On the Formation of Radical Dications of Protonated Amino Acids in a "Microsolution" of Water or Acetonitrile and Their Reactivity Towards the Solvent

Martin Sørensen,[a] James S. Forster,[c] Preben Hvelplund,*[a] Thomas J. D. Jørgensen,[b] Steen Brøndsted Nielsen,*[a] and Shigeo Tomita[a]

Abstract: In high-energy collisions (50 keV) between O_2 and protonated amino acids AH^+, radical dications $AH^{2+\bullet}$ are formed for A = Phe, His, Met, Tyr, and Trp. When solvated by water or acetonitrile (S), $AH^{2+\bullet}(S)_{1,2}$ are formed for A = Arg, His, Met, Tyr, and Trp. The stability of the hydrogen-deficient $AH^{2+\bullet}$ in the "microsolution" depends on the energetics of the electron transfer reaction $AH^{2+\bullet}+S \rightarrow AH^+ + S^{+\bullet}$, the hydrogen abstraction reaction $AH^{2+\bullet}+S \rightarrow AH_2^{2+}+[S-H]^\bullet$, and the proton transfer reaction $AH^{2+\bullet}+S \rightarrow A^{+\bullet}+SH^+$. Using B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d) model chemistry, we describe these three reactions in detail for A = Tyr and find that the first two reactions are unfavorable whereas the third one is favorable. However, energy is required for the formation of $Tyr^{+\bullet}$ and SH^+ from $TyrH^{2+\bullet}(S)$ to overcome the Coulomb barrier, which renders the complex observable with a life-time larger than 5 μs. The ionization energy, IE, of $TyrH^+$ is calculated to be 11.1 eV in agreement with an experimental measurement of 10.1 ± 2.1 eV ([IE(CH_3CN)+IE(Tyr)]/2); hydration further lowers the IE by 0.3 eV [IE($TyrH^+(H_2O)$ = 10.8 eV, calculated]. We estimate the ionization energies of $TrpH^+$, $HisH^+$, and $MetH^+$ to be 10.1 ± 2.1 eV, 12.4 ± 0.2 eV, and 12.4 ± 0.2 eV, and that of $PheH^+$ to be larger than 12.6 eV.

Keywords: amino acids · density functional calculations · high-energy collisions · solvation

Introduction

The chemical behavior of biological molecules in the gas phase has attracted considerable attention in recent years, and both experimental and theoretical studies have been carried out to better understand the function of proteins, protein folding as well as how the protein conformation is affected by hydration.[1, 2] The experiments rely on a range of mass spectrometric and laser spectroscopic tools, several of which were developed over the last decade. In a controlled experiment, reactions can be initiated by collisions between ions and gases or by irradiation of ions. Ionic biomolecules such as protonated amino acids and multiply protonated proteins are easily transferred from solution phase to gas phase by electrospray ionization (ESI).[1] Desolvation usually occurs in a heated capillary resulting in "naked" (unsolvated) ions, but under "gentle" source conditions (i.e., low temperature of the capillary) not all of the solvent is removed.[2] This makes it possible to form ions in a "microsolution" where the ionic environment may closely resemble that in biological systems since the number of water molecules in protein cavities is limited. Thus, the term microsolution simply means an environment that is somewhere between bulk solution and vacuum.

Solvent molecules can also be added afterwards to the naked ions in a high-pressure chamber as described by Kebarle and co-workers[3] or by a free jet expansion of a gaseous mixture containing the naked ions and the solvent in an inert carrier gas as described by Fenn and co-workers.[4] If ionic reactions reach equilibrium in the chamber, ΔH, ΔS, and ΔG values can be obtained from a simple measurement of the ionic composition.[3] Knowledge of the thermodynamics of specific reactions is crucial for the development of protein models and for understanding the very complicated processes taking place in nature.

[a] Prof. P. Hvelplund, Dr. S. B. Nielsen, M. Sørensen, Dr. S. Tomita
Institute of Physics and Astronomy
Aarhus Center for Atomic Physics, University of Aarhus
Ny Munkegade, 8000 Aarhus C (Denmark)
Fax: (+45) 86 12 07 40
E-mail: hvelplun@ifa.au.dk
sbn@ifa.au.dk

[b] Dr. T. J. D. Jørgensen
Department of Biochemistry and Molecular Biology
University of Southern Denmark (Odense University)
5230 Odense M (Denmark)

[c] Prof. J. S. Forster
Department of Engineering Physics
McMaster University Hamilton, Ontario, L8S 4L7 (Canada)

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  0947-6539/01/0715-3214 $ 17.50+.50/0

It is commonly said that the study of solvated ions bridges the gap between gas-phase chemistry (intrinsic chemistry) and solution chemistry. Surprisingly, however, there are few studies of hydrated biomolecules.

In this preliminary paper, it is shown that protonated amino acids AH^+ and micro-solvated ones $AH^+(S)_{1,2}$ ($S = H_2O$, CH_3CN) are further ionized to form radical dications $AH^{2+\bullet}(S)_{0,1,2}$ in high energy collisions (50 keV) with O_2. To our knowledge, this is the first report of the existence of such species in the gas phase. The energetics of electron transfer, proton transfer, and hydrogen atom transfer between $TyrH^{2+\bullet}$ and the solvent S are discussed based on density functional theory (DFT) calculations [B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d) model chemistry]. These three processes and their control are ubiquitous in biochemistry. Reactions that are normally not energetically feasible may become so; for example, in photosystem II the well-identified manganese cluster lowers the energy required to break the OH bond in water by approximately 1.3 eV thereby allowing a tyrosyl radical to abstract a hydrogen atom from water.[5] Transient or stable tyrosine and tryptophan radicals play key roles in electron transfer/hydrogen transfer processes (often involving nearby water molecules); for example, a tyrosyl radical is essential for the function of the iron-containing ribonucleotide reductase isolated from *E. coli*, and a tryptophan amino acid residue of the peptide chain in cytochrome c peroxidase stabilizes high iron oxidation states [O=Fe^{V}–Trp → O=Fe^{IV}–$Trp^{+\bullet}$].[6] In addition, glycine and cysteine radicals have been identified within proteins.[7] For more details about the utilization of amino acid radicals by enzyme systems and the mechanistic details, we refer the reader to a comprehensive review by Stubbe and van der Donk.[7]

This paper is divided in two parts; in the first part experimental results are presented and in the second part theoretical results for tyrosine systems are given and discussed in the context of the first part. This division reflects the two independent approaches to 1) obtain ionization energies of protonated amino acids and 2) elucidate the stability of solvated radical dications of protonated amino acids, that is, how do radical dications interact with surrounding solvent molecules?

