Gas-phase regiocontrolled generation of charged amino acid and peptide radicals†

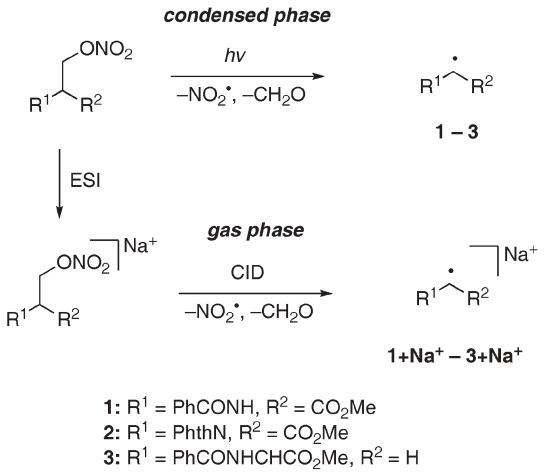
Sheena Wee,[a] Adam Mortimer,[bc] Damian Moran,[cd] Adam Wright,[b] Christopher K. Barlow,[ace] Richard A. J. O'Hair,*[ace] Leo Radom*[cd] and Christopher J. Easton*[bc]

Received (in Cambridge, UK) 20th June 2006, Accepted 7th July 2006
First published as an Advance Article on the web 29th August 2006
DOI: 10.1039/b608724h

The combined use of advanced mass spectrometry experiments, condensed-phase synthesis of serine and homoserine nitrate ester radical precursors, and high-level *ab initio* calculations provides a powerful way of examining the fundamental reactivity of radicals derived from peptides.

Peptide radicals play important roles in chemistry[1] and biology.[2] In the condensed phase, much of the information about these species is inferred from studies of reactants or products, or obtained more directly *via* spectroscopy (*e.g.*, ESR). Mass spectrometry, on the other hand, allows the direct study of radicals and their fundamental reactivity in the gas phase.[3] Electrospray ionization (ESI) has overcome the limits of volatility of peptides and typically yields protonated or deprotonated species. Attention has thus focused on developing new ESI-based methods of forming radical ions of peptides. Examples include one-electron reduction of multiply-protonated peptides *via* either electron-capture dissociation[4] or electron-transfer dissociation,[5] and dissociation of ternary transition metal complexes, which sometimes leads to the formation of peptide radical cations.[6] Although a range of methods have been developed to generate peptide radicals regiospecifically in solution,[1] these have been rarely exploited for the gas-phase generation of peptide radicals.[7] Here we utilize serine and homoserine nitrate esters, previously developed for the regiospecific generation of amino acid and peptide radicals *via* photolysis in the condensed phase (Scheme 1),[8] to generate analogous gas-phase radicals. We also demonstrate that these radicals undergo unimolecular and bimolecular reactions consistent with the results of high-level *ab initio* calculations.

Protonated and sodiated nitrate esters of serine and homoserine derivatives are easily formed *via* ESI.[9] While the protonated systems tend to fragment *via* even-electron reactions, low-energy collision-induced dissociation (CID) of the sodiated species readily results in formation of the amino acid radicals **1+Na$^+$**, **2+Na$^+$** and **3+Na$^+$**, and related peptide radicals, through homolytic cleavage



1: R^1 = PhCONH, R^2 = CO_2Me
2: R^1 = PhthN, R^2 = CO_2Me
3: R^1 = $PhCONHCHCO_2Me$, R^2 = H

Scheme 1 Method for regiospecific radical generation from nitrate esters.

involving the combined losses of $NO_2^{\bullet}$ and CH_2O (Scheme 1). The site of radical formation is therefore predetermined.

Although several studies have examined the gas-phase fragmentation reactions of amino acid and peptide radicals,[3–7] their bimolecular reactivity towards neutral reagents that probe their radical character has not been previously investigated. Thus we have examined the ion–molecule reactions of the sodiated amino acid derivatives **1+Na$^+$**–**3+Na$^+$** with the radical probes[10] allyl iodide, dimethyl disulfide, 4-fluorothiophenol and tributyltin hydride. Only the β-centred alanyl radical **3+Na$^+$** reacts with allyl iodide and dimethyl disulfide, by iodine-atom abstraction and homolytic substitution on the disulfide, respectively. In contrast, all three radicals abstract hydrogen from 4-fluorothiophenol and tributyltin hydride. As illustrated by the spectra shown in Fig. 1, the order of their reactivities is **3+Na$^+$** > **2+Na$^+$** > **1+Na$^+$** with 4-fluorothiophenol [Fig. 1(a)–(c)], and **2+Na$^+$** > **1+Na$^+$** > **3+Na$^+$** with tributyltin hydride [Fig. 1(d)–(f)].

