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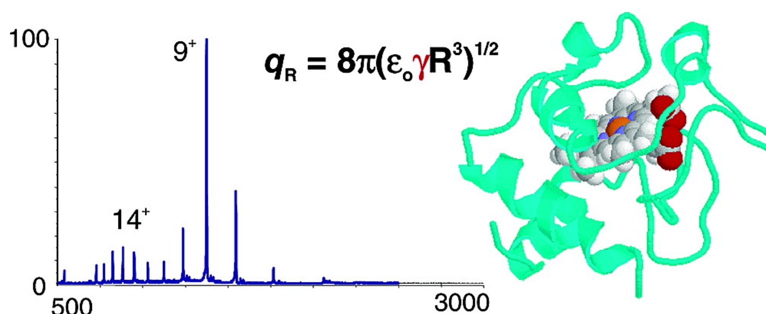

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Protein Charge-State Distributions in Electrospray-Ionization Mass Spectrometry Do Not Appear To Be Limited by the Surface Tension of the Solvent

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Applications of electrospray-ionization mass spectrometry (ESI-MS) to protein studies have developed rapidly,[1−3] but the mechanism controlling protein charge-state distributions (CSDs) is not yet well understood.[3,4] According to a current model of protein ESI,[5−7] the observed charge states (CSs) are limited by the Rayleigh charge of the droplets that generate the gas-phase ions:

$$q_R = z_R e = 8\pi(\epsilon_o \gamma R^3)^{1/2} \quad (1)$$

Here, q_R is the total charge of the droplet at the point of Rayleigh instability (at which the electrostatic repulsion among surface charges equals the surface tension of the droplet), e is the elementary charge, ϵ_o is the permittivity of vacuum, γ is the surface tension of the solvent, and R is the radius of the droplet. This hypothesis is based on the observation that the maximum CS of folded proteins in ESI-MS is between 65% and 110% of the z_R value calculated by eq 1 for water droplets of the same radius as the globular protein structure.[7] A testable prediction of this model is that protein CSDs in ESI-MS should respond to changes in the surface tension of the droplets according to the Rayleigh equation. A recent paper points to an influence of the surface tension of the solvent on the CSD of unfolded Cyc in the presence of acetic acid.[5] However, this conclusion is not supported by comparison between acetic acid and HCl solutions.[8] In this work, we investigate the effect of solvent surface tension on protein CSDs by means of additives with lower vapor pressure and lower surface tension than water. The results indicate that the CSDs of either folded or unfolded proteins are not affected by the surface tension of the solvent as predicted by the Rayleigh equation.

We present data by nano-ESI-MS[9] on the effect of 1-propanol (prOH) and 1,2-propylene glycol (PG) on the CSDs of folded and unfolded proteins. Both compounds have lower vapor pressure and lower surface tension than water (Table 1). The vapor pressure of prOH is below that of water up to ~70 °C.[10] It is generally argued that late ESI droplets contain almost exclusively the solvent component with the lowest vapor pressure.[5,11] However, the relative evaporation rate might also play a role, because the system never reaches equilibrium between the liquid and vapor phase. PG also has a lower evaporation rate than water, thus providing an even more stringent test for surface tension effects in late ESI droplets. prOH forms an azeotrope with water at 72% (v/v, here and below) alcohol.[12] The surface tension of a 30% prOH solution in water at 25 °C is ~26 × 10^{-3} N/m,[13] very close to that of pure prOH. If surface tension were the limiting factor for protein ionization, these compounds would be expected to reduce the maximum CS, presumably along with the main CS, to 60−70% of their value in water (Table 1).[5,7] When the protein radius R_m reported in ref 9 for folded proteins is used, the calculated z_R in prOH (PG) is 4.2 (5.2) for ubiquitin (Ubq), 5.0 (6.2) for cytochrome *c* (Cyc), 5.4 (6.7) for lysozyme (Lyz), and 6.0 (7.5) for myoglobin (Mb). This calculation is done assuming the same protein radius in the different solvents and the surface tension of the least volatile component. Protein-bound water might cause a 10% increase of the effective protein radius.[7] This possible effect is assumed to be similar in the samples employed here.[14] The limit charge of micrometer-size droplets in the presence of organic solvents can be 100−120% of z_R, but this discrepancy decreases together with the droplet size.[15]