Results and Discussion

Experimental results

Spectra from collisions between protonated amino acids and helium or dioxygen: First we observed the collision of protonated common α-amino acids (50 keV in the laboratory system) with helium and O_2. Figure 1 shows the results for protonated tyrosine, $TyrH^+$. Interestingly, for collisions with O_2 the radical dication $TyrH^{2+\bullet}$ is formed in very high abundance by electron loss from the $TyrH^+$ precursor.[8] The peak at m/z 68 is assigned to a doubly charged fragment, $[TyrH - H_2O, CO]^{2+\bullet}$, formed by loss of H_2O and CO from $TyrH^{2+\bullet}$ (Scheme 1).[9] Doubly charged ions, $AH^{2+\bullet}$ or fragments, were detected for other amino acids with O_2 as collision gas (Table 1), for example, $TrpH^{2+\bullet}$ and $[TrpH - H_2O, CO]^{2+\bullet}$ (see Figure 3).

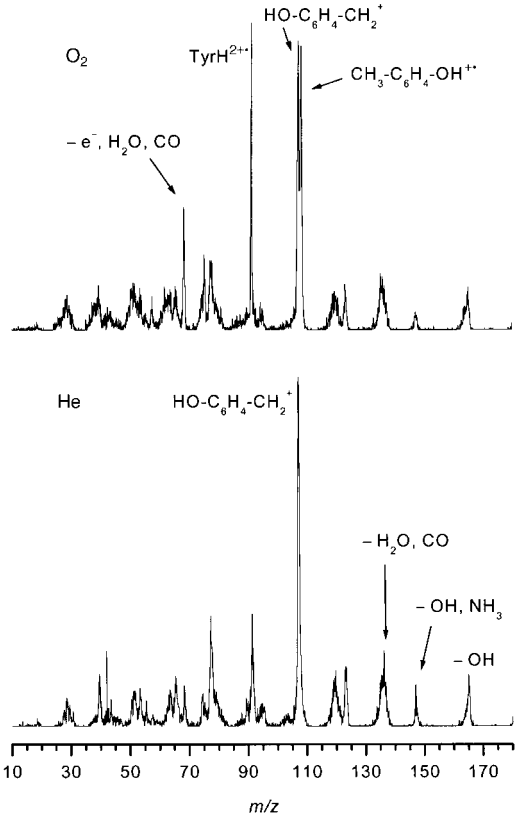
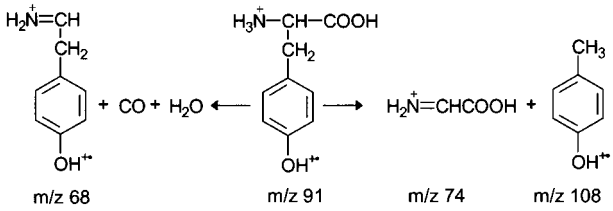


Figure 1. Spectra obtained from high energy collisions between $TyrH^+$ (m/z 182) and either helium or O_2.



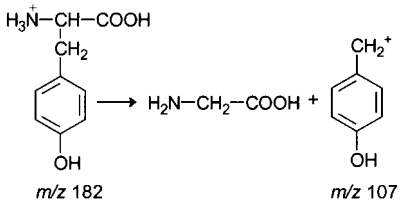
Scheme 1.

In the spectra of $TyrH^+$, peaks at both m/z 107 ($HO\text{-}C_6H_4\text{-}CH_2^+$) and 108 ($CH_3\text{-}C_6H_4\text{-}OH^{+\bullet}$) are observed with O_2 whereas only the m/z 107 peak is observed with He (Figure 1). Similar results were found for $PheH^+$: peaks at m/z 91 ($C_7H_7^+$, tropylium ion) and m/z 92 ($C_6H_5\text{-}CH_3^{+\bullet}$) are observed with O_2 but only m/z 91 with He.[10] We believe m/z 107 to result from a heterolytic C–C cleavage in vibrational excited $TyrH^+$ (Scheme 2). The selective enhancement of m/z 108 by O_2 is more difficult to explain. First, we can assume that the ion originates from the radical dication $TyrH^{2+\bullet}$ and is formed by a homolytic C–C cleavage, involving hydrogen atom transfer to avoid the formation of a biradical $^{\bullet}CH_2\text{-}C_6H_4\text{-}OH^{+\bullet}$ (Scheme 1). Likely, the dissociation co-produces the quite stable α-glycyl cation, $^+H_2N{=}CHCOOH$ (m/z 74),[11] with a slightly higher relative abundance, when O_2 is used. However, the m/z 108 peak is quite narrow and similar to the m/z 107 peak in shape. Actually, if the m/z 108 ion is formed together with m/z 74, the charge-dissociation reaction is only associated with a kinetic energy release (KER) of approximately 0.1 eV based on the peak width of m/z 108.[12] This KER is

Table 1. Summary of results obtained from high energy collisions between O_2 and protonated amino acids, AH^+, and solvated protonated amino acids, $AH^+(S)_{1,2}$ ($S = H_2O$ or CH_3CN).

Amino acid	RH	PA(RH)[a] [kJ mol^{-1}]	IE(RH)[a] [eV]	$AH^{2+\cdot}$	$AH^{2+\cdot}(S)_{1,2}$
group I					
Gly	H_2	422.3	15.4	no	
	HCOOH		11.3		
Ala	CH_4	543.5	12.6	no	
	HCOOH		11.3		
Val	$CH_3CH_2CH_3$	625.7	10.94	no	
Pro	$CH_3CH_2CH_2^{\cdot}$			no	
Leu	$CH(CH_3)_3$	677.8	10.68	no	
Ile	$CH_3CH_2CH_2CH_3$		10.57	no	
Ser	CH_3OH	754.3	10.84	no	
Thr	CH_3CH_2OH	776.4	10.48	no	
Asp	CH_3COOH	783.7	10.65	no	
Phe	CH_3-Ph	784.0	8.83	yes	no
Glu	CH_3CH_2COOH	797.2	10.44	no	
Tyr	p-CH_3-PhOH	$>$ 817.3 (phenol)	8.34	yes	yes
Met	$CH_3CH_2SCH_3$	846.5	8.46	yes	yes (H_2O), no (CH_3CN)
Asn	CH_3CONH_2	863.6	9.69	?	
Gln	$CH_3CH_2CONH_2$	876.2	$<$ 9.69 (acetamide)	?	
Trp	3-methyl-indole	$>$ 933.4 (indole)	$<$ 7.76 (indole)	yes	yes
group II					
Lys	$CH_3CH_2CH_2CH_2NH_2$	921.5	8.73	2+ ions	no
His	5-methyl-imidazole	$>$ 942.8 (1H-imidazole)	$<$ 8.81 (1H-imidazole)	yes	yes (H_2O), no (CH_3CN)
Arg	$CH_3CH_2CH_2NH$-C(NH)(NH_2)	$>$ 986.3 (guanidine)	$<$ 9.10 (guanidine)	2+ ions	yes

[a] Taken from ref. [20].



