LETTER

Femtosecond laser vaporization that preserves protein-folded structure: An unproven idea

Electrospray ionization (ESI) made the transfer of nonvolatile molecules, such as proteins, from solution into the gas phase without covalent bond dissociation possible, revolutionizing MS for biological applications. However, whether the gaseous ions from ESI can preserve their biologically active native structure for characterization by MS is still controversial. The method described in ref. 1 claimed this preservation using femtosecond laser pulses to vaporize native proteins from solution into the electrospray plume used for conventional ESI/MS; the low net charge value measured for the resulting molecular ions was used as preservation evidence. Instead, however, charge value data has only been used to indicate protein conformation in solution before ESI, not during or after ESI. The claim (1) that ions of the lowest charge states produced by femtosecond laser desorption and ESI of a protein have preserved their native structure conflicts with extensive evidence (2–4).

For cytochrome *c*, femtosecond laser vaporization of a pH 7 solution into the ESI plume of a pH 7.18 solution yielded 3:1 7+:8+ ions, whereas conventional ESI from 1:1 CH_3OH:H_2O at pH 7.18 yielded ~1:1 7+:8+ ions and some higher charge states (1). However, these latter ESI conditions apparently were not optimized; several reports since 1997 have found that ESI from solutions at pH > 6 yield no higher charge states. The gaseous 7+ cytochrome *c* ions have also been shown to have lost their native structure; ion mobility measurements from ESI of cytochrome *c* in 1:1 CH_3OH:H_2O gave three conformers of cross-sections 1,600–2,000 Å^2, with a value of 1,100 Å^2 calculated for the native protein (2). In agreement, the solution to gas phase stability order of cytochrome *c* is essentially the reverse of its unfolding in solution, and therefore, the newly desolvated native ions should be thermodynamically unstable (3). The method used in ref. 1 may also produce byproducts; the claimed “6, 5, and 4+ charge states” (ref. 1, figure 2a) actually correspond to 6+, *m/z* 2,250, and 5+, respectively.

For lysozyme, inexplicably, its ions were first collisionally activated by 50–390 V acceleration at ~1.2 Torr (1), which should cause substantial unfolding without significant increase in charge. However, structural preservation again was claimed for the method, which yielded 10+ and 9+ ions from H_2O solution, whereas their ESI from pH 3.25 solution showed 12+ ions as the most intense (1). However, ESI under less denaturing conditions (4) yielded 8+ and 9+ charge states, suggesting less complete structure preservation from femtosecond vaporization than ESI alone.

Is a softer desorption method than ESI necessary to improve structural preservation? Even if desorption completely preserves the incipient native conformation, protein dehydration can collapse extended basic side chains in ~10^{-11} s, destroy hydrophobic noncovalent bonds, and rearrange others (3). In contrast, extensive retention of the native conformation in conventional ESI/MS is possible if the protein has similar solution and gas phase stabilities (5).

ACKNOWLEDGMENTS. This work was supported by the Austrian Science Fund Grant Y372 (to K.B.) and National Institutes of Health Grant GM16609 (to F.W.M.).

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Author contributions: K.B. and F.W.M. designed research; K.B., O.S.S., and F.W.M. performed research; and K.B. and F.W.M. wrote the paper.

The authors declare no conflict of interest.

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www.pnas.org/cgi/doi/10.1073/pnas.1115475109