The effect of prOH is illustrated in Figure 1.[19] Addition of prOH up to 30% for Ubq, and up to 20% for Lyz and Cyc, does not shift

Table 1. Physicochemical Properties of Employed Solvents

	v.p. (mmHg)[a]	γ (N/m)[b]	e.r.[c]	z_R (c)/z_R (w)[d]
water	23.8	72.0 × 10^{-3}	0.30 (0.26)	
acetic acid	15.7	27.4 × 10^{-3}	0.97 (0.8)	0.6
PrOH	21.0	23.4 × 10^{-3}	1.30 (1.42)	0.6
PG	0.13	36.5 × 10^{-3}	0.01 (0.009)	0.7

[a] Vapor pressure at 25 °C.[10,16] [b] Surface tension at 25 °C.[16,17] [c] Evaporation rate relative to *n*-butyl acetate, as mass (volume) flow.[12,18] [d] Ratio between z_R calculated with γ of the cosolvent or of water for any given radius.

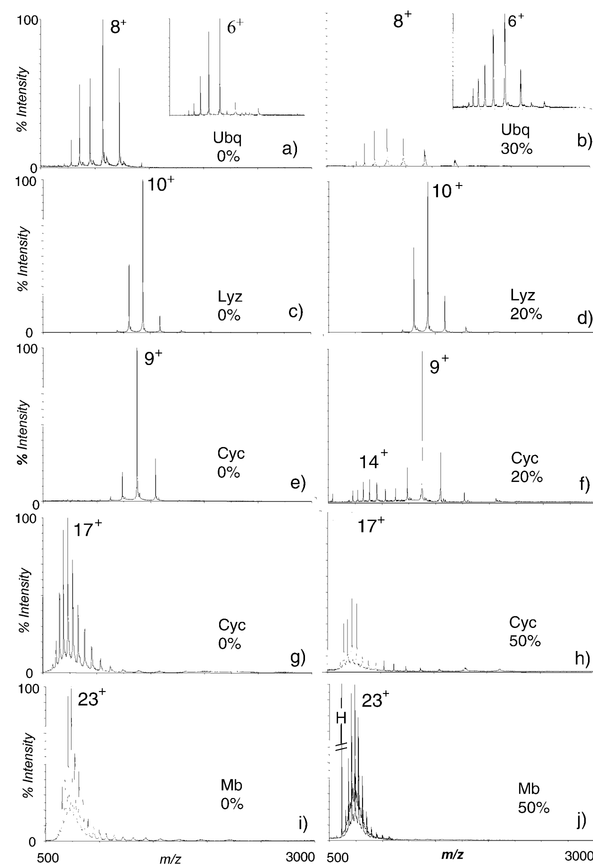

Figure 1. Effect of prOH. The prOH content (%) and main CS are indicated on each panel. The proteins are 5 μM. Other solvent components and final pH values: water pH ~7 (a−f); water/HCl pH 2.2 (g−h); water/HCl pH 2.8 (i−j). Curtain-gas flow rate: 0.6−0.7 L/min. Nozzle temperature: 85 °C. The insets show alternative CSDs. H, heme.

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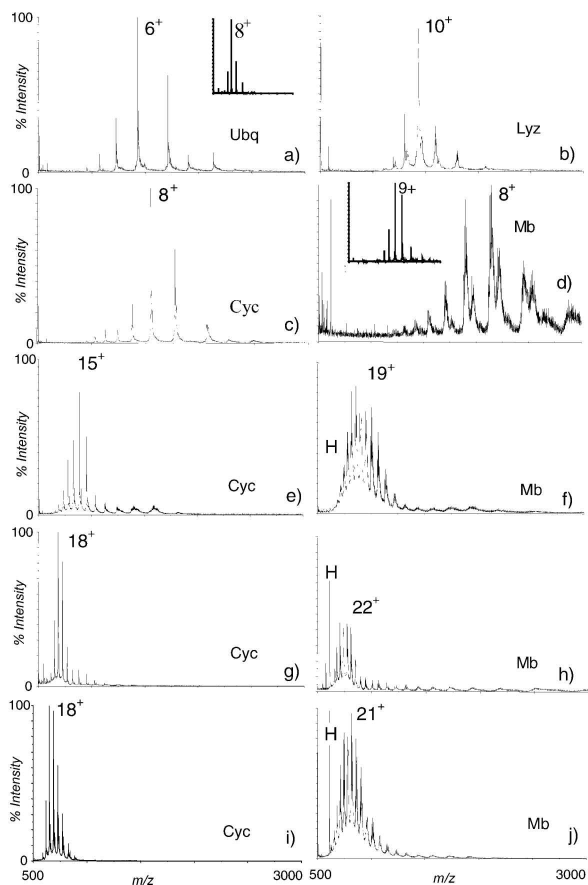