Scheme 2.

lower than that typically measured for "coulombic explosions" of molecular dications (compared: $Ru(bipy)_3^{2+} \rightarrow [Ru(bipy)_2 - H]^+ + bipyH^+$ (bipy = 2,2′-bipyridine): KER = 0.3 eV, and $Ni(H_2O)_4^{2+} \rightarrow Ni(OH)(H_2O)_3^+ + H_3O^+$: KER = 0.8 eV).[13] Also, several of the peaks in the helium spectrum, which correspond to fragment ions formed from singly charged precursor ions, are broader than the m/z 108 peak. Hence, the step-wise mechanism through $TyrH^{2+\cdot}$ seems unlikely for the formation of the m/z 108 ion. Another mechanism involving an electronic excited state of $TyrH^+$ could be involved. Earlier, Flammang et al.[14] and Aubry and Holmes[15] have reported on the selective enhancement of particular fragment ions by O_2. Aubry and Holmes[15] proposed a resonance electronic excitation process to explain the O_2 specific reactions. In this process, the ions are formed with only a narrow band of excess internal energies; reactions that have activation energies within this band are preferred, or the ions dissociate nonergodically from the initial excited states.

It is well known from charge stripping and neutral reionization experiments that collisions with O_2 produce multiply or singly charged cations.[16] However, to emphasize the relatively high ionization efficiency with this technique, a comparison with electron irradiation (EI) is worthwhile: The EI (30 eV) of protonated polypeptides was previously reported to result in radical dications for large systems such as Substance P [mass 1347 Da] but not for smaller compounds such as [D-Ala^2]-leucine enkephalin-Arg [mass 726 Da] although these are much larger than the amino acids in this study.[17] Our ionization technique using O_2 collisions at high energy is efficient for protonated peptides and proteins, for example, $[Bradykinin+nH]^{n+}$ ($n = 1, 2$) [mass 1060 Da], $[gramicidin\text{-}S + nH]^{n+}$ ($n = 1, 2$) [mass 1140 Da], and $[lysozyme+nH]^{n+}$ ($n = 7-10$) [mass 14305 Da] were further ionized to $[bradykinin+nH]^{(n+1)+\cdot}$, $[gramicidin\text{-}S+nH]^{(n+1)+\cdot}$, and $[lysozyme+nH]^{(n+1)+\cdot}$.[18]

Ionization energies of protonated amino acids: Before addressing the factors which determine the ionization energy, we discuss the site of protonation in AH^+ which is either the α-amino group [$^+H_3NCH(R)COOH$, denoted group I amino acids] or the side chain [$H_2NCH(RH^+)COOH$, denoted group II amino acids]. The side chain is the preferred site for protonation if its proton affinity, PA(RH), is high. Theoretical calculations indicate that the common amino acids belong to group I with the exception of Arg, Lys, and His which have high PA(RH) values (Table 1).[19] We classify Arg, Lys, and His as group II amino acids even though there is most likely a favorable hydrogen bond interaction between RH^+ and the α-amino group (i.e., cyclic structure).[19, 21] However, in the ion beam several isomers may be present.

If we assume that for group I amino acids the electron is removed from the side-chain,[22] and that for group II amino acids the electron is removed from the glycine backbone, the vertical ionization energies (IE) of group I and II amino acids are calculated from Equations (1) and (2).

$$IE(AH^+)_I \approx IE(RH) + \frac{14.4\,eV \cdot Å}{\varepsilon_r \cdot r} \quad (1)$$

$$IE(AH^+)_{II} \approx IE(glycine) + \frac{14.4\,eV \cdot Å}{\varepsilon_r \cdot r} \quad (2)$$

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R is the amino acid side chain, *r* is the distance between the two positive charges, and ε_r is the effective dielectric constant (not to be confused with the macroscopic dielectric constant) of the amino acid. For the case of CH_2 spacer groups between two charges ε_r is approximately 1 (the vacuum limit).[23] However, for cyclic systems ε_r is larger (1.4 for benzene and 1.7 for cyclohexane)[24] and increases with solvation.[25] For large R groups, with low IE(RH) values, the Coulomb term is low as well, and $IE(AH^+)_I$ is therefore smallest for amino acids with a small IE(RH). It is clear from Table 1 that only when IE(RH) is less than about 10 eV, ionization with the formation of doubly charged ions occurs (group I amino acids). Our experiment is inconclusive for the smaller $AsnH^+$ and $GlnH^+$ ions due to interfering fragment ions; however, Coulomb repulsion is most probably too high in $AsnH^{2+\bullet}$ thus preventing ionization. For group II amino acids, glycine approximates the backbone from which the electron is removed. The IE of glycine (8.90 eV) is almost equal to the IE of toluene (8.83 eV, cf. side chain of Phe),[20] and indeed ionization occurs for $HisH^+$, $LysH^+$, and $ArgH^+$ as observed for $PheH^+$.

Ionization of solvated AH^+: Our aim was to form solvated $AH^{2+\bullet}$ radical dications as such species may be generated in biological systems under UV radiation. In fact, UV induced inactivation of proteins is caused partly by the degradation of photoionized tryptophan.[26] The procedure we have used is to collide $AH^+(S)_{1,2}$ [S = water or acetonitrile] with O_2. Water was chosen because it is an essential ingredient in biological environments, and acetonitrile because it is only a hydrogen acceptor in hydrogen bond formation and thereby represents a different case system. Also, the C–H bond strength in acetonitrile is smaller than the O–H bond strength in water (by 1 eV, cf. part II), facilitating possible hydrogen abstraction reactions. Note, that in general the solvation of a molecule decreases its ionization energy.