These results are consistent with the calculated radical stabilization energies (RSEs, the computed enthalpies for the reaction $R^{\bullet}$ + $CH_4 \rightarrow RH + {}^{\bullet}CH_3$ at 0 K) of the radicals **1+Na$^+$**–**3+Na$^+$** of 51.9, 50.2 and 1.5 kJ mol^{-1}, respectively, and their computed enthalpies of reaction with dimethyl disulfide and 4-fluorothiophenol (Table 1).[11] The β-centred alanyl radical **3+Na$^+$** has the lowest RSE and is the only one to react with allyl iodide and dimethyl disulfide, and is the most reactive towards 4-fluorothiophenol. The glycyl radicals **1+Na$^+$** and **2+Na$^+$** are calculated to have much higher RSEs and much less exothermic reactions with

[a]*School of Chemistry, University of Melbourne, Victoria 3010, Australia*
[b]*Research School of Chemistry, Australian National University, ACT, 0200, Australia*
[c]*ARC Centre of Excellence in Free Radical Chemistry and Biotechnology, Australia*
[d]*School of Chemistry, University of Sydney, NSW 2006, Australia*
[e]*Bio21 Institute of Molecular Science and Biotechnology, University of Melbourne, Victoria, 3010, Australia*

† Electronic supplementary information (ESI) available: computed geometries and energies for all species required for Table 1 and Fig. 3. See DOI: 10.1039/b608724h

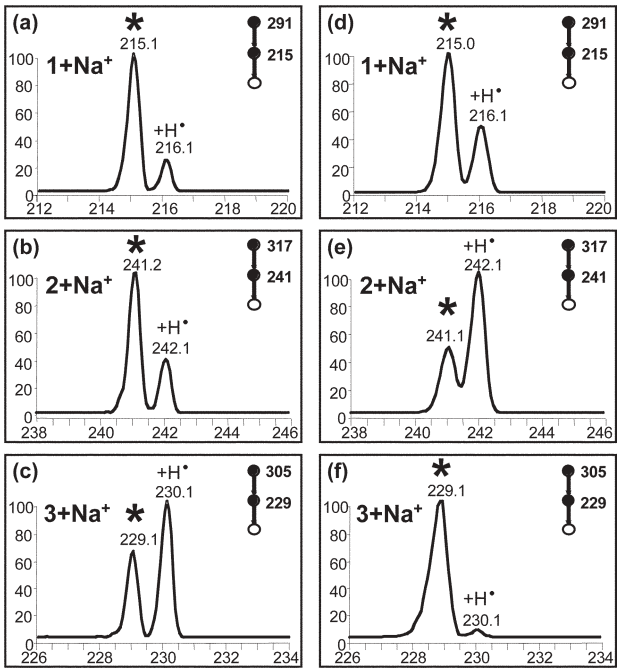


Fig. 1 Spectra showing the reactant (*) and product (+H•) ions of hydrogen-atom transfer reactions of the sodiated amino acid radicals **1+Na$^+$**, **2+Na$^+$** and **3+Na$^+$** with 4-fluorothiophenol [spectra (a), (b) and (c), reaction time = 10 s, pressure in trap $\approx 1.9 \times 10^{-4}$ Pa] and tributyltin hydride [spectra (d), (e) and (f), reaction time = 100 ms, pressure in trap $\approx 8.2 \times 10^{-4}$ Pa].[9]

4-fluorothiophenol and dimethyl disulfide, and they are generally less reactive, more so for the amide **1+Na$^+$** than for the corresponding phthalimide **2+Na$^+$**. The different pattern of reactivity with tributyltin hydride may reflect the ability of the substituents attached to the radical centres of **1+Na$^+$** and **2+Na$^+$** to stabilize the polar transition state for hydrogen-atom transfer.[1]

