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Multiple substitution of protons by sodium ions in sodiated oligoglycines

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Abstract

Multiply-sodiated ions were observed by electrospraying oligoglycines and their N-acetylated and O-amidated derivatives in the presence of sodium hydroxide. These ions have all their hydrogens in the peptide linkages replaced by sodiums; one hydrogen in the C-terminal amide and the hydrogen in the C-terminal carboxylic group are also replaced. The N-terminal amine hydrogens are unreactive. These results are consistent with earlier postulations [Int. J. Mass Spectrom. 192 (1999) 303, J. Am. Soc. Mass Spectrom. 11 (2000) 967], and apparently confirm the gas-phase origin of these ions (formed in the electrospray source and/or the lens region). Collision-induced dissociation of multiply-sodiated oligoglycines showed that the major product ions are C-terminal ions. (Int J Mass Spectrom 219 (2002) 89–99)

Keywords: Sodium substitutions; Gas-phase reactions; Oligoglycines

1. Introduction

Sodium ions play a biological role by interacting with a number of important peptides and proteins [1–3]. The sodium ion participates in energy regulation as well as signaling [1,4–6]. The interaction between a sodium ion and a peptide or protein is specific and is essential for the biological function. Furthermore, the binding can be quite complex as there are multiple and competing binding sites on a given molecule [1–9].

Unlike transition metal ions, sodium ions do not appear to bind non-specifically to peptides and proteins [10]. There has been no report on general binding of sodium ions to peptides either in vivo or in vitro. By contrast, it is known that transition metal ions have high affinities for peptides in solution; anchoring a transition metal ion at the N-terminus is believed to be the first step leading to subsequent deprotonation of an amide linkage and chelation of the metal ion [10]. The resulting species may be described as $[M–H + X]^+$, where M–H is the peptide minus a proton and X is the doubly charged transition metal ion. Also, investigations on Cu(II) complexes with dipeptides and sodium hydroxide also show deprotonation of an amide linkage to form [Cu(II)(dipeptide–2H + Na)(bpy)]$^+$ where bpy is 2,2′-bipyridyl [11,12].

Sodiated peptides of the type, $[M–nH+mNa]^{(m-n)+}$, are observed when a peptide is electrosprayed in the presence of sodium hydroxide or a sodium salt [13–15]. Rodriquez et al. [14,15] postulated that these

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multiply-sodiated ions are formed in the lens region of the mass spectrometer as a result of gas-phase reactions. As micromolar concentration of peptide is electrosprayed with a millimolar concentration of sodium hydroxide or sodium chloride, it is expected that any peptide-bearing droplets or clusters will contain many sodium ions and their counter ions (anions). These droplets and clusters shrink in the ion source by solvent evaporation; consequently, the peptide and sodium hydroxide or chloride are brought into close contact effectively making a very concentrated solution and can react. Incomplete 'drying' of the droplets and clusters may be brought to completion in the lens region via collisional heating. Furthermore, reaction may also take place between a peptide ion and sodium hydroxide or chloride upon their collision in the ion source or in the lens region. The presence of alkali hydroxide within the species that are sampled into the mass spectrometer was firmly established in a collision-induced dissociation experiment of the ions in the continuous background, which yielded alkali-metal-containing peptide and alkali hydroxide cluster ions [14,16]. Possible reactions between alkali (sodium, potassium and lithium) hydroxide or chloride and the peptide can take place on a number of functional groups. Those that have been considered include the amide, the carboxylic and the amine groups. It was argued that because the number of protons abstracted and the number of sodium or potassium ions gained are relatively large, even for oligopeptides such as gramicidin s and bradykinin, most of the reactions must involve the amide group in the peptide linkage [14,15].

Using *N*-methylacetamide, acetic acid, the acetate anion and 1-propanamine as models for peptide functional groups, Rodriquez et al. [14,15] were able to show, via density functional theory (DFT) molecular orbital calculations, that many reactions between these model compounds and alkali hydroxide and alkali chloride are exothermic or slightly endothermic. Furthermore, using the model reactions between formamide and sodium hydroxide, and formamide and sodium chloride, they showed that these reactions have small barriers and are largely driven by their exothermicity [14,15]. The experimental difference in reactivity between alkali hydroxide and alkali chloride is in accord with results of the DFT calculations in that more reactions with the former are found to be exothermic or slightly endothermic than with the latter. It was judged that reactions that are endothermic by less than 20 kcal/mol are probably accessible because of collisional activation in the lens region [14,15].

It has been pointed out that peptides such as gramicidin s and bradykinin are too complicated in that they contain a number of competing functional groups, which makes interpretation unnecessarily difficult and the conclusions less than convincing. This study aims to improve our understanding of the substitution chemistry by reducing the number of variables that have to be considered by using only oligoglycines (thereby eliminating all possible side-chain functional group reactions). Furthermore, some of these oligoglycines are N-acetylated and O-amidated derivatives; thus they contain only one type of functional group, an amide.