In high energy collisions, dissociation of the weakly hydrogen-bound complex is the most important reaction channel, but $AH^{2+\bullet}(S)_{1,2}$ was also observed for several of the amino acids (Table 1). The abundance of $AH^{2+\bullet}(S)$ is highest for $AH^+ = TrpH^+$, $TyrH^+$, and $MetH^+$, and the dications $TrpH^{2+\bullet}(S)_{1,2}$, $TyrH^{2+\bullet}(H_2O)$, $TyrH^{2+\bullet}(CH_3CN)_{1,2}$, and $MetH^{2+\bullet}(H_2O)$ were observed. Spectra obtained for $TyrH^+$ and $TrpH^+$ are shown in Figures 2–4. The spectra of $TrpH^+(S)_2$ reveal that the $TrpH(S)_n^{2+\bullet}$ distribution ($n = 0$, 1, or 2) depends on S; for $S = H_2O$ the distribution peaks at $TrpH^{2+\bullet}$ whereas it peaks at $TrpH^{2+\bullet}(S)_2$ for $S = CH_3CN$; this indicates that acetonitrile binds more strongly to the radical dication than water. Interestingly, the $[TrpH^{2+\bullet} - H_2O, CO](S)_n$ distribution peaks at $[TrpH^{2+\bullet} - H_2O, CO]$ ($n = 0$, the unsolvated fragment ion of $TrpH^{2+\bullet}$). Either solvation prohibits loss of H_2O and CO, or $[TrpH^{2+\bullet} - H_2O, CO](S)_{1,2}$ is formed with enough internal energy to dissociate into $[TrpH^{2+\bullet} - H_2O, CO]$ and S or $2S$.

In the collisions between protonated amino acid dimers $TyrH^+ \cdots Tyr$, $TrpH^+ \cdots Tyr$, and $TrpH^+ \cdots Trp$ (S now being an amino acid) and O_2, no peaks in the spectra could be assigned to doubly charged ions (Figure 5). This is not a surprising result as IE(A) is smaller than $IE(AH^+)$. Therefore, any

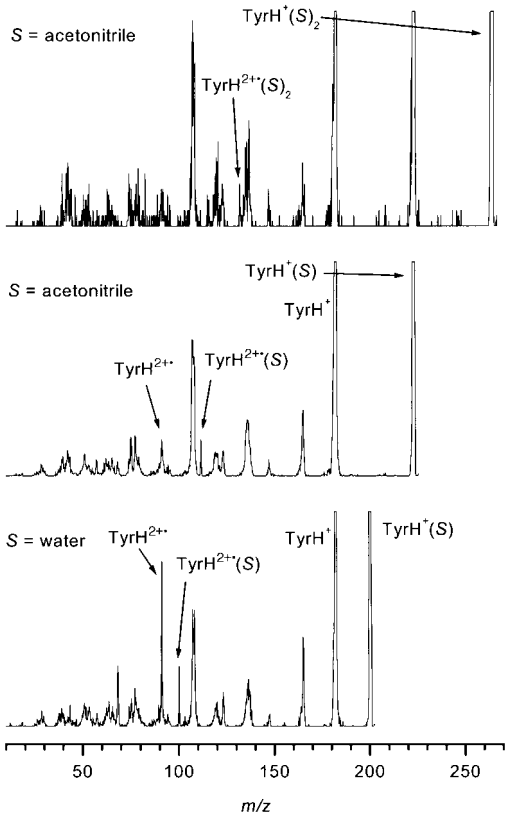

Figure 2. Spectra obtained from high energy collisions between O_2 and $TyrH^+(H_2O)$ (m/z 200) and $TyrH^+(CH_3CN)_{1,2}$ (m/z 223 and 264, respectively).

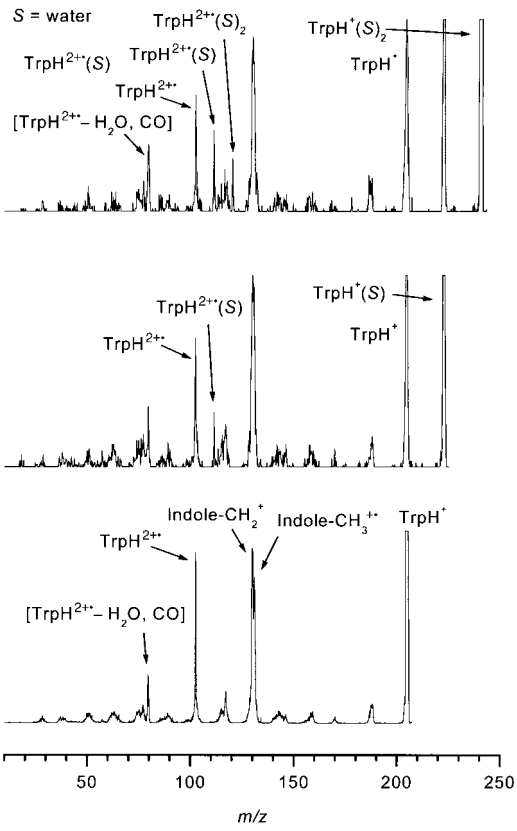

Figure 3. Spectra obtained from high energy collisions between $TrpH^+(H_2O)_{0,1,2}$ (m/z 205, 223, and 241, respectively) and O_2.

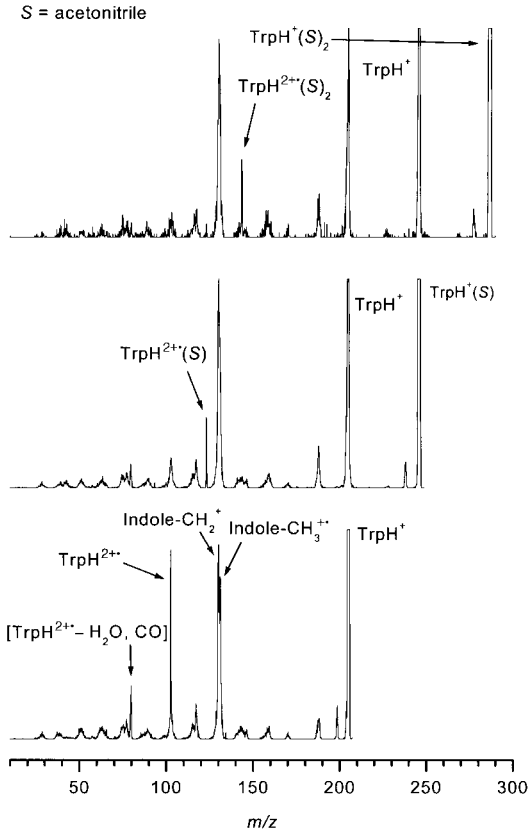

Figure 4. Spectra obtained from high energy collisions between $TrpH^+(CH_3CN)_{0,1,2}$ (m/z 205, 246, and 287, respectively) and O_2.

