Conformation of Gas-Phase Myoglobin Ions

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The development of electrospray mass spectrometry, allowing the production of intact gas-phase protein ions,[1] has naturally stimulated discussion of the structures of these ions. The observations of noncovalent complexes of proteins with substrates and prosthetic groups[2] suggest that the folded tertiary structure of proteins and the interactions binding noncovalent complexes survive the ionization process and that the gas-phase protein may retain some memory of the solution structure. Clues to the structures of gas-phase protein ions come from measurements of H/D exchange of trapped ions,[3] the relative reactivity[4] or basicity[5] of different charge states, shapes and sizes of impact damage from ions on surfaces,[6] and collision cross sections measured by ion energy loss[7] or ion mobility,[8] a measure of ion "size".

In myoglobin (Mb), a heme prosthetic group binds noncovalently to a crevice in the compact folded apoprotein (apoMb) by van der Waals interactions, hydrogen bonds, and coordination of the heme iron to protein ligands.[9] Several groups have reported observation of the heme−apoMb complex by electrospray mass spectrometry.[10] Preliminary data[10e] suggest that gas-phase Mb ions may have a more compact structure than apoMb ions. Here we report collision cross sections for gas-phase ions of Mb, and for ions of apoMb formed by dissociating the heme−apoMb complex by collisionally activated dissociation (CAD) in the orifice-skimmer region of an electrospray mass spectrometer.[10b−e] The results here show that gas-phase Mb ions have a relatively compact structure that unfolds moderately when heme is lost. It is also shown that Mb when activated in collisions may unfold significantly while retaining heme, at least on the millisecond time scale of the mass spectrometry experiment. The moderate increase in cross section on loss of heme from gas-phase Mb ions is qualitatively similar to the moderate increase in cross section observed on loss of heme from Mb in solution as determined by small-angle X-ray scattering[11] and size exclusion chromatography.[12] This suggests that the gas-phase ion may have a degree of folding and a structure similar to those of the solution ion.

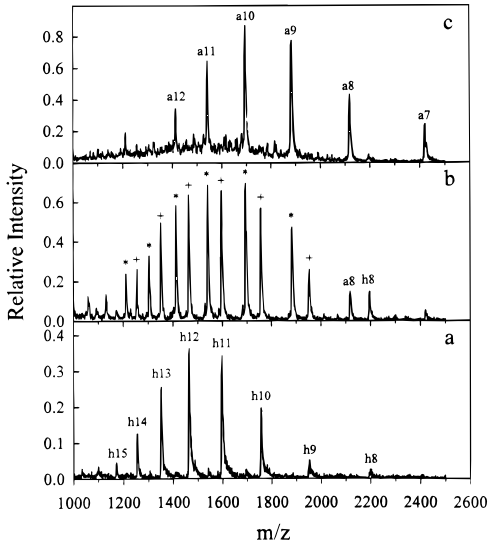

Figure 1. (a) Mass spectra of Mb measured with an orifice to skimmer voltage difference (ΔOS) of (a) 30 V, (b) 110 V giving a mixture of Mb (+) and apoMb(*), and (c) 180 V. Notation: h10 is Mb + $10H^+$, a8 is apoMb + $8H^+$, etc.

Experiments were performed on a triple quadrupole mass spectrometer constructed in house. Ions formed by pneumatically assisted electrospray (Sigma horse heart myoglobin no. M-1882, 5 μM in 10% MeOH/90% water) passed through a dry nitrogen "curtain" gas, a 0.25 mm diameter sampling orifice in a flat plate, a skimmer (0.75 mm orifice diameter) to a radio frequency (RF) quadrupole, and then to the first quadrupole of the triple quadrupole mass spectrometer system. Similar ion sampling arrangements have been described previously.[13] Collision cross sections with argon (Praxair, 99.999%) were determined by measuring the ratio of energies of ions leaving the collision cell, E, to the incident energy, E_o.[7a] The energy E_o was determined by the difference in rod offset potential between the first RF quadrupole and the collision cell quadrupole. This energy was independent of the orifice-skimmer voltage difference because collisions of ions with the background gas in the first RF quadrupole moderated the ion energies and energy spreads to <1 eV.[13a] The data were interpreted using the collision model of ref 7a (hard sphere collisions at a 90° center-of-mass scattering angle). Modeling the energy loss as an aerodynamic drag shows cross sections within 20% of this simple collision model.[7b] Tandem mass spectrometry (MS/MS) experiments confirmed that no dissociation or change of charge state of Mb occurred in the collision cell, indicating that the injection energy at the collision cell entrance was sufficiently low to preserve the heme−apoMb complex throughout the experiment. The pressure in the enclosed collision cell was