2. Experimental

Experiments were conducted on an AB-SCIEX API 3000 prototype triple-quadrupole mass spectrometer (Concord, Ont.). All peptides and reagents were commercially available from Sigma (St. Louis, MO). Samples were 20 μM peptide + 1 mM sodium hydroxide (when desired, sodium chloride, potassium hydroxide or potassium chloride substituted for sodium hydroxide) in 50/50 methanol/water. To form deuterated peptides, samples were prepared in CH_3OD/D_2O and NaOD. The sample solution was continuously infused at a rate of 4 μL/min into the pneumatically-assisted electrospray probe with air being used as the nebulizer gas. Mass spectra were acquired in the range of 20–500 Th, at a step size of 0.2 Th, and at a dwell time of 10 ms. Typically 10 scans were summed to produce a mass spectrum. Tandem mass spectra were produced with nitrogen being the collision gas and at a typical pressure of 3 mTorr; centre-of-mass collision energies (E_{cm}) were typically 1–4 eV.

3. Computational methods

DFT employing the hybrid B3LYP method, which uses Becke's three-parameter exchange functional [17,18] and the correlation functional of Lee et al. [19], plus the 6–311 + +G(d,p) basis set [20–23] in Gaussian 98 [24], was used to calculate the optimized geometries and vibrational frequencies of the ions. All reported structures were characterized to be at minima. Inclusion of diffuse functions [20–23] allows a more accurate description of the outermost electrons. Splitting the valence shell into three parts increases the flexibility in the bonding region and enhances the description of the valence orbitals. The split-valence basis set was augmented by d-polarization functions

Table 1
Energetics of sodiated oligoglycines

Molecule	Total energy[a]	Thermal correction[b]
I	−264.65223	61.7
II	−426.81881	64.4
III	−588.57465	58.2
IV	−588.55874	58.1
V	−426.39098	50.7
VI	−426.35977	48.9
VII	−903.02121	76.8
VIII	−903.01423	77.0
IX	−903.00135	76.7
H_2O	−76.45853	15.1

[a] In hartrees.
[b] $(H_{298} - H_0)$ + ZPE (zero-point energy), in kcal/mol.

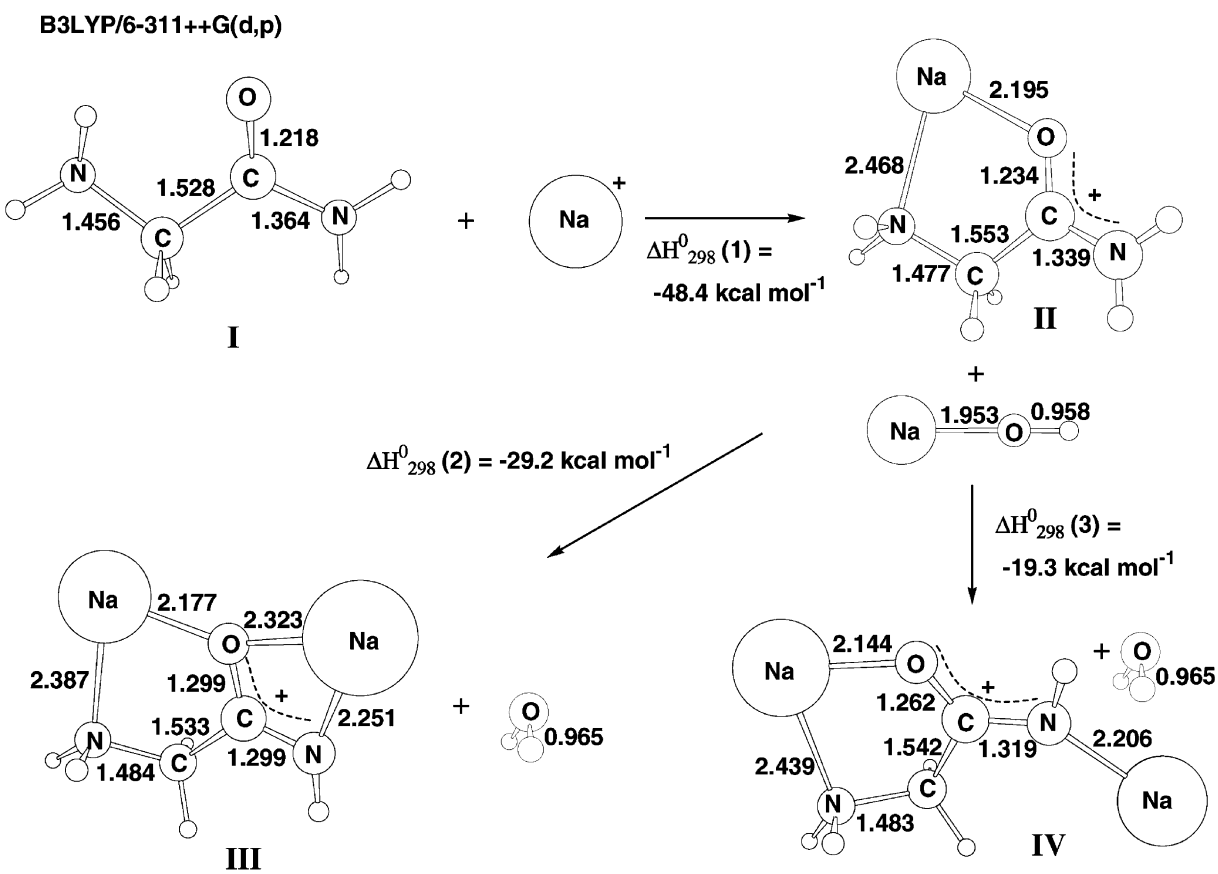

Fig. 1. Glycinamide and sodium: structures at minima as determined using B3LYP/6-311 + +G(d, p).

on the heavy atoms, as well as p-polarization functions on hydrogen to further improve the molecular description. Table 1 lists the energetics of molecules investigated.