Although the coordinated sodium impacts on the properties of the radicals **1–3**, the trends in their behaviour are all maintained (Table 1). Thus, like the sodiated analogue **3+Na$^+$**, the non-sodiated β-centred alanyl radical **3** has the lowest RSE of **1–3**, and its reactions are calculated to be the most exothermic. The greater gas-phase reactivity of the phthalimide **2+Na$^+$** compared with the benzamide **1+Na$^+$** towards both dimethyl disulfide and 4-fluorothiophenol parallels the behaviour of acyl- and phthaloyl-glycyl radicals in solution,[12] and appears to correlate more closely with the relative RSEs and enthalpies of reactions of **2** and **1** rather than those of the sodiated analogues **2+Na$^+$** and **1+Na$^+$** (*cf.* reactions **A+Na$^+$/A** and **C+Na$^+$/C**). Although the reasons for this are unclear, it is apparent that there are parallels between the bimolecular reactivity of charged amino acid radicals in the gas phase and that of the uncharged analogues in solution, as well as with the calculated RSEs and reaction enthalpies of the radicals. The regiocontrolled gas-phase generation of charged amino acid and peptide radicals thus provides a useful method to investigate their reactivity with neutral reagents.

Table 1 Computed reaction enthalpies (0 K, kJ mol^{-1})[11]

Reaction	1	2	3
A+Na$^+$: R$^\bullet$ + CH_4 → RH + $^\bullet CH_3$	51.9	50.2	1.5
A: R$^\bullet$ + CH_4 → RH + $^\bullet CH_3$	81.2	30.8	1.6
B+Na$^+$: R$^\bullet$ + MeSSMe → MeSR + MeS$^\bullet$	−25.7	−36.3	−68.1
B: R$^\bullet$ + MeSSMe → MeSR + MeS$^\bullet$	19.6	−18.0	−57.8
C+Na$^+$: R$^\bullet$ + 4-FC_6H_4SH → RH + 4-$FC_6H_4S^\bullet$	−50.1	−51.8	−100.5
C: R$^\bullet$ + 4-FC_6H_4SH → RH + 4-$FC_6H_4S^\bullet$	−20.8	−71.3	−100.5

The controlled generation of radicals also provides the opportunity to examine their fundamental unimolecular fragmentation reactions, including simple radical scission processes. Loss of PhCO$^\bullet$ through a standard β-scission is the major fragmentation of **1+Na$^+$**. In contrast, **2+Na$^+$** loses the combined elements of CH_2O and CO, while **3+Na$^+$** fragments *via* loss of HNCO.

Having a technique for the regiospecific production of radicals also allows study of more complex rearrangements between degenerate species. We therefore examined some intramolecular hydrogen-atom migrations, a process that has been used to rationalize the fragmentation reactions of peptide radical cations[6d] and which is conceptually related to the "mobile proton" model for fragmentation of protonated peptides.[13] Thus we used the nitrate esters of *N*-benzoyl-α-methylserylglycine methyl ester, *N*-benzoylalanylserine methyl ester and *N*-benzoylhomoserylglycine methyl ester, to produce each of the sodiated radicals **4+Na$^+$**–**6+Na$^+$** in a regiocontrolled manner. If the fragmentation reactions were solely derived from these initial radicals, then each CID spectrum would be unique. An examination of Fig. 2 reveals that this is not the case. Thus the CID spectra of **4+Na$^+$** and **5+Na$^+$** are almost identical, and show ions characteristic of each species, indicating that these radicals readily interconvert by 1,4-hydrogen-atom transfer in the gas phase. The spectrum of the β-centred alanyl radical **6+Na$^+$** is very similar to those of **4+Na$^+$** and **5+Na$^+$**, the major difference being the greater relative intensity of the peak with *m*/*z* 170, which corresponds to β-scission of

