placement. Our attempts to form the thiyl radical anion of Hcy, deprotonated at the C terminus, were unsuccessful. Herein we explore alkali metal ions as an alternative means of introducing charge and their effect on the HAT in the Hcy radical.



Our approach relies on the hypothesis that using metal ions as charge carriers greatly reduces the positive charge on the amino group. In this scenario some combination of the carbonyl O, S• and amino N is coordinated to the metal ion thereby distributing the positive charge (structure B). Detachment of the amino group from the metal ion in structure B is a relatively low-energy process and the lone pair on the free NH_2 assists in stabilising the incipient C_α-based radical through the captodative effect.[6b]

Building on the earlier successful characterisation of both C_α[10] and thiyl[11] amino acid radicals by infrared multiple

[a] M. Lesslie, J. T. Lawler, Dr. V. Ryzhov
Department of Chemistry and Biochemistry, Northern Illinois University
DeKalb, IL 60115 (USA)
E-mail: ryzhov@niu.edu

[b] Dr. J. K.-C. Lau, Dr. K. W. M. Siu, Dr. A. C. Hopkinson
Department of Chemistry and Centre for Research in Mass Spectrometry
York University, Toronto, Ontario, M3J 1P3 (Canada)
E-mail: ach@yorku.ca

[c] Dr. J. K.-C. Lau, Dr. K. W. M. Siu
Department of Chemistry and Biochemistry, University of Windsor
Windsor, Ontario, N9B 3P4 (Canada)

[d] Dr. J. Oomens, Dr. G. Berden
Institute for Molecules and Materials, FELIX Laboratory
Radboud University, Toernooiveld 7c, 6525 ED Nijmegen (The Netherlands)

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photon dissociation (IRMPD) action spectroscopy, our group has developed a three-pronged approach to studying radicals in the gas phase. We have shown that a combination of gas-phase ion molecule reactions (IMR), IRMPD spectroscopy, and density functional theory (DFT) calculations has been effective in the characterisation of several cysteine derivatives and cysteine-containing peptides.[8,12] In this work, S-based radical cations are initially formed by the well-documented S–NO homolytic bond cleavage,[13] which is independent of the charging species (proton (structure A) or metal ion (structure B)). These ions are then subjected to regiospecific IMRs for initial screening of radical location in our modified ion trap MS.[14] Full structural elucidation is accomplished by IRMPD spectroscopy and compared to theoretically predicted IR absorption spectra. This produces full characterisation (reactivity and structure) of the radical cations. In this study, we first compare the reactivity of Hcy radical cation $[Hcy]^{\bullet+}$ and alkali metal (Li^+, Na^+, and K^+, generally indicated as M^+) bound Hcy ions, $[Hcy-H+M]^{\bullet+}$. We then present IRMPD spectroscopy data and DFT calculations on the structure of metal-ion-bound Hcy radicals not previously studied.

Nitric oxide (NO) has previously been shown to be highly reactive with sulfur-based cysteine radicals and unreactive with C_α-based radicals in the gas-phase.[15] This regioselectivity makes NO a suitable indicator of radical location in Hcy radical cations. Gas-phase reactions with NO revealed high reactivity for $[Hcy]^{\bullet+}$ and no reaction for any metallated species $[Hcy-H+M]^{\bullet+}$ (M = Li, Na, or K). Full kinetic profiles for these ion types are displayed in Figure 1. The linear trend for $[Hcy]^{\bullet+}$ decay indicates pseudo-first-order kinetics and is clear evidence that the radical remains localised on the sulfur atom. This agrees with the previously determined structure of the protonated Hcy radical.[8] The lack of any reactivity of the metal ion/radical complexes suggests that there is either a fast hydrogen atom transfer converting the S$^\bullet$-radical to the C_α-radical or, possibly, a sizeable reduction in S-radical reactivity due to sulfur-metal coordination.