4. Results and discussion

4.1. Glycinamide

Electrospraying glycinamide in the presence of sodium hydroxide produced the $[M + Na]^+$ ion at 97.0 Th and the $[M–H + 2Na]^+$ ion at 119.1 Th, but no $[M–2H + 3Na]^+$ or any other more extensively sodiated ions. Fig. 1 shows the DFT structures and energetics of the reactions between sodiated glycinamide and sodium hydroxide. As sodium hydroxide was present in large excess of glycinamide, the most prominent glycinamide cation was sodiated glycinamide. The most stable structure of sodiated glycinamide II, has sodium dicoordinated by the amino nitrogen and the carbonyl oxygen of the amide. The Na–O bond (2.195 Å) is considerably shorter than the Na–N bond (2.468 Å); the C–N bond of the sodiated amide at 1.339 Å is considerably shorter than the same bond of glycinamide I, at 1.364 Å, whereas the C–O bond of II at 1.234 Å is considerably longer than that of I at 1.218 Å, as a result of an increase in double bond character of the C–N bond in expense of the C–O bond. Reacting sodiated glycinamide with NaOH leads to substitution of an amide hydrogen on sodiated glycinamide by sodium and formation of H_2O. Fig. 1 shows two possible isomers of the resulting doubly sodiated derivative. Structure III,

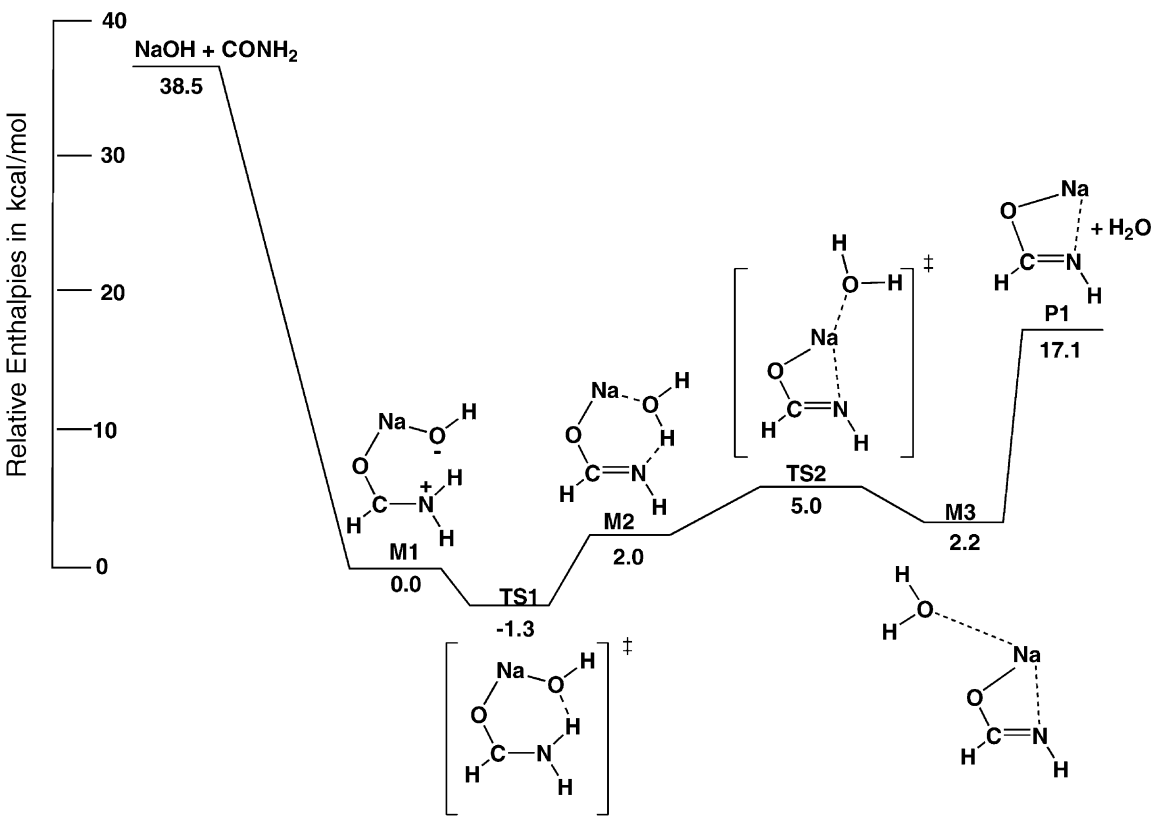

Fig. 2. Potential energy surface at B3LYP/6-311 + +G(d, p) for the reaction of NaOH with $HCONH_2 \rightarrow HCONH(Na)H + H_2O$ [15].

with the second sodium diccordinated by the amide oxygen and the amide nitrogen, is lower in enthalpy by 9.9 kcal/mol than structure IV, in which the second sodium is bound only by the amide nitrogen. Thus it may be assumed that doubly sodiated glycinamide exists almost exclusively as III. The ΔH°_{298} of both substitution reactions are exothermic (−29.2 and −19.3 kcal/mol for the formation of III and IV, respectively) and, as discussed before [14,15], these reactions should proceed efficiently. Although in reality these reactions take place in the lens region at an unknown temperature *T*, as a consequence of supersonic jet expansion and collisional heating [25], the *RT* term is small, thus ΔH°_{T} differs from ΔH°_{298} by, at the most 1–2 kcal/mol, and the use of ΔH° at the standard temperature of 298 K is justified in face of the unknown reaction temperature of *T*.

