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APPLYING ION-MOLECULE REACTIONS TO STUDIES OF GAS-PHASE PROTEIN STRUCTURE

R. R. Ogorzalek Loo
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Pacific Northwest Laboratory
Richland, Washington 99352

The 40th ASMS Conference on Mass Spectrometry and Allied Topics

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The question of whether remnants of solution phase differences in protein higher order structure persist in the gas phase is examined by carrying out proton transfer reactions on ions generated by electrospray ionization (ESI) of different solution conformations. If dramatic differences were observed in the reactivities of *equally* charged ions generated from two solution conformers, those differences could provide evidence that some structural differences persist.

Ion-molecule reactions were carried out in the atmosphere-vacuum interface of a quadrupole mass spectrometer by employing a "Y"-shaped capillary inlet-reactor.[1,2] The inlet-reactor was fabricated from 1 mm i.d. stainless steel tubing soldered to a post through which a Y-shaped channel had been drilled. To aid in desolvation and in examining temperature dependences, the reactor was electrically heated. Countercurrent gas flow was not employed. Dimethylamine (DMA), trimethylamine (TMA), or diethylamine (DEA) were delivered to one inlet arm in a controlled manner. Because this quadrupole-based experiment does not directly measure reaction rates for individual charge states, several assumptions pertaining to relative reaction rates are important. Those assumptions are discussed in reference 2.

Cysteine-cysteine disulfide-reduced and disulfide-intact proteins were compared as systems for which it is known that structural differences persist in the gas phase. Our recent studies[2] reacting DEA with bovine pancreatic trypsin inhibitor (M_r 6512), hen lysozyme (M_r 14 306), and bovine albumin (M_r 66 430) showed dramatic differences in charge state distributions between disulfide-intact and disulfide-reduced proteins to which equal amounts of DEA had been added. It was argued that increased electrostatic repulsion causes a disulfide-intact protein to be more reactive to proton transfer than an *equally* charged, disulfide-reduced protein. In extending those studies, we have observed similar behavior in DMA reactions with bovine albumin, hen lysozyme, and other disulfide-containing proteins such as α-lactalbumin, (M_r 14 175) and bovine proinsulin (M_r 8681).

The reactivities of bovine cytochrome *c* (M_r 12 231) ions sprayed from denatured (5% HOAc/H_2O, methanol sheath) and native (H_2O, H_2O sheath) solutions were examined with DMA in a 150°C capillary inlet (Figs. 1 and 2). In contrast to the α-lactalbumin results, ions generated from both cytochrome *c* solutions shifted to approximately the same charge states after addition of equal amounts of DMA. Similar behavior has been observed in cytochrome *c* reactions with DEA and with TMA in a 150°C reactor and also with DMA in a 95°C reactor. Differences in relative intensities of the charge states remaining after proton transfer reaction (e.g., the $(M+8H)^{8+}$ charge state is the most intense in Fig. 1b, but $(M+7H)^{7+}$ is most intense in Fig. 2b) may simply reflect the large differences in the charge states before reaction (Figs. 1a and 2a). However, we cannot rule out the possibility that small differences in proton transfer rates for equally charged ions from different conformers may also contribute to these differences.

Addition of equal amounts of DMA (150°C or 100°C inlet reactor) or DEA (150°C) to ions generated from different solution conformations of bovine ubiquitin (M_r 8565) also yielded similar final charge states. However, in contrast to the temperature dependence observed for DMA reacting with

cytochrome *c* (i.e., average charge state of ion *population* decreases with increasing temperature), reactions of DMA and DEA with ubiquitin all proceeded farther at lower temperatures (i.e., average charge state increases with increasing temperature).

Reactions of myoglobin (M_r 17 568) and apomyoglobin (M_r 16 951) with DMA (150°C, 100°C) or TMA (150°C) yielded similar final charge states, as well, despite the fact that myoglobin in the native solution retained its noncovalently associated heme.

These results suggest that for the non-disulfide linked proteins studied under these experimental conditions, either there are not significant differences in gas phase higher order structure, or proton transfer reactions are not a sufficiently sensitive probe of higher order structural differences arising from noncovalent interactions. It is possible that direct measurement of proton transfer reaction rates for individual charge states may show differences attributable to higher order structure.

References

1. R. R. Ogorzalek Loo, H. R. Udseth, and R. D. Smith, *Proc. 39th ASMS Conference on Mass Spectrometry and Allied Topics*, Nashville, TN, 266-267 (1991).

2. R. R. Ogorzalek Loo, J. A. Loo, H. R. Udseth, J. L. Fulton, and R. D. Smith, *Rapid Commun. Mass Spectrom.* **6**, 159-165 (1992).