In order to determine the nature of reactivity loss for the Hcy radical/alkali metal ion complexes, IRMPD action spectroscopy was employed to ascertain the structure of the ions. The experimental gas-phase IR action spectrum in the fingerprint region was obtained for each of the metal complexes $[Hcy-H+M]^{\bullet+}$ (M = Li, Na, K; Figure 2). The similar positions and intensities of absorption bands in these spectra strongly suggest analogous structures for all three radical/metal ion complexes. While the $[Hcy-H+K]^{\bullet+}$ species suffered from a decreased signal-to-noise ratio due to low ion counts, the absorption bands are still clearly discernible. Interestingly, in all of the experimental IRMPD spectra there is a notable absence of a strong absorption above 1700 cm^{-1} typically ascribed to the stretching of a free carbonyl group. We have previously used this band to identify S-based radical structures, including the protonated homocysteine radical where it occurs at 1780 cm^{-1}, as opposed to C_α-based ones where it was significantly red-shifted due to protonation of the carbonyl and conjugation of the backbone.[8,12b] The presence of the metal ion complicates the empirical interpretation of these spectra, as coordination of the metal to the carbonyl would similarly red-shift the absorption band.

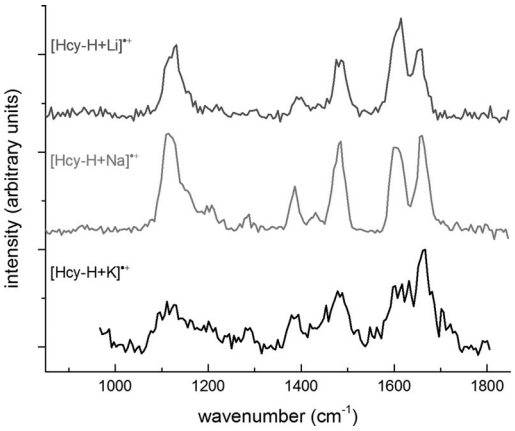

Figure 2. Experimental IRMPD spectra of $[Hcy-H+Li]^{\bullet+}$, $[Hcy-H+Na]^{\bullet+}$, and $[Hcy-H+K]^{\bullet+}$ collected at the FELIX facility (Nijmegen, The Netherlands).

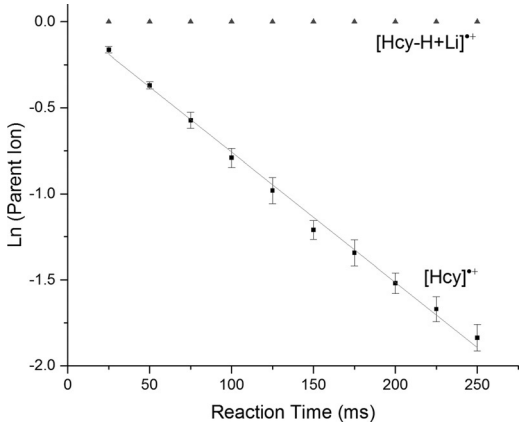

Figure 1. Decay of the radical cation during reaction with NO. None of the metal ion (Li^+, Na^+, or K^+)/radical adducts produced measurable reactivity.

To better understand the experimental IR spectra, theoretical calculations were performed to predict absorption bands of possible metal-bound homocysteine radical structures (Figure 3 and Figures S1 and S2 in the Supporting Information). For all three metals, the C_α-based radicals with bidentate (S,O) metal configuration (C_α-1) were found to be at the global minima lower in energy by approximately 40–50 kJ mol^{-1} relative to the best sulfur radical species (Table 1, S-1). A second low-energy bidentate (O,O) C_α-based radical structure (C_α-2) was also found. A third bidentate (N,O) structure (C_α-3) was consistently higher in energy; this is attributed to weak electron donation to the radical centre from the amino group resulting in a reduction in captodative stabilisation. The lowest energy S-based radical (S-1) had a tridentate metal binding configuration (S,N,O). Therefore, as the ion is initially formed on the sulfur, it can be assumed that S-1 represents the most likely S-based radical structure of the ion.

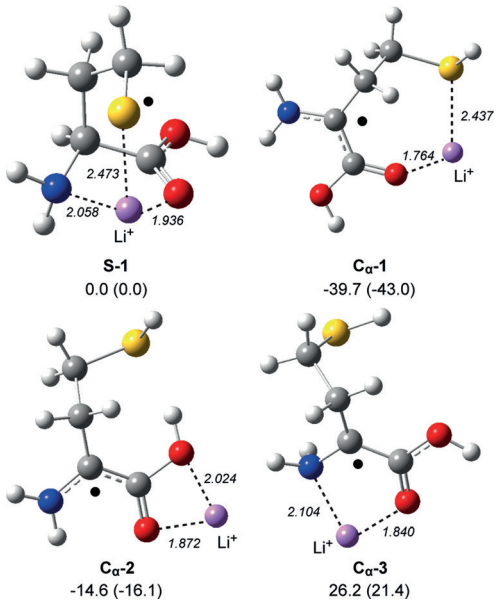

Figure 3. Low-energy structures of $[Hcy-H+Li]^{\bullet+}$. Enthalpies (ΔH°_{0}) and free energies (ΔG°_{298}, in parenthesis) in kJ mol^{-1} are relative to the S-1 ion. All distances are in Å.