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(1) (a) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64−71. (b) Smith, R. D.; Loo, J. A.; Edmonds, C. G.; Barinaga, C. J.; Udseth, H. R. *Anal Chem.* **1990**, *62*, 882−899.

(2) Recent reviews: (a) Smith, D. L.; Zhang, Z. *Mass Spectrom. Rev.* **1994**, *13*, 411−429. (b) Smith, R. D.; Light-Wahl, K. J. *Biol. Mass Spectrom.* **1993**, *22*, 493−501.

(3) (a) Suchan, D.; Shi, Y.; Ben, S. C.; Senko, M. W.; Quinn, J. P.; Wampler, F. M., III; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 790−793. (b) Wood, J. D.; Choush, R. A.; Wampler, F. M., III; Little, D. P.; O'Conner, P. B.; McLafferty, F. W. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2451−2454.

(4) (a) McLuckey, S. A.; Van Berkel, G. J.; Glish, G. L. *J. Am. Chem. Soc.* **1990**, *112*, 5668−5669. (b) Winger, B. E.; Light-Wahl, K. J.; Rockwood, A. L.; Smith, R. D. *J. Am. Chem. Soc.* **1992**, *114*, 5897−5898. (c) Loo, R. R.; Smith, R. D. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 207−220.

(5) (a) Schnier, P. D.; Gross, D. S.; Williams, E. R. *J. Am. Chem. Soc.* **1995**, *117*, 6747−6757. (b) Gross, D. S.; Schnier, P. D.; Rodriguez-Cruz, S. E.; Fagerquist, C. K.; Williams, E. R. *Proc. Natl. Acad. Sci. U.S.A.*, submitted for publication.

(6) (a) Quist, A. P.; Ahlbom, J.; Reimann, C. T.; Sundqvist, B. U. R. *Nucl. Instrum. Methods Phys. Res.* **1994**, *88*, 164−169. (b) Reimann, C. T.; Quist, A. P.; Kopniczky, J.; Sundqvist, B. U. R. *Ibid.* **1994**, *88*, 29−34. (c) Sullivan, P. A.; Axelsson, J.; Altmann, S.; Quist, A. P.; Sundqvist, B.U. R.; Reimann, C. T. *J. Am. Soc. Mass Spectrom.* **1996**, *7*, 329−341.

(7) (a) Covey, T. R.; Douglas, D. J. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 616−623. (b) Douglas, D. J. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 17−18. (c) Cox, K. A.; Julian, R. K.; Cooks, R. G.; Kaiser, R. E. *J. Am. Soc. Mass Spectrom.* **1994**, *5*, 127−136.

(8) (a) von Helden, G.; Wyttenbach, T.; Bowers M. T.; *Science* **1995**, *267*, 1483−1485. (b) Clemmer, D. E.; Hudgins, R. R.; Jarrold, M. F. *J. Am. Chem. Soc.* **1995**, *117*, 10141−10142.

(9) Stryer, L. *Biochemistry*, 3rd ed.; W. H. Freeman and Co.: New York, 1988; pp 143−150.