Figure 2. Effect of PG. Each sample contains 40% PG and 5 μM protein. Other solvent components and final pH values: water pH ~7 (a−d); water/HCl pH 2.2 (e, g); water/HCl pH 2.8 (f, h); water/acetic acid pH 2.2 (i−j). Curtain-gas flow rate (L/min): 1 (d), 1.2 (g, h), 0.6−0.7 (others). Nozzle temperature: 100 °C. The inset shows an alternative Ubq CSD (a) and a reference spectrum of Mb in water (b), which was not part of Figure 1.

the CSDs of the folded proteins. These are shifted toward lower *m*/*z* values at higher prOH concentrations (data not shown), consistent with reduced compactness of the protein structures. Spectra of Ubq in water are shown in Figure 1a.[8] A main CS of 8+ is observed most frequently. The CSD centered on the 6+ ion correlates with lower total ESI current and higher relative intensity of dimer-specific peaks. The same features are observed in 30% prOH. Cyc in 20% prOH gives rise to a bimodal distribution that shows the onset of the unfolding transition, in agreement with fluorescence data.[20] The high-*m*/*z* component is very similar to the envelope of the folded protein in water. The low-*m*/*z* component, with a main CS of 14+, seems to correspond to the partially folded form of this protein previously identified in the presence of other alcohols.[21] We then used HCl-unfolded Cyc and Mb to analyze the effect of prOH on the CSDs of proteins in the denatured state. The addition of 50% prOH leaves the CSDs almost unchanged. The only remarkable effect of the hydrophobic cosolvent is that it increases the relative intensity of the peak of free heme (*m*/*z* 616.4) in Mb spectra. Reduction of the main CS by two units, along with intensity loss, was observed at 75% prOH, that is, above the azeotropic point (data not shown).

Figure 2 shows spectra from 40% PG solutions. To obtain any signal at all, it was necessary to either increase the curtain-gas flow rate above 0.7 L/min or increase the nozzle temperature to 100 °C. The reported spectra were recorded at low curtain-gas flow rates (exceptions discussed below) and a nozzle temperature of 100 °C, because high gas flow rates can induce protein unfolding.[8] Raising the nozzle temperature from 85 to 100 °C did not affect the reference CSDs of the folded proteins in water, which remained the same as those reported in Figure 1 (data not shown). The CSD of Ubq is stabilized on a main CS of 6+, but spontaneous fluctuations to 8+ can still be observed. The CSD of Lyz is centered on the 10+ ion, as in pure water. The main CS of Cyc and Mb is shifted from 9+ to 8+ as compared to pure water. Mb gives very faint signals. Even the low-intensity spectrum shown in Figure 2 required higher curtain-gas flow rates than those used for the other samples. The approximate intensity loss caused by 40% PG relative to pure water ranges from 0% (Ubq) to 90% (Mb).