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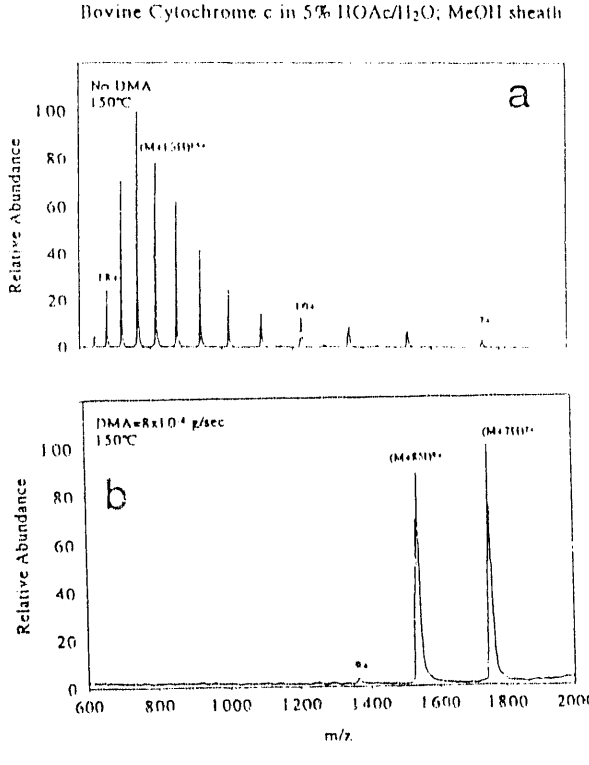

Figure 1

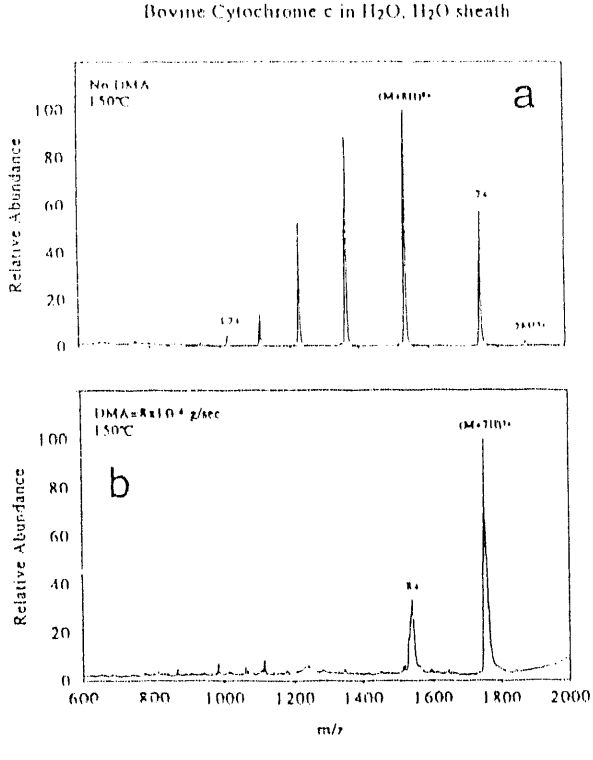

Figure 2

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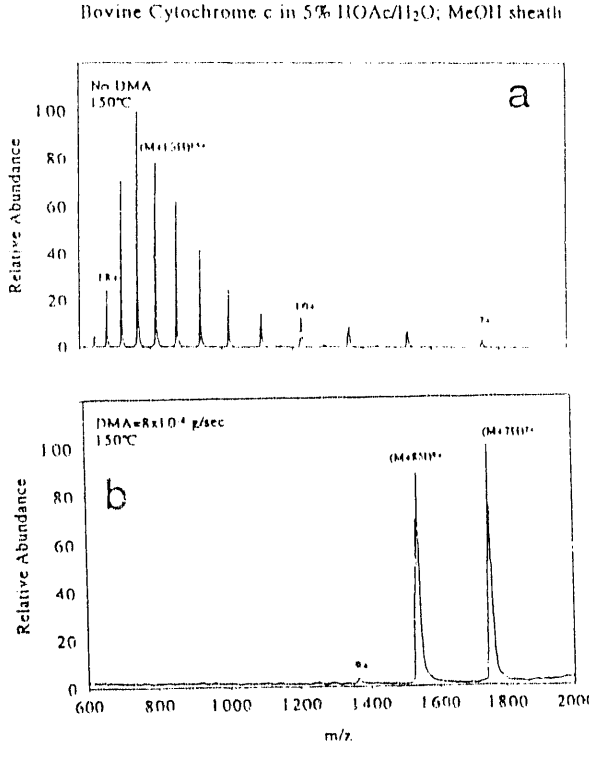

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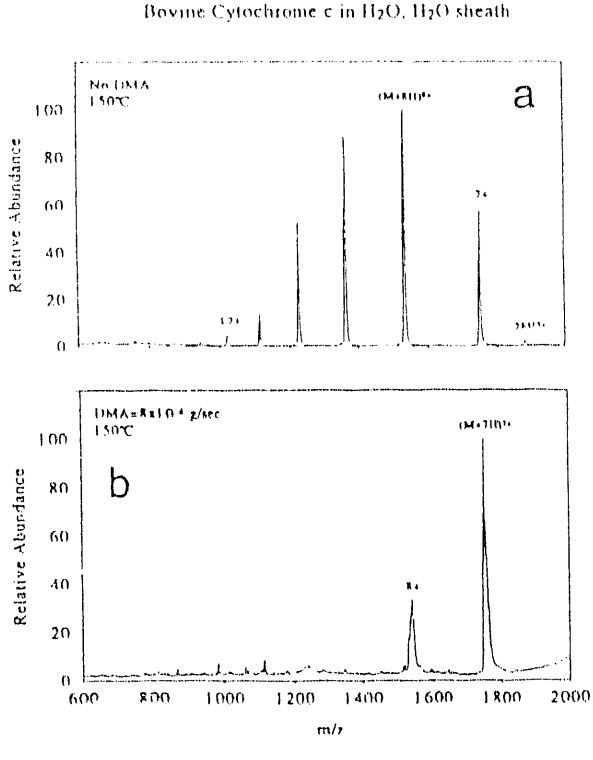

Figure 2

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