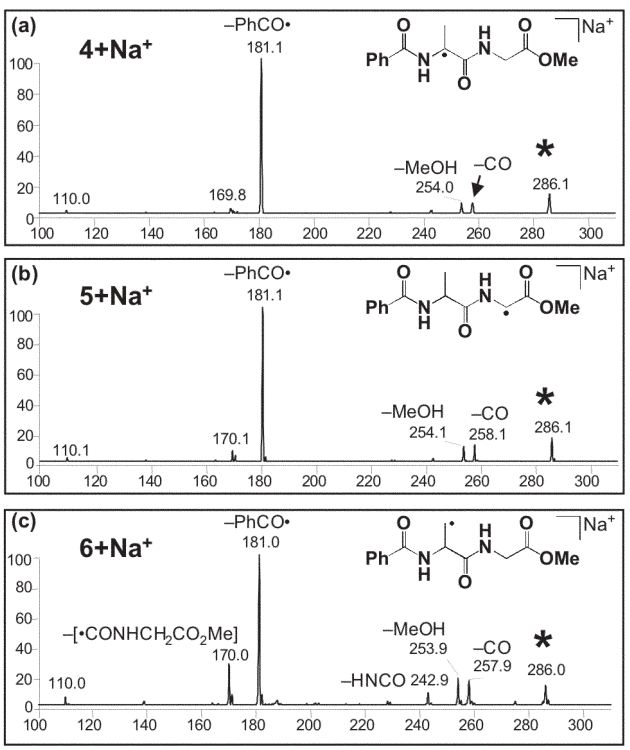


Fig. 2 CID spectra illustrating the rearrangements and fragmentations of the sodiated peptide radicals **4+Na$^+$** (a), **5+Na$^+$** (b), and **6+Na$^+$** (c).

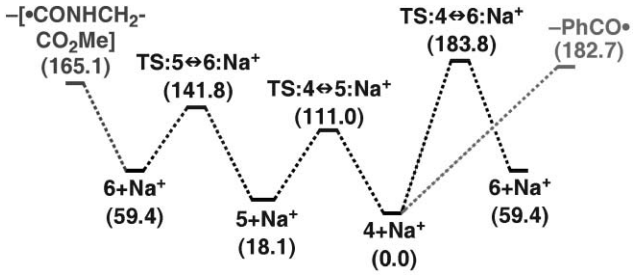


Fig. 3 Energy profile for rearrangements connecting **4+Na$^+$**, **5+Na$^+$** and **6+Na$^+$**, and the fragmentation of **4+Na$^+$** and **6+Na$^+$** (0 K, kJ mol^{-1}).[14]

6+Na$^+$. This suggests that the radical **6+Na$^+$** either fragments or rearranges to a mixture of **4+Na$^+$** and **5+Na$^+$**, presumably through an initial 1,5-hydrogen-atom transfer to give **5+Na$^+$**.

To gain further insights into the experimental results, we have extended our theoretical calculations[14] to probe aspects of the potential energy surfaces for the rearrangement and fragmentation reactions of the radicals **4+Na$^+$**–**6+Na$^+$**. An examination of the computed energy profile in Fig. 3 shows an energy ordering of **4+Na$^+$** < **5+Na$^+$** ≪ **6+Na$^+$**. Furthermore, the 1,5-shift from **6+Na$^+$** to **5+Na$^+$** has a barrier of 82.4 kJ mol^{-1} and the 1,4 shift from **5+Na$^+$** to **4+Na$^+$** requires 92.9 kJ mol^{-1}, so these processes should occur fairly readily. In contrast, rearrangement of **4+Na$^+$** and **5+Na$^+$** to the least stable radical **6+Na$^+$** requires 141.8 and 123.7 kJ mol^{-1}, respectively (*via* **TS:5↔6:Na$^+$**). The *ab initio* results are thus consistent with (a) the easy conversion of **6+Na$^+$** to **5+Na$^+$**, (b) the easy interconversion of **5+Na$^+$** and **4+Na$^+$**, and (c) the more difficult reverse rearrangements of **5+Na$^+$** or **4+Na$^+$** to **6+Na$^+$**. Finally the fragmentations of **4+Na$^+$** (with a barrier of 182.7 kJ mol^{-1}) or **6+Na$^+$** (with a barrier of 105.7 kJ mol^{-1}) require somewhat greater energy than rearrangement but are favoured entropically because they are more direct processes.

Preliminary studies suggest that these radical rearrangements also occur in larger peptides. Thus the radical formed from the sodiated nitrate ester of *N*-acetylserylpentaglycine methyl ester yields a series of similar types of fragment ions. These are indicative of multiple 1,4-hydrogen-atom migrations including those from the *N*-terminus to the *C*-terminus. Further studies are under way to probe the fundamental gas-phase reactivity of radicals derived from peptides. In particular, we will examine how other amino acid residues influence the radical rearrangements described here.

The authors thank the Australian Research Council for generous support, the University of Sydney for a Sesqui Postdoctoral Fellowship to DM, and APAC, ANUSF and AC3 for generous allocations of computing time.