We consider the substitution reaction as formally 'ionic' in that it is essentially a sodium ion that replaces an amide proton and not a sodium atom replacing an amide hydrogen atom. DFT calculations of the model reaction between formamide and sodium hydroxide [15] show that the sodium atom carries a large amount of positive charge in all adducts of the two molecules. (For example, in structure III the Mulliken charges are +0.845 and +0.709 for the N-terminal sodium and the C-terminal sodium, respectively.) In Fig. 2, we provide the potential energy hypersurface for the reaction of $HCONH_2$ with NaOH calculated at B3LYP/6-311 + +G(d, p) [15]. Attachment of the sodium of NaOH to the carbonyl oxygen (M1) leads subsequently to elongation of the Na–O bond (M2) following transfer of an amide proton to the OH of NaOH via TS1, and dicoordination of the Na by the anionic

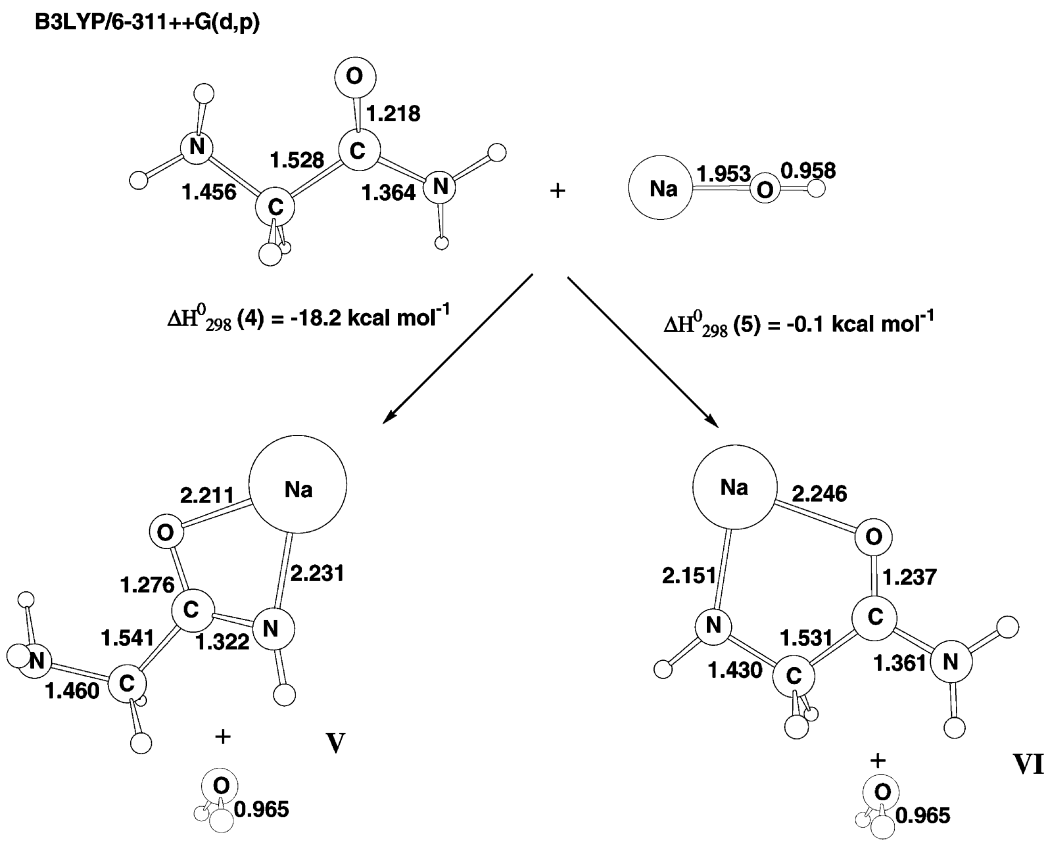

Fig. 3. Amide vs. amine substitution: structures at minima as determined using B3LYP/6-311 + +G(d, p).

amide nitrogen (in addition to the carbonyl oxygen) in TS2 and M3. Further elongation of the Na–OH_2 bond ensures that water is eliminated. Performing the experiments in deuterated solvents proved that the protons substituted by sodium ions are H/D exchangeable hydrogens, i.e., N–H or O–H hydrogens, rather than C–H hydrogens. Also we have clearly shown from these model reactions the presence of small activation barriers in the reaction steps and the reaction is driven entirely by its exothermicity.