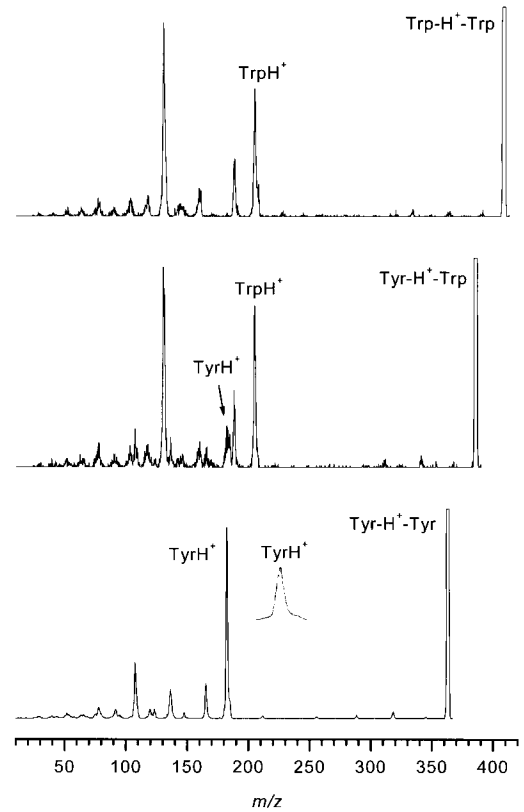

Figure 5. Spectra obtained from high energy collisions between $O_2$and Tyr-H^+-Tyr (m/z 365), Tyr-H^+-Trp (m/z 387), and Trp-H^+-Trp (m/z 409). The inset gives the enlarged Tyr H^+ peak.

ionization leading to $AH^+ \cdots A^{+\bullet}$ results in immediate dissociation into AH^+ and $A^{+\bullet}$ since the strength of the hydrogen bond is smaller than the Coulomb repulsion between the two charged amino acids.[27] In contrast, when IE(S) is larger than IE(AH^+), the solvated radical dication $AH^{2+\bullet}(S)$ is formed. Hence the observation of $AH^{2+\bullet}(S)$ when $S = H_2O$ or CH_3CN indicates that it is AH^+ which is ionized and not S implying an upper limit for IE(AH^+) of IE(CH_3CN): 12.20 eV [IE(H_2O) = 12.62 eV > IE(CH_3CN)].[20] As a result, the Coulomb term in Equation (1) for $TyrH^+$ is less than 3.86 eV [the difference between IE(p-CH_3-C_6H_4-OH), 8.34 eV, and IE(CH_3CN), 12.20 eV]. A rough estimate of IE($TyrH^+$) [and IE($TrpH^+$)] would be the average of IE(Tyr) (lower limit) and IE(CH_3CN) (upper limit) on account of the observance of $TyrH^{2+\bullet}(CH_3CN)$ [and $TrpH^{2+\bullet}(CH_3CN)$] and the lack of $TyrH^{2+\bullet}$(Tyr) [and $TrpH^{2+\bullet}$(Tyr)] and is (8.0+12.2) eV/2 = 10.1 eV with an uncertainty of (12.2 – 8.0) eV/2 = 2.1 eV.[28] It is interesting to note that for $MetH^+(S)$ and $HisH^+(S)$ ionization occurs when $S = H_2O$ but not when $S = CH_3CN$ which may be due to the lower IE of CH_3CN compared with that of H_2O. This would imply that IE($MetH^+$) ≈ IE($HisH^+$) ≈ (IE(CH_3CN)+IE(H_2O))/2 = (12.2+12.6) eV/2 = 12.4 eV (±0.2). No solvated radical dications were observed for Phe, which indicate that IE($PheH^+$) > IE(H_2O) = 12.6 eV. Such "bracketing-type" experiments are in progress to obtain better estimates of IE(AH^+).

The singly charged SH^+ and $S^{+\bullet}$ ions are only formed in very low abundance compared with that of $AH^{2+\bullet}(S)_{1,2}$, that is, $AH^{2+\bullet}(S)_{1,2}$ is robust or stable with respect to internal proton or electron transfer and subsequent dissociation.

Earlier attempts to achieve hydrogen transfer in reactions between multiply charged peptide radicals and H_2, NH_3, H_2O, and CH_3CH_2OH failed.[17] Also, in our work where the adduct between the potential hydrogen donor and the hydrogen-deficient radical dication is formed (an adduct was not observed in the ion-molecule reaction experiment), we could not observe any AH_2^{2+} formation in our MIKE spectra.

Ionization mechanism: An important question to pose is whether the ionization mechanism is vertical or adiabatic. To answer this an estimate of the collision interaction time is required. An ion with the mass of 300 Da and a kinetic energy of 50 keV moves with a speed of 1.8×10^5 m s^{-1}, which gives a collision interaction time of 6×10^{-15} s assuming an interaction length of 10 Å. Since the vibrational periods of C–C, C–O, and C–N bonds ($\approx 3 \times 10^{-14}$ s) and of C–H, O – H, and N–H bonds ($\approx 1 \times 10^{-14}$ s) are longer than the collision interaction time, only partial relaxation of X–H bonds can occur.[29] Hence, the ionization mechanism is nearly vertical, and $AH^{2+\bullet}(S)_{0,1,2}$ is most likely formed with several quanta of vibrational excitation determined by the Franck – Condon factors (but with less internal energy than that required for breaking of the weakly hydrogen-bound complex).

Model chemistry

Geometry-optimized structures: Optimized structures of Tyr, $Tyr^{+\bullet}$, $TyrH^+$, $TyrH^{2+\bullet}$, and $TyrH_2^{2+}$ are shown in Figure 6. As there are several possible isomers of $TyrH_2^{2+}$ due to the high