Notes and references

1 C. J. Easton, *Chem. Rev.*, 1997, **97**, 53.
2 (*a*) M. J. Davies and R. T. Dean, *Radical-Mediated Protein Oxidation: From Chemistry to Medicine*; Oxford University Press: Oxford, 1997; (*b*) C. L. Hawkins and M. J. Davies, *Biochim. Biophys. Acta*, 2001, **1504**, 196.
3 K. W. M. Siu and A. C. Hopkinson, Peptide Radical Cations, in *Principles of Mass Spectrometry Applied to Biomolecules*, ed. C. Lifshitz and J. Laskin, Wiley Interscience, 2006.
4 (*a*) R. A. Zubarev, N. L. Kelleher and F. W. McLafferty, *J. Am. Chem. Soc.*, 1998, **120**, 3265; (*b*) R. A. Zubarev, *Mass Spectrom. Rev.*, 2003, **22**, 57.
5 (*a*) J. E. P. Syka, J. J. Coon, M. J. Schroeder, J. Shabanowitz and D. F. Hunt, *Proc. Natl. Acad. Sci. USA*, 2004, **101**, 9528; (*b*) H. P. Gunawardena, M. He, P. A. Chrisman, S. J. Pitteri, J. M. Hogan, B. D. M. Hodges and S. A. McLuckey, *J. Am. Chem. Soc.*, 2005, **127**, 12627.
6 (*a*) I. K. Chu, C. F. Rodriquez, T. C. Lau, A. C. Hopkinson and K. W. M. Siu, *J. Phys. Chem. B*, 2000, **104**, 3393; (*b*) C. K. Barlow, S. Wee, W. D. McFadyen and R. A. J. O'Hair, *Dalton Trans.*, 2004, 3199; (*c*) C. K. Barlow, W. D. McFadyen and R. A. J. O'Hair, *J. Am. Chem. Soc.*, 2005, **127**, 6109; (*d*) S. Wee, R. A. J. O'Hair and W. D. McFadyen, *Int. J. Mass Spectrom.*, 2004, **234**, 101.
7 (*a*) D. S. Masterson, H. Yin, A. Chacon, D. L. Hachey, J. L. Norris and N. A. Porter, *J. Am. Chem. Soc.*, 2004, **126**, 720; (*b*) R. Hodyss, H. A. Cox and J. L. Beauchamp, *J. Am. Chem. Soc.*, 2005, **127**, 12436.
8 (*a*) C. J. Easton, A. J. Ivory and C. A. Smith, *J. Chem. Soc., Perkin Trans. 2*, 1997, 503; (*b*) H. A. Headlam, A. Mortimer, C. J. Easton and M. J. Davies, *Chem. Res. Toxicol.*, 2000, **13**, 1087.
9 All experiments were completed in a modified Finnigan LCQ quadrupole ion-trap mass spectrometer (QITMS): G. E. Reid, R. A. J. O'Hair, M. L. Styles, W. D. McFadyen and R. J. Simpson, *Rapid Commun. Mass Spectrom.*, 1998, **12**, 1701. The initially-formed sodiated radicals are expected to be thermalized *via* collisions with the helium bath gas. Previous studies by Gronert suggest that ions in the QITMS are essentially at room temperature during ion–molecule reactions: S. Gronert, *J. Am. Soc. Mass Spectrom.*, 1998, **9**, 845.
10 (*a*) S. E. Tichy, K. K. Thoen, J. M. Price, J. J. Ferra, C. J. Petucci and H. I. Kenttamaa, *J. Org. Chem.*, 2001, **66**, 2726; (*b*) J. L. Heidbrink, L. E. Ramirez-Arizmendi, K. K. Thoen, L. Guler and H. I. Kenttamaa, *J. Phys. Chem. A*, 2001, **105**, 7875.
11 Radical stabilization energies (RSEs) and reaction enthalpies have been computed at the RMP2/G3MP2Large level of theory, with geometries and (scaled) ZPVE corrections determined at the B3-LYP/6-31G(2df,p) level. The RMP2 energies of the sodiated species were calculated with the inner-valence electrons on Na included in the correlation space (*i.e.*, RMP2(riv)/G3MP2Large). A positive RSE corresponds to relative stabilization of the radical. For a recent discussion of RSEs, see: D. J. Henry, C. J. Parkinson, P. M. Mayer and L. Radom, *J. Phys. Chem. A*, 2001, **105**, 6750.
12 C. J. Easton and C. A. Hutton, *Synlett*, 1998, 457, and references cited therein.
13 V. H. Wysocki, G. Tsaprailis, L. L. Smith and L. A. Breci, *J. Mass Spectrom.*, 2000, **35**, 1399.
14 Calculations were carried out at the RMP2(riv)/G3MP2Large//B3-LYP/6-31G(*d*) level.