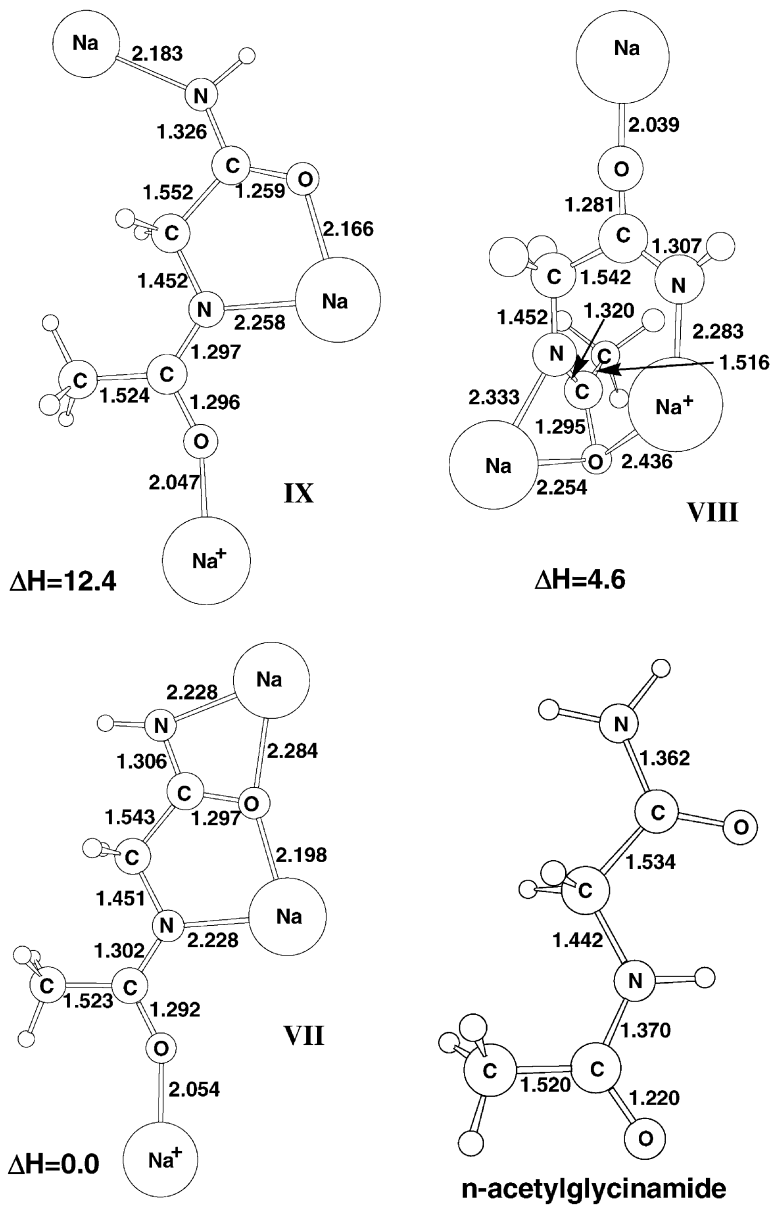

Fig. 4. The $[M–2H + 3Na]^+$ ion of *N*-acetylglycinamide: ΔH values are relative enthalpies (in kcal/mol) relative to the lowest energy structure VII.

Fig. 3 shows a comparison of DFT results for Na^+ substituting an amide proton (structure V) or an amino proton (structure VI) in glycinamide. It is apparent that the former reaction is exothermic by about 18 kcal/mol, whereas the latter is thermoneutral. Assuming the reactions to have small barriers, we would expect the substitution reaction for the amide proton to proceed at a much higher rate than that for the amine proton. This conclusion is in accord with experimental observations—the most extensively sodiated species for glycinamide is $[M–H + 2Na]^+$ whereas that for *N*-acetylglycinamide is $[M–2H + 3Na]^+$. The three lowest energy structures for the $[M–2H + 3Na]^+$ ion of *N*-acetylglycinamide are shown in Fig. 4. The relative enthalpies of the three structures are: VII, 0.0; VIII, 4.6; and IX, 12.4 kcal/mol. Both structures VII and VIII have two dicoordinated Na and one monocoordinated Na, while their higher energy isomer IX has only one dicoordinated Na. Structure VIII is higher in enthalpy than VII, perhaps reflecting the shorter distance (and hence larger Coulombic repulsion) between the two dicoordinated strongly-electropositive sodium species in VIII than in VII (3.526 vs. 4.380 Å, respectively).

4.2. Oligoglycines

Fig. 5 shows the electrospray mass spectrum of hexaglycine; six multiply-sodiated peptide ions are evident, from $[M–H + 2Na]^+$ to $[M–6H + 7Na]^+$,