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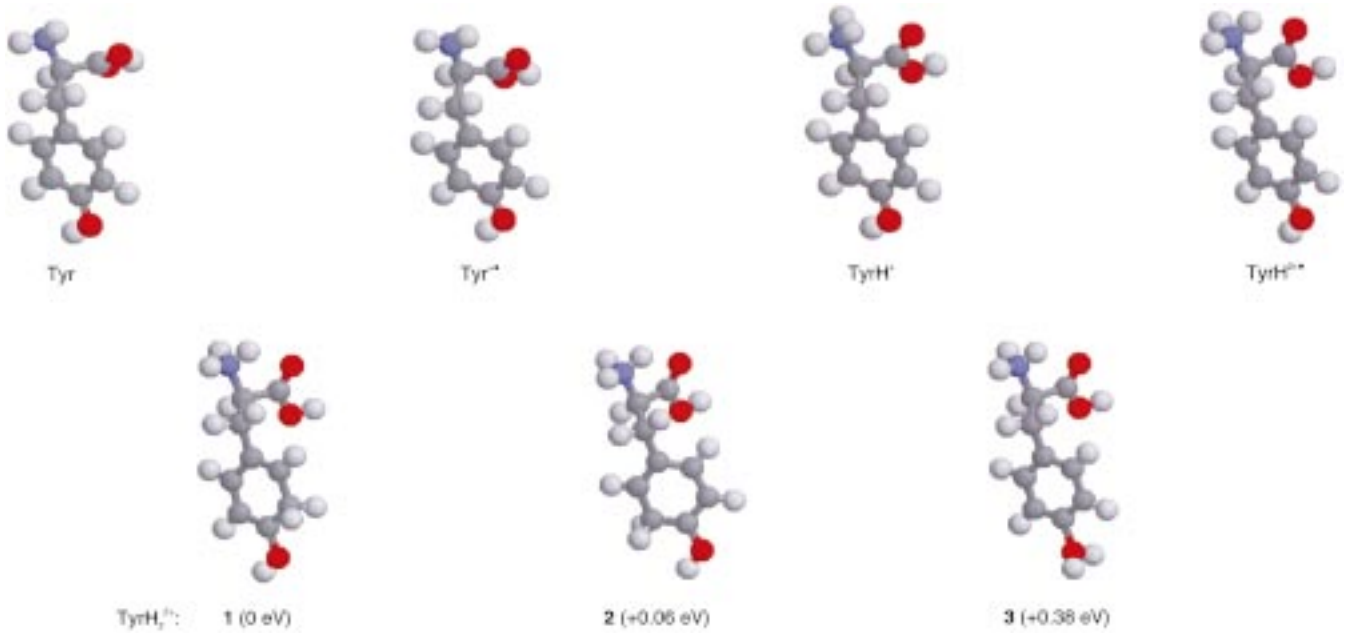

Figure 6. Optimized structures of tyrosine systems: Tyr, $Tyr^{+\bullet}$, $TyrH^+$, $TyrH^{2+\bullet}$, and $TyrH_2^{2+}$ (relative energies given) at the B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d) level.

number of protonation sites on the phenol ring it is a very time-consuming task to determine all of them. We have therefore limited our search to those isomers we expect to be of lowest energy. For example, the lowest energy isomer of $[p\text{-}CH_3\text{-}PhOH]H^+$ is the one in which the proton is bound to the second carbon in the benzene ring (denoted C_2 when C_1 is defined as *C*-OH), and hence calculations were carried out for the corresponding $TyrH_2^{2+}$ complex. Two C_2-protonated isomers were located, denoted **1** and **2** in Figure 6. An O-protonated structure of $TyrH_2^{2+}$ (**3**) was also found which lies higher in energy by 0.38 eV relative to **1**. In the parent ion complex $TyrH^+(H_2O)$ the water molecule is hydrogen bound to the ammonium group (Figure 7), and this must be so also for the radical dication complex $TyrH^{2+\bullet}(H_2O)$ if the ionization process is vertical (cf. last Section, first part).

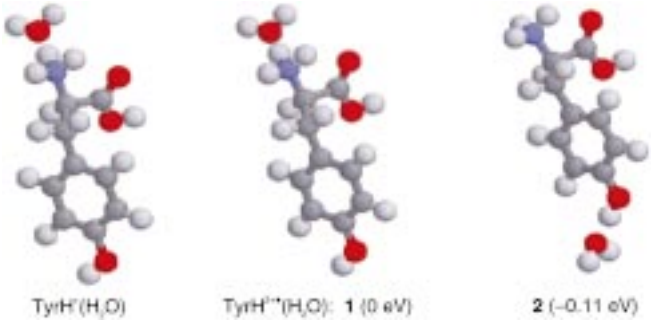

Figure 7. One optimized structure of $Tyr^+(H_2O)$ and two of $Tyr^{2+\bullet}(H_2O)$ (relative energies given) at the B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d) level.

Energetics: Individual ionization energies (IEs), hydrogen atom affinities (HAs), and proton affinities (PAs) are summarized in Table 2. Also, the energy change for loss of water from $TyrH^+(H_2O)$ and $TyrH^{2+\bullet}(H_2O)$ is calculated. For

Table 2. B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d) calculated reaction energies (zero-point corrected), ΔE. Included are also some experimental values (enthalpies), taken from ref. [20]. All values are in eV.

Reaction	Calcd	Exptl.
ionization		
$TyrH^+ \rightarrow TyrH^{2+\bullet} + e^-$	11.09	10.1 ± 2.1[a]
$TyrH^+(H_2O) \rightarrow TyrH^{2+\bullet}(H_2O)$ (**1**) $+ e^-$	10.84	
$Tyr \rightarrow Tyr^{+\bullet} + e^-$	7.71	8.0
$p\text{-}CH_3\text{-}PhOH \rightarrow p\text{-}CH_3\text{-}PhOH^{+\bullet} + e^-$	7.93	8.34
$PhOH \rightarrow PhOH^{+\bullet} + e^-$	8.30	8.49
$H_2O \rightarrow H_2O^{+\bullet} + e^-$	12.60	12.62
$CH_3CN \rightarrow CH_3CN^{+\bullet} + e^-$	11.87	12.20
hydrogen release[b]		
$TyrH_2^{2+}$ (**1**) $\rightarrow TyrH^{2+\bullet} + H^\bullet$	3.05	
$TyrH^+ \rightarrow Tyr^{+\bullet} + H^\bullet$	3.48	
$[PhOH]H^+ \rightarrow PhOH^{+\bullet} + H^\bullet$	3.16[c]	3.36
$[p\text{-}CH_3\text{-}PhOH]H^+ \rightarrow p\text{-}CH_3\text{-}PhOH^{+\bullet} + H^\bullet$	2.82[d]	
$H_2O \rightarrow HO^\bullet + H^\bullet$	4.94	5.17
$CH_3CN \rightarrow CH_2CN^\bullet + H^\bullet$	3.94	
proton release[b]		
$TyrH_2^{2+}$ (**1**) $\rightarrow TyrH^+ + H^+$	5.62	
$TyrH^{2+\bullet} \rightarrow Tyr^{+\bullet} + H^+$	6.06	
$TyrH^+ \rightarrow Tyr + H^+$	9.44	9.60
$[PhOH]H^+ \rightarrow PhOH + H^+$	8.52[c]	8.47
$[p\text{-}CH_3\text{-}PhOH]H^+ \rightarrow p\text{-}CH_3\text{-}PhOH + H^+$	8.55[d]	
$H_3O^+ \rightarrow H_2O + H^+$	7.04	7.16
$CH_3CNH^+ \rightarrow CH_3CN + H^+$	8.09	8.08
dissociation		
$TyrH^+(H_2O) \rightarrow TyrH^+ + H_2O$	0.66	
$TyrH^{2+\bullet}(H_2O)$ (**1**) $\rightarrow TyrH^{2+\bullet} + H_2O$	0.90	
rearrangement		
$TyrH^{2+\bullet}(H_2O)$ (**1**) $\rightarrow TyrH^{2+\bullet}(H_2O)$ (**2**)	−0.11	