Table 1. Energies of barriers to isomerisation and C_{α}-1 structures relative to S-1. The enthalpies and free energies (in parentheses) as calculated at the B3LYP/6-311++G(d,p) level.

Radical Species	Critical TS [kJ mol^{-1}]	C_{α}-radical [kJ mol^{-1}]
$[Hcy]^{\bullet+}$	82.0 (82.0)	−28.6 (−27.1)
$[Hcy-H+Li]^{\bullet+}$	62.5 (64.4)	−39.7 (−43.0)
$[Hcy-H+Na]^{\bullet+}$	48.0 (49.5)	−43.3 (−48.0)
$[Hcy-H+K]^{\bullet+}$	42.2 (44.0)	−51.7 (−54.0)

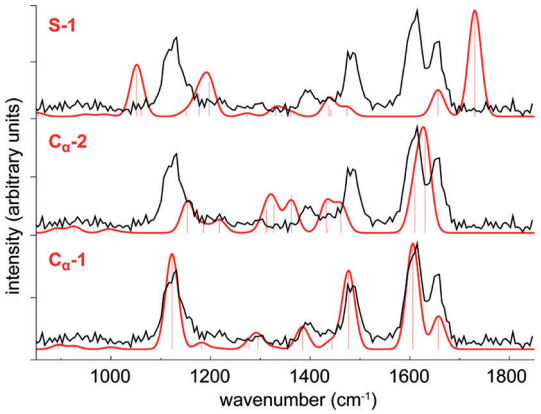

Figure 4. Experimental (black) and theoretical (red) IR spectra for $[Hcy-H+Li]^{\bullet+}$. Theoretical spectra were calculated at the B3LYP/6-311++G(d,p) level and with a scaling factor of 0.976.

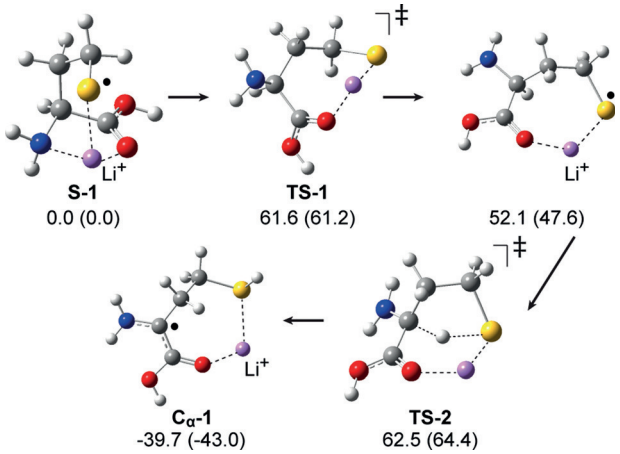

Figure 5. Energy profile for the S-to-C_{α} radical isomerisation of $[Hcy-H+Li]^{\bullet+}$. All other metal ion/Hcy radical complexes investigated resulted in the same pathway. Enthalpies (free energies in parentheses) are given in kJ mol^{-1} and are relative to the S-1 ion.

Comparison of the experimental and theoretical IR spectra in the 850–1850 cm^{-1} region reveals that the C_{α}-1 species gives the best and only satisfactory agreement for all metal ions (Figure 4 for Li^{+} and Figures S1 and S2). A contribution from the S-1 species can be entirely excluded based on the absence of a carbonyl stretch band predicted at approximately 1725 cm^{-1}. Contributions from the C_{α}-2 structure are also unlikely due to the poor fit. It is apparent that for all the $[Hcy-H+M]^{\bullet+}$ ions the C_{α}-1 structure is the only one present in significant amounts in the gas phase.