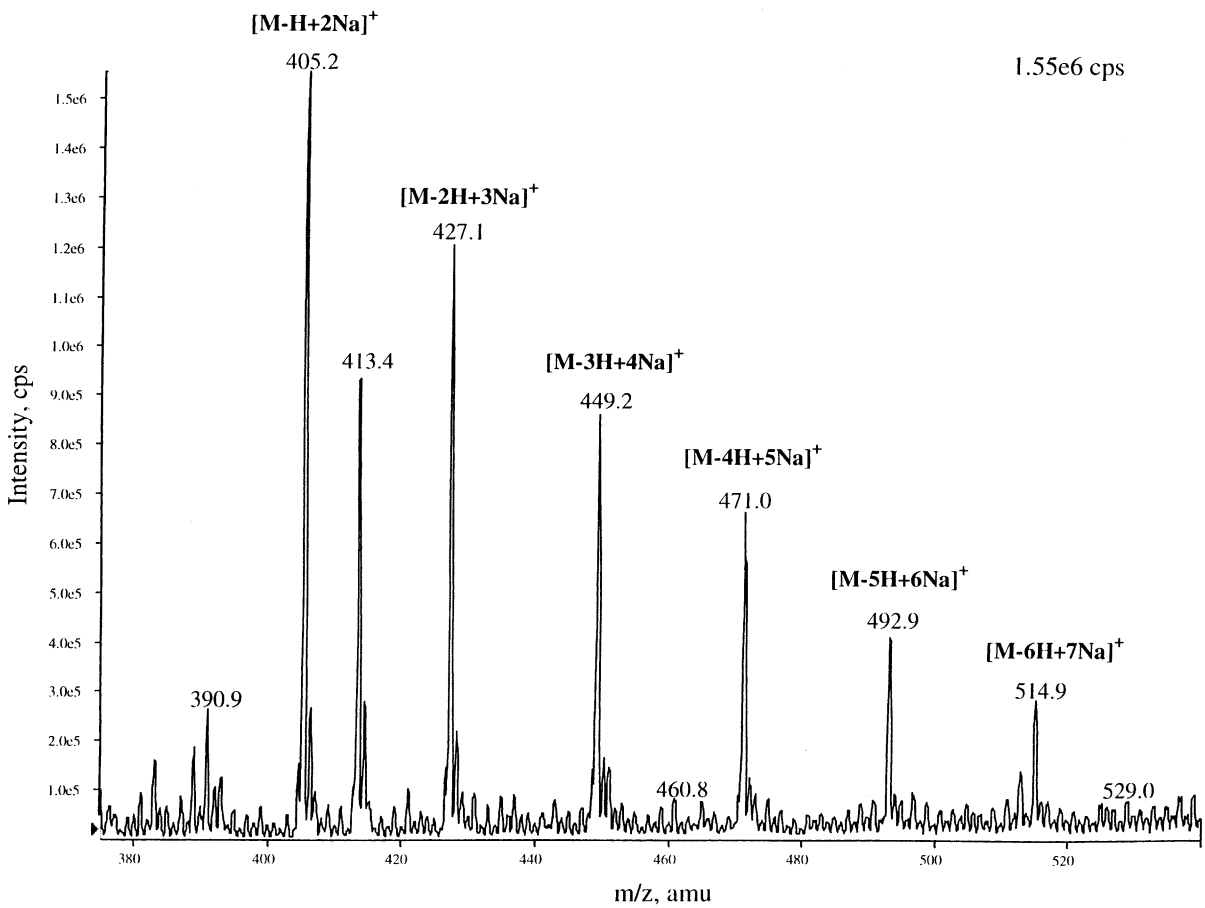

Fig. 5. Electrospray mass spectrum of hexaglycine with sodium hydroxide. The ions at *m/z* 391 and 413 are sodiated solvent clusters.