[a] This work. [b] HA or PA $= \Delta E + \Delta E_{int} + \frac{5}{2} RT$, where ΔE_{int} is the difference in internal energy which is less than or equal to 0.01 eV, and the $\frac{5}{2} RT$ term corresponds to the loss of three degrees of freedom plus the pV term (p = pressure, V = volume) ($= RT$) and is 0.06 eV at 298 K. [c] C_4 protonated (lowest energy structure according to ref. [31]). [d] C_2 protonated with *C*-OH defined as C_1 (this structure is lower in energy than the C_1, C_3, C_4, and *O* protonated ones by 0.08, 0.38, 0.88, and 0.74 eV, respectively).

those reactions where experimental values are known the validity of our theoretical model can be tested. All calculated values are within 0.3 eV of the experimental values with the largest deviations for ionization energies which are underestimated. A systematic underestimation should only have a small effect on the reaction energies given in Table 3. However, the ionization energy of $TyrH^+$, calculated to be 11.09 eV, is then probably 0.3 eV higher but within the limits of the experimental estimate of 10.1 ± 2.1 eV. Hydration of $TyrH^+$ decreases its ionization energy (by 0.25 eV) as expected. The reactions of tyrosine systems are summarized in Figure 8.

Table 3. B3LYP/6-311+(2d,p)//B3LYP/6-31+G(d) calculated reaction energies [eV]. Values are corrected for zero-point motion.

Reaction	$S = H_2O$	$S = CH_3CN$
electron transfer		
$TyrH^{2+\bullet} + S \rightarrow TyrH^+ + S^{+\bullet}$	1.51	0.78
hydrogen atom transfer		
$TyrH^{2+\bullet} + S \rightarrow TyrH_2^{2+}$ (**1**) $+ [S-H]^\bullet$	1.89	0.89
proton transfer		
$TyrH^{2+\bullet} + S \rightarrow Tyr^{+\bullet} + SH^+$	− 0.98	− 2.03

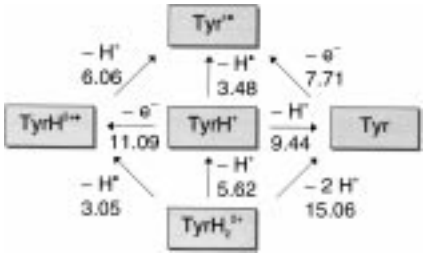

Figure 8. Electron transfer, proton transfer, and hydrogen transfer reactions of tyrosine systems. Values [eV] are based on B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d) model chemistry.

An estimate of the Coulomb term in Equation (1) for tyrosine is obtained from the difference between IE($TyrH^+$) and IE(CH_3-C_6H_4-OH) and is $(11.09 - 7.93)\ eV = 3.16$ eV [B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d) values used]. In the first section on the ionization of solvated AH^+ (first part) we estimated the Coulomb term to be less than 3.86 eV based on experimental observations in good agreement with our theoretical estimate of 3.16 eV.

Based on the individual reaction energies in Table 2, the energetics of the fundamental Reactions (3), (4), and (5) are calculated (Table 3).

$TyrH^{2+\bullet} + S \rightarrow TyrH^+ + S^{+\bullet}$ electron transfer (3)

$TyrH^{2+\bullet} + S \rightarrow TyrH_2^{2+} + [S-H]^\bullet$ hydrogen atom transfer (4)

$TyrH^{2+\bullet} + S \rightarrow Tyr^{+\bullet} + SH^+$ proton transfer (5)

Electron transfer: The ionization energy of $TyrH^+$ is lower than that of both H_2O and CH_3CN by 1.51 and 0.78 eV, respectively, which renders Reaction (3) unfavorable. This conclusion was also reached from the experimental data.

Hydrogen atom transfer: The hydrogen atom affinity of both $^\bullet OH$ and $^\bullet CH_2CN$ is higher than that of $TyrH^{2+\bullet}$, and the energy change of Reaction (4) is 1.89 eV for H_2O and 0.89 eV for CH_3CN, which explains the lack of $TyrH_2^{2+}$. The favored site for $H^\bullet$ capture in proteins is Trp on the indole ring,[30] but transfer of a hydrogen atom from H_2O to $indole^{+\bullet}$ is endothermic by 1.3 eV.[20] Thus, the formation of $AH_2^{2+}(OH^\bullet)$ is unfavorable for both Tyr and Trp.

Proton transfer: Transfer of a proton from $TyrH^{2+\bullet}$ to H_2O or CH_3CN liberates 0.98 eV and 2.03 eV, respectively, but the formation of two monocations from the adduct is associated with a high Coulomb barrier (cf. Section on the ionization of solvated AH^+).

Reactions of $TyrH^{2+\bullet}(H_2O)$: Based on the calculated structure of $TyrH^{2+\bullet}(H_2O)$ (isomer **1**, Figure 7) and its energy, we have determined the energy terms for the different product channels (Figure 9). The formation of $TyrH^{2+\bullet}(H_2O)$ (**1**) from $TyrH^+(H_2O)$ requires 10.84 eV.