The spectra of acid-unfolded Cyc and Mb show a more remarkable shift toward higher *m*/*z* values upon addition of 40% PG. The main CS is reduced from 17+ to 15+ for Cyc, and from 23+ to 19+ for Mb, and the maximum CS changes from 20+ to 19+ for Cyc, and from 28+ to 25+ for Mb. However, the spectra revert to the typical[8,22] CSDs of these unfolded proteins when either the curtain-gas flow rate is increased or acetic acid is employed as a denaturant instead of HCl (Figure 2). These control experiments rule out that the effect is due to the surface tension of the solvent, because this is even lower upon addition of acetic acid (Table 1). Rather, it could be due to more compact protein conformations in 40% PG.[23] Higher gas flow rates or higher hydrophobicity of the solvent could promote more extensive protein unfolding.[8] Alternatively, this effect could be due to suboptimal electrospray conditions caused by the additive.[24] In conclusion, protein CSDs in the presence of low-surface-tension, low-vapor-pressure additives either are the same as those in the control samples or present much smaller changes than those calculated by the Rayleigh equation. Thus, they do not seem to be limited by the surface tension of the solvent and, rather, appear to be quite protein-specific.

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References

(1) Chowdhury, S. K.; Katta, V.; Chait, B. T. *J. Am. Chem. Soc.* **1990**, *112*, 9012−9013.
(2) Konermann, L.; Simmons, D. A. *Mass Spectrom. Rev.* **2003**, *22*, 1−26.
(3) Grandori, R. *Curr. Org. Chem.* **2003**, *7*, 1−15.
(4) Kebarle, P. *J. Mass Spectrom.* **2000**, *35*, 804−817.
(5) Iavarone, A. T.; Williams, E. R. *J. Am. Chem. Soc.* **2003**, *125*, 2319−2327.
(6) Peschke, M.; Blades, A.; Kebarle, P. *J. Am. Chem. Soc.* **2002**, *124*, 11519−11530.
(7) de la Mora, J. F. *Anal. Chim. Acta* **2000**, *406*, 93−104.
(8) Šamalikova, M.; Matečko, I.; Müller, N.; Grandori, R. *Anal. Bioanal. Chem.* **2003**, in press.
(9) Mann, M. *Org. Mass Spectrom.* **1990**, *25*, 575−587.
(10) Perry, R. H., Chilton, C. H., Eds. *Chemical Engineers' Handbook*; McGraw-Hill Kogakusha, Ltd.: Tokyo, Japan, 1973.
(11) Zhou, S.; Cook, K. D. *Anal. Chem.* **2000**, *72*, 963−969.
(12) Flick, E. W., Ed. *Industrial Solvent Handbook*; Noyes Data Corp.: Park Ridge, NJ, 1991.
(13) Vazquez, G.; Alvarez, E.; Navaza, J. M. *J. Chem. Eng. Data* **1995**, *40*, 611−614.
(14) Gekko, K.; Morikawa, T. *J. Biochem. (Tokyo)* **1981**, *90*, 39−50.
(15) Smith, J. N.; Flagan, R. C.; Beauchamp, J. L. *J. Phys. Chem. A* **2002**, *106*, 9957−9967.
(16) http://solvdb.ncms.org.
(17) Lide, D. R., Ed. *CRC Handbook of Chemistry and Physics*, 82nd ed.; CRC Press: Boca Raton, FL, 2001.
(18) http://hazard.com/msds/.
(19) Bovine Ubq, horse Cyc, chicken Lyz, and horse Mb were from Sigma. Spectra were recorded on a time-of-flight instrument (Mariner, Perkin-Elmer) with Protana capillaries ("medium") in positive-ion mode, spray-tip potential 1600−1700 V, nozzle potential 20−200 V (set at the lowest value compatible with a stable spray), 3 s per spectra. The reported traces are averaged over at least 10 spectra. Samples were sprayed at ~25 °C.
(20) Rodriguez-Cruz, S. E.; Khoury, J. T.; Parks, J. H. *J. Am. Soc. Mass Spectrom.* **2001**, *12*, 716−725.
(21) Grandori, R. *Protein. Sci.* **2002**, *11*, 453−458.
(22) Dobo, A.; Kaltashov, I. A. *Anal. Chem.* **2001**, *73*, 4763−4773.
(23) Kamiyama, T.; Sadahide, Y.; Nogusa, Y.; Gekko, K. *Biochim. Biophys. Acta* **1999**, *1434*, 44−57.
(24) King, R.; Bonfiglio, R.; Fernandez-Metzler, C.; Miller-Stein, C.; Olah, T. *J. Am. Soc. Mass Spectrom.* **2000**, *11*, 942−950.

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