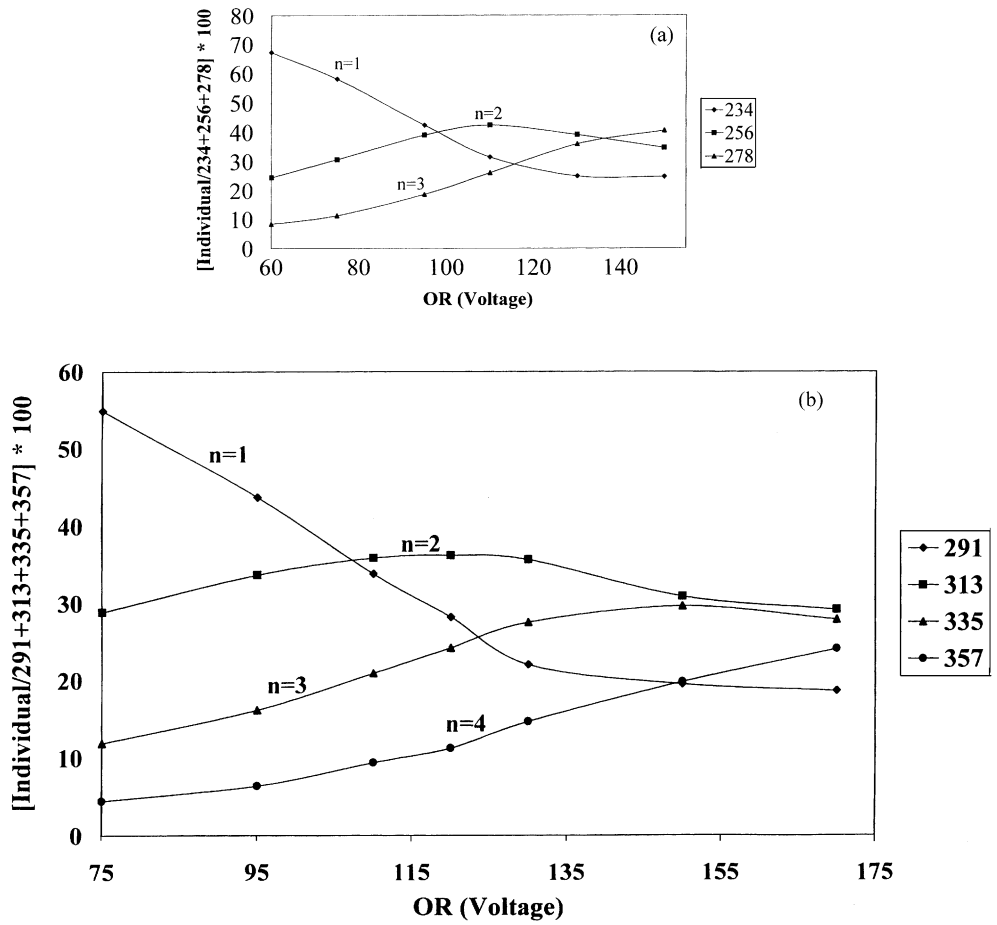

Fig. 6. Abundances of $[M–nH + (n + 1)Na]^+$ ions of (a) triglycine and (b) tetraglycine vs. orifice potential (OR): (◆) $n = 1$; (■) $n = 2$; (▲) $n = 3$; and (●) $n = 4$.

the last of which has all of its five amide protons and one carboxylic proton replaced by sodium ions. The relative abundance of the sodiated peptide ions is influenced by the collision energy in the lens region. More energetic collision conditions typically result in a decrease in the relative abundance of the less extensively sodiated peptides and an increase in those of the more extensively sodiated peptides. This is illustrated in Fig. 6, where the relative abundances of the $[M–nH + (n + 1)Na]^+$ ions for triglycine and tetraglycine are plotted vs. the orifice potential (OR), with higher OR value corresponding to more energetic collision conditions. These trends are consistent with the hypothesis [14,15] that the multiply-sodiated ions are formed in the lens region of the mass spectrometer as a result of gas-phase collisional heating.

A number of experiments were conducted with potassium hydroxide, which produces $[M–nH + (n + 1)K]^+$ ions that are entirely analogous to their sodiated counterparts. The use of the chlorides of sodium and potassium instead of the hydroxides results in a much lower extent of metal–ion incorporation. This is in apparent accord with the endothermic nature of

the reaction between formamide (amide group model) and alkali chloride, and the exothermic nature of the reaction between formamide and alkali hydroxide, which led to the postulation of slower rates for the reactions with alkali chloride than with alkali hydroxides [14,15].

The gas-phase fragmentations of monosodiated peptides have been examined by many groups [26–29]. A reoccurring observation is that the dissociation is mediated by sodium and results in elimination of the C-terminal residue with formation of truncated sodiated peptide ion, the $[b_n + OH + Na]^+$ ion. Repeated eliminations of the C-terminal residues in consecutive collisional-induced dissociation reactions produces a series of $[b_n + OH + Na]^+$ ions that may be used for peptide sequencing [29]. Fig. 7 shows a comparison of the fragmentation of the $[M–3H + 4Na]^+$ ions of (a) GGG and (b) LGG under two collision energy conditions, $E_{cm} = 2.5$ and 3.5 eV. It is evident that most of the major fragmentation products of the two peptides, i.e., the ions at 221, 197 and 142 Th, are identical, thus suggesting strongly that they are C-terminal fragment ions. These are to be contrasted with the N-terminal $[b_n + OH + Na]^+$ ions produced in the fragmentation of $[M + Na]^+$ peptide ions. H/D-exchange experiments showed that for GGG the precursor ion at 278 Th contains two exchangeable hydrogen atoms, and that one of them is lost in the dissociation to the product ions at 221, 197 and 142 Th. It would appear that the product ions at 221 and 142 Th are best described as y_2- and y_1-type ions with their carboxylic, amide, and one of the two amine hydrogens replaced by sodiums, and with an additional sodium ion added.