(10) (a) Katta, V.; Chait, B. J. *J. Am. Chem. Soc.* **1993**, *113*, 8534−8535. (b) Li, Y. T.; Hsieh, Y. L.; Henion, J. D.; Ganem, B. *J Am. Soc. Mass Spectrom.* **1993**, *4*, 631−637. (c) Jaquinod, M.; Leize, E., Potier, J.; Albrecht, A.-M.; Shauzer, A.; Dorsselaer, A. *Tetrahedron Lett.* **1993**, *34*, 2771−2774. (d) Feng, R.; Konishi, Y. *J. Am. Soc. Mass Spectrom.* **1993**, *4*, 638−645. (e) Konishi, Y.; Feng, R. *Biochemistry* **1994**, *33*, 9706−9711.

(11) Nishii, I.; Kataoka, J.; Tokunaga, F.; Goto, Y. *Biochemistry* **1994**, *33*, 4903−4904.

(12) Goto, Y.; Calciano, L. J.; Fink, A. L. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 573−577.

(13) (a) Douglas, D. J.; French, J. B. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 398−408. (b) Bruins, A. P. *Mass Spectrom. Rev.* **1991**, *10*, 53−77.

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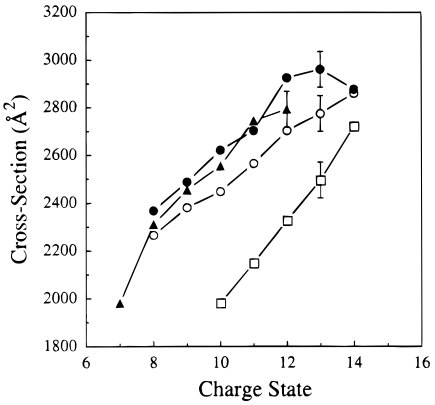

Figure 2. Collision cross section as a function of charge state for Mb (□) at ΔOS = 30 V, Mb (○) and apoMb (●) at ΔOS = 110 V, and apoMb (▲) at ΔOS = 180 V. Representative error bars are ±75 Å².

measured with a precision capacitance manometer (manufacturer's stated accuracy 0.12% of the reading). The slope of a linear plot of $\ln(E/E_o)$ vs target gas thickness, S (number density × cell length), gives the collision cross section. Simple linear plots were observed with correlation coefficients typically 0.994 or better, giving a maximum uncertainty of ±75 Å² (±3%) in the measured cross sections.

Recently it has been shown that gas-phase cytochrome *c* ions of a given charge state may have several coexisting conformers.[3,8b] Such conformers might be expected to have different cross sections and hence give different energy losses in our experiment. The concentrations of any coexisting conformers in our experiment is unknown. A mix of conformers can be expected to lead to complex nonlinear plots of $\ln(E/E_o)$ vs S. The linearity of our plots suggests however either that the Mb and apoMb ions exist substantially as conformers with similar cross sections or that a single conformer is the dominant species.

Figure 1a shows the mass spectrum of Mb measured with an orifice-skimmer voltage difference (ΔOS) of 30 V. Figure 1b shows that at a higher ΔOS of 110 V additional peaks appear from apoMb, that is, Mb which has lost a heme group by CAD in the interface. Figure 1c shows that at the highest ΔOS used, 180 V, only apoMb peaks remain. Loss of heme from a protonated Mb ion is expected to give a net loss of one charge, although MS/MS here and elsewhere[10b] shows some loss (ca. 25%) of neutral heme as well. Ions of apoMb then are formed in charge states similar to those of the original Mb ions. At the highest ΔOS, apoMb can lose an additional charge to produce ions in lower charge states. Figure 1c thus shows an average charge ca. 2 less than Figure 1a. Collision cross sections for each of the major ion species of Figure 1 were measured and are shown in Figure 2.