As the initial sulfur radical is generated by S–NO homolytic cleavage, there must be a subsequent facile rearrangement to form the C_{α}-based species. Examination of the potential energy surfaces yielded a two-step isomerisation pathway (Figure 5) common for the Li^{+}, Na^{+} and K^{+} adducts. In the initial step, coordination with the NH_2 is broken via the first transition state (TS-1); this results in a bidentate (O,S) sulfur radical intermediate. Subsequently, the hydrogen atom transfer from C_{α} to S$^{\bullet}$ occurs with a slightly larger barrier resulting in the C_{α}-1 structure. The isomerisation was also calculated with the direct 1,4 HAT occurring before the metal migration. However, this pathway was found to be markedly higher in energy (Figure S3 in the Supporting Information).

The barriers against which $[Hcy-H+M]^{\bullet+}$ ions convert from S-1 to C_{α}-1 are lower by at least approximately 20 kJ mol^{-1} than the relevant barrier on the $[Hcy]^{\bullet+}$ potential energy surface (Table 1). Furthermore, the mechanisms by which these rearrangements occur are distinctly different. In the critical transition state (TS-2) for the $[Hcy-H+M]^{\bullet+}$ systems the metal ion formally carries the charge and is strongly coordinated to both S$^{\bullet}$ and the carbonyl oxygen, leaving the NH_2 free to assist in the stabilisation of the nascent captodative C_{α}-1 structure. By contrast, the S-1 conformer of $[Hcy]^{\bullet+}$ that undergoes the 1,4-HAT has the charge formally located on the NH_3^{+} and this is stabilised by hydrogen bonding with the S$^{\bullet}$ and the carbonyl oxygen (Figure S4 in the Supporting Information). In this rearrangement a proton is initially transferred to the sulfur and then in the step with the highest barrier (82 kJ mol^{-1}) the $SH^{\bullet+}$ group rotates away from the NH_2 to a structure in which it is close to the carbonyl oxygen. In the final step the hydrogen atom on the α-carbon is transferred to the carbonyl oxygen with the migration being assisted by the sulfur atom. A similar

isomerisation mechanism was found in the methionine radical cation.[16] For isomerisation of the homocysteine radical cation, the straightforward pathway of a 1,4 HAT followed by proton migration to the carboxyl group resulted in a substantially higher barrier (132 kJ mol^{-1}).[8]

In conclusion, the combination of IMR, IRMPD, and theoretical calculations reveals facile S-to-C_α radical rearrangement in alkali metal complexes of the homocysteine radical (structure B). This is in contrast with the equivalent rearrangement in $[Hcy]^{\bullet+}$ (structure A) where hydrogen migration from C_α-to-S is not observed in the gas phase under thermal conditions.[8] Theoretical calculations reveal both thermodynamic and kinetic rationale for this difference. Alkali metal ion coordination provides substantially greater stabilisation to the C_α-based species versus the S-based radical cation, as compared to $[Hcy]^{\bullet+}$ (indicated by an additional approximately 11–23 kJ mol^{-1}; Table 1). The calculated isomerisation pathways clearly show a significantly lower barrier for C_α-to-S hydrogen atom transfer by the introduction of an alkali metal ion (42–63 kJ mol^{-1} for metallated species vs. 82 kJ mol^{-1} for the protonated radical; Table 1).

While there has been a considerable amount of work examining metal ion/amino acid complexes in the gas phase,[17] few studies have looked at the effect of metal ions on radicals. Alkali metals have been shown to stabilize polyglycol radicals,[18] and have been used to eliminate the mobile proton in peptide radicals.[19] In this work we show that metal ion complexation to the Hcy thiyl radical induces facile HAT. To our knowledge, this is the first example of a gas-phase study where conversion of an S$^{\bullet}$-radical into a C_α- radical via hydrogen atom transfer occurs within a single amino acid residue. This opens the possibility of using metal ions as charge carriers to modulate the C_α-to-S hydrogen atom transfer barrier. Preliminary IMR data indicate that alkali metal ions lower the rearrangement barrier not only in Hcy, but also in several peptide systems (glutathione, GluCys, see Figure S5 in the Supporting Information). Future studies will focus on investigating those systems in greater detail.

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Keywords: alkali metal ions · homocysteine · hydrogen transfer · ion-molecule reactions · radical ions

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