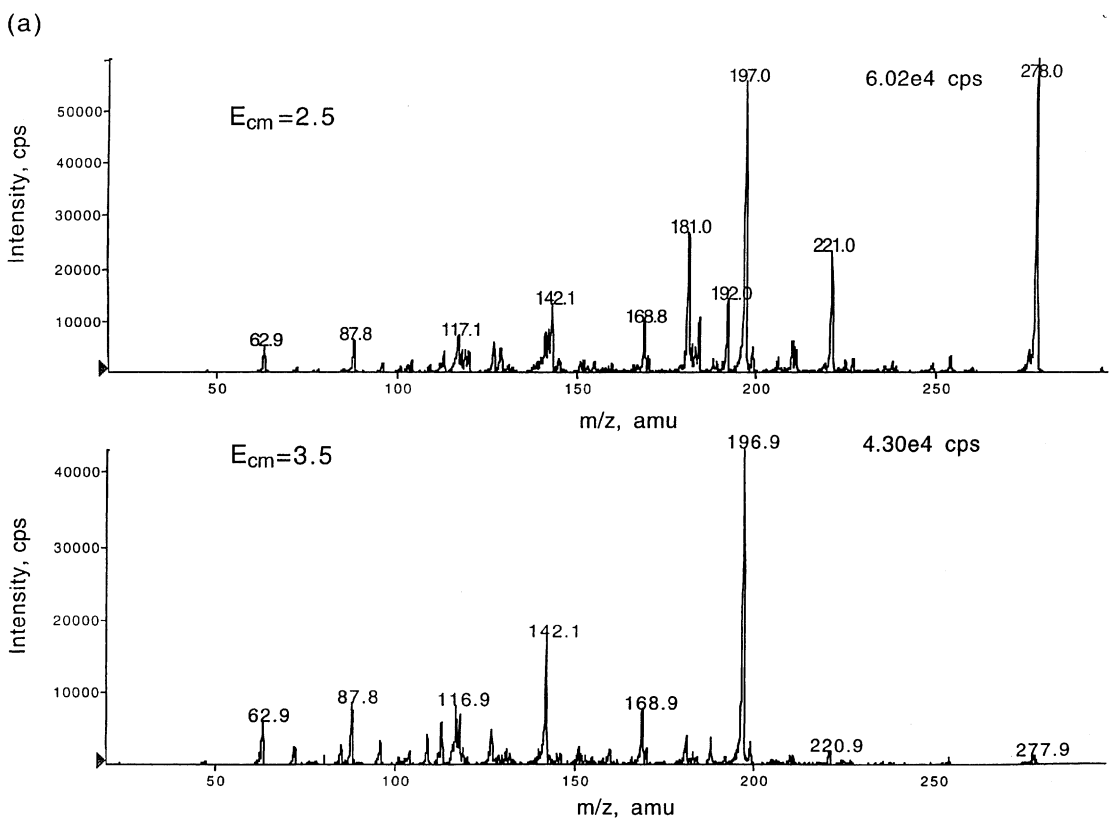

Fig. 7. Product ion spectra of (a) GGG and (b) LGG at $E_{cm} = 2.5$ and 3.5 eV: the common ions at 221, 197 and 142 Th are C-terminal ions.

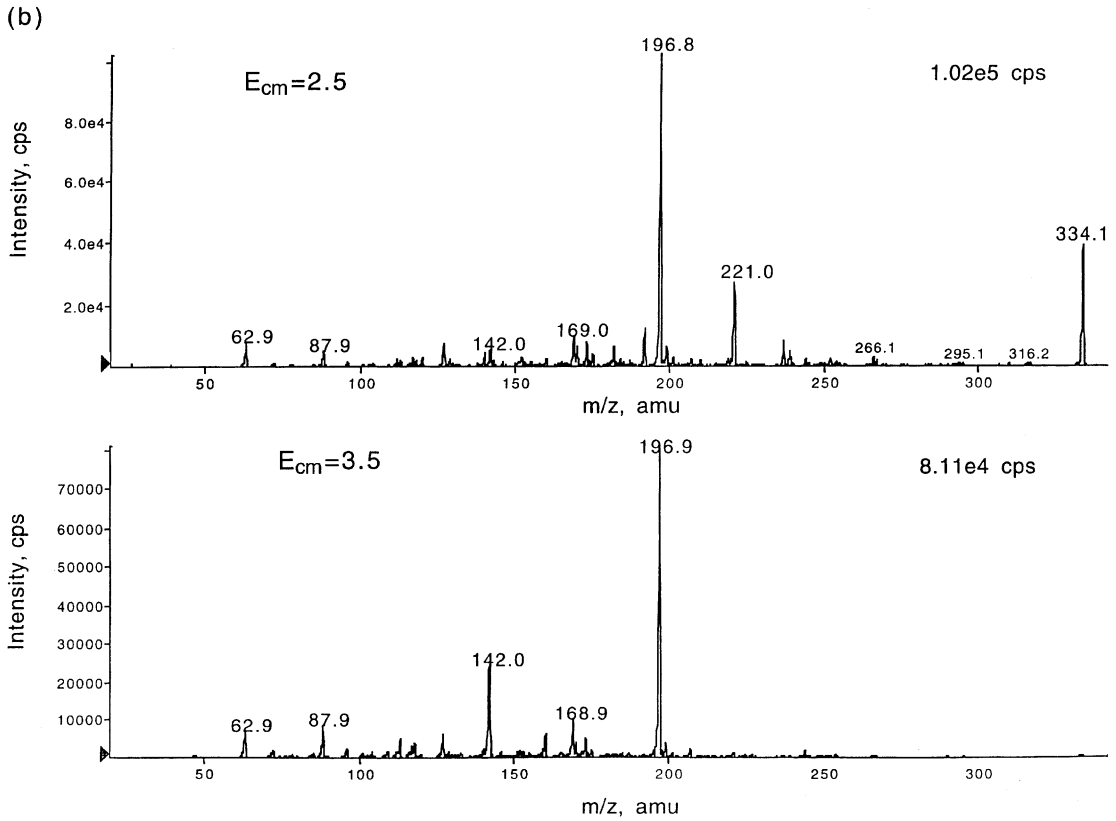

Fig. 7. (*Continued*).

5. Conclusion

The results in this study confirm that sodium ions can indeed substitute for amide protons in gas-phase reactions taking place in the front-end of an electrospray mass spectrometer. The resulting ions are unique in that they have not been observed to exist in solution. Collision-induced dissociation of the fully sodiated peptide ions reveals that the resultant fragment ions are C-terminal ions rather than the N-terminal ions, such as the b + OH, b and a ions, commonly observed in the dissociation of monosodiated peptide ions.

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