In contrast to the electron transfer plus charge dissociation reaction, $TyrH^{2+\bullet}(H_2O)$ (**1**) $\rightarrow TyrH^+ + H_2O^{+\bullet}$, which is unfavorable (by 2.41 eV), the proton transfer plus charge

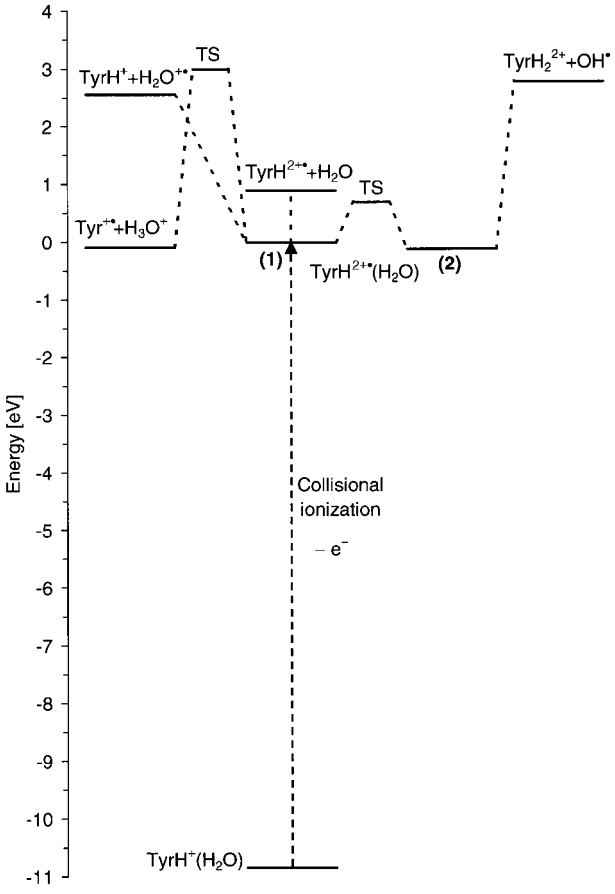

Figure 9. The parent ion $TyrH^+(H_2O)$ is ionized through a collision to $TyrH^{2+\bullet}(H_2O)$, which costs 10.84 eV. Electron transfer, proton transfer, and hydrogen transfer between $TyrH^{2+\bullet}$ and H_2O followed by dissociation requires more energy than dissociation into $TyrH^{2+\bullet}$ and H_2O. All energies are relative to $TyrH^{2+\bullet}(H_2O)$ (isomer **1**: 0 eV). TS = transition state. Model chemistry: B3LYP/6-311+G(2d,p)//B3LYP/6-31+G(d).

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dissociation reaction, $TyrH^{2+\bullet}(H_2O)$ (**1**) $\rightarrow Tyr^{+\bullet}+H_3O^+$, is slightly favorable (by 0.09 eV). However, there is an intrinsic Coulomb barrier that must be overcome for release of a proton with the formation of two monocations; that is, if the distance between the two charges is about 5 Å the barrier height is about 3 eV.

The hydrogen transfer plus dissociation reaction, $TyrH^{2+\bullet}(H_2O)$ (**1**) $\rightarrow TyrH_2^{2+}+{}^{\bullet}OH$, is highly unfavorable (by 2.79 eV). In addition, the distance between the H_2O and the phenol ring is large in $TyrH^{2+\bullet}(H_2O)$ (isomer **1**), which renders any hydrogen atom transfer reaction unlikely even if it were energetically favorable. Therefore, the water molecule would have to break its bond to the ammonium group [cf. isomer **1**, $H_2O\cdots{}^+H_3NCH(COOH)CH_2PhOH^{+\bullet}$] and instead form a new hydrogen bond to the phenol radical cation [cf. isomer **2**, $^+H_3NCH(COOH)CH_2PhOH^{+\bullet}\cdots OH_2$]. The energies of isomer **1** and **2** are almost identical, **2** being lower than **1** by only 0.11 eV. The barrier for the rearrangement reaction (**1** $\rightarrow$ **2**) is probably less than the energy required for water loss (0.90 eV), and in Figure 9 a value of 0.7 eV is used. The barrier for internal hydrogen atom transfer in **2** would be close to the reaction energy according to Siegbahn et al.[32] Hydrogen bonded groups such as water provide a low activation energy pathway for hydrogen atom transfer,[33] and the reaction becomes more feasible when two water molecules are attached as in $TyrH^{2+\bullet}(H_2O)_2$ though still highly endothermic.

The reaction which requires least energy (0.90 eV) is simple dissociation into $TyrH^{2+\bullet}$ and H_2O so this process is most likely to dominate [as experimentally observed].

The findings described above clearly indicate that the lifetime of the hydrated hydrogen-deficient radical dication is long in agreement with the experimental observation (lifetime $>5\,\mu$s, the flight time from the collision region to the detector).

Conclusion

"Naked" and solvated protonated amino acids were ionized (loss of one electron) in high-energy collisions with O_2. The method proved very efficient for ionization including the formation of weakly bound solvated amino acid radical dications. From the experimental data, we have deduced ionization energies of protonated amino acids in good agreement with model chemistry calculations on tyrosine systems.

Experimental Section

A detailed description of our instrumental set-up was given previously.[34] Briefly, ions formed by ESI were accelerated to a kinetic energy of 50 keV, mass selected by a magnet and subjected to collisional activation in a 3 cm long collision region (2 mTorr He or O_2, corresponding to single-collision conditions). Mass-analyzed ion kinetic energy (MIKE) scans were measured for the product ions in a 180° hemispherical electrostatic analyzer. The spray solutions were made by dissolving amino acids in a water/methanol 1:1 (*v*/*v*) solution with 1% acetic acid (*v*/*v*) or water/acetonitrile 1:1 (*v*/*v*) solution with 1% acetic acid (*v*/*v*) to concentrations of about 50 μM. Charged droplets formed in the ESI process passed through a heated capillary (180 °C) where desolvation occurred. To generate protonated amino acids with solvent molecules attached, the capillary was heated to only 100 °C.

Computational methods: Geometry optimizations of Tyr, $Tyr^{+\bullet}$, $TyrH^+$, $TyrH^{2+\bullet}$, $TyrH_2^{2+}$, $TyrH^+(H_2O)$, $TyrH^{2+\bullet}(H_2O)$, ${}^{\bullet}OH$, H_2O, $H_2O^{+\bullet}$, H_3O^+, ${}^{\bullet}CH_2CN$, CH_3CN, $CH_3CN^{+\bullet}$, CH_3CNH^+, PhOH, $PhOH^{+\bullet}$, $[PhOH]H^+$, *p*-CH_3-PhOH, *p*-CH_3-$PhOH^{+\bullet}$, and [*p*-CH_3-PhOH]H^+ at the B3LYP/6-31+G(d) level of theory were made using the Gaussian 98 program package.[35] For each molecule vibrational frequencies were calculated to confirm that the calculated stationary point is a minimum on the potential energy surface. Next, energies were calculated at the B3LYP/6-311+G(2d,p) level and corrected for zero-point motion [using non-scaled B3LYP/6-31+G(d) values].

Acknowledgements

We highly appreciate helpful discussions with Prof. Jens Ulrik Andersen (University of Aarhus) and Prof. Roman A. Zubarev (University of Southern Denmark). This work has been supported by the Danish National Research Foundation through the Aarhus Center for Atomic Physics (ACAP).

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Received: December 21, 2000 [F2957]

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