Comparison of the cross sections for Mb at ΔOS = 30V with those for apoMb at ΔOS = 180V shows that, for a given charge state, the Mb ions have smaller cross sections, corresponding to a more compact structure while the apoMb ions have larger cross sections corresponding to a more extended or unfolded structure. This provides direct physical evidence that the gas-phase ion has unfolded on loss of heme. Clues to the mechanism of this unfolding come from measurements of cross sections for Mb and apoMb at the intermediate ΔOS of 110 V (Figure 1b). These cross sections show (i) that both apoMb and Mb ions formed at this ΔOS have similar cross sections and (ii) that the cross sections for both are similar to those of the unfolded apoMb formed at the highest ΔOS. Activated Mb ions can unfold significantly to a structure similar in size to apoMb before losing heme on the experimental time scale (estimated to be <2 ms from ion activation to detection). The mechanism of heme loss appears to be

$$\text{Mb} \rightarrow \text{unfolded Mb} \rightarrow \text{apoMb}$$

Unfolding of Mb can also be partially induced by increased charging of the ion. The cross sections for $Mb^{13,14+}$ for example are greater than those for $apoMb^{7,8+}$, formed by CAD of $Mb^{8,9+}$. (This increase in cross section with charge, which has been seen for several proteins,[7,8b] is unlikely to be from an increased ion-induced dipole potential. Calculation of the ion-dipole cross sections for the conditions of the experiment here gives values 4.3−5.8 times less than observed. The different cross sections observed for Mb and apoMb in the same charge states indicate that the properties of the protein and not solely the charge determine the collision cross section. Nevertheless, additional work with different collision gases to elucidate any effects of target size or polarizability would be of interest.)

Table 1. Comparison of Cross Sections (Å²)

species	r_g	r_s	cross section[a]	A_g	A_s
n Mb[b]	17.5	2239	1603		
n apoMb[c]	20.1	21.5	2638	2114	1451
u apoMb[d]	29.3[e]	43	3496[f]	4492	5804

[a] This work. [b] Native Mb. [c] Native apoMb. [d] Denatured apoMb. [e] Acid-denatured Mb. [f] From ref 7a, denatured in 1:1 acetonitrile/water 0.1% acetic acid, averaged over charge states.

Measurements of the size of Mb in solution come from small-angle X-ray scattering[11] which gives the radius of gyration, r_g, and from size exclusion chromatography[12] which gives the Stokes radius, r_s. For a sphere of radius r_o, $r_g^2 = (3/5)r_o^2$.[14] Table 1 compares our cross sections, averaged over charge state, with those calculated from r_g, i.e., $A_g = (5/3)\pi r_g^2$, and from r_s, i.e., $A_s = \pi r_s^2$. In solution Mb the cross section increases ca. 1.3× on loss of heme and a further 2−4× when the Mb is fully denatured. Gas-phase ions show a ca. 1.2× increase in cross section on loss of heme. This increase is moderate compared to the additional increase seen for the higher charge states that are formed from denatured apoMb in solution, a ca. 2× increase from the +9 to the +21 charge state.[7a] The data in Table 1 for gas-phase cross sections for denatured Mb (from ref 7a) are averaged over charge states of +8 to +21 to give some indication of this additional increase in cross section. (Detailed comparison between solution and gas-phase cross sections is difficult, particularly for apoMb, because the mass spectral data describe individual charge states and it is not clear how to compare these directly with the solution measurements. For example, where the titration curve[15] of Mb indicates a net charge on the protein of +2 at pH 7, we see an average charge of +11.) Consistent with our results, Mb and apoMb in low charge states produce compact "hillocks" when impacted on surfaces whereas denatured apoMb in high charge states shows elongated hillocks, suggesting a more unfolded structure.[6] It is intriguing that the gas-phase ions and solution myoglobin show similar unfolding behavior. It suggests that, in the gas phase, the protein remains folded around the heme group. In broader terms studies of folding of gas-phase ions may eventually allow elucidation of the relative contributions of solvent and protein internal interactions[16] to protein folding.

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(14) Glatter, O.; Kratzky, O. *Small Angle X-Ray Scattering*; Academic Press: New York, 1982, p 156.
(15) Shire, S. J.; Hanania, G. I. H.; Gurd, F. R. N *Biochemistry* **1974**, *13*, 2967−2974.
(16) (a) Dill, K. A. *Biochemistry* **1990**, *29*, 7133−7155. (b) Wolynes, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2426−2427.