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Minireview

Is arginine a protein-denaturant?

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Abstract

Arginine is a useful solvent additive for many applications, including refolding and solubilization of proteins from insoluble pellets, and suppression of protein aggregation and non-specific adsorption during formulation and purification. However, there is a concern that arginine may be a protein-denaturant, which may limit the expansion of its applications. Such concern arises from the facts that arginine decreases melting temperature and perturbs the spectroscopic properties of certain proteins and contains a guanidinium group, which is a critical chemical structure for denaturing activity of guanidine hydrochloride. Here, we show that although arginine does lower the melting temperatures of certain proteins, the extent is insufficient to cause denaturation of proteins at or below room temperature. The proteins described here show enzymatic activity and folded structure in the presence of arginine, although the local structure around aromatic amino acids is perturbed by arginine. Arginine differs from guandinine hydrochloride in the mode of interactions with proteins, which may be a primary reason why arginine is not a protein-denaturant.
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Arginine is a useful solvent additive for many applications in the biotechnology and pharmaceutical industries. It assists refolding of recombinant proteins, solubilizes proteins from certain inclusion bodies (IBs)[1] [1–10], facilitates elution of antibodies from Protein-A columns [11], reduces non-specific interactions with solid surface (D. Ejima, R. Yumioka, T. Arakawa, and K. Tsumoto, manuscript submitted), and reduces aggregation of proteins during storage [12]. However, there is a concern, based on the following observations, that arginine may be a protein-denaturant, which limits the expansion of its applications. In their elegant work on osmolytes, Yancey et al. [13] showed that the activity and stability of certain enzymes were perturbed by arginine and concluded that arginine is a protein-destabilizer and hence is not used by nature as an osmolyte. Xie et al. [14] have made a similar observation that fluorescence properties of aminoacylase are perturbed by arginine. They interpreted the observed effects of arginine in terms of denaturing property of the guanidinium group, which makes guanidine hydrochloride (GdnHCl) a strong denaturant. Here, we will summarize the effects of arginine on the stability, enzymatic activity, and spectroscopic property of proteins and show that arginine affects the melting temperature of certain proteins and their conformation, but not to such an extent that it

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[1] *Abbreviations used:* IBs, inclusion bodies; GdnHCl, guanidine hydrochloride; RNase, ribonuclease; BSA, bovine serum albumin.

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causes protein denaturation and that arginine differs from GdnHCl in the mode of interactions with proteins.

Arginine is a weak protein-destabilizer

A clear evidence exists that arginine decreases the melting temperature of certain proteins, but to the extent much less than that by GdnHCl. For example, bovine pancreatic ribonuclease (RNase) showed a ~1 °C decrease in melting temperature by the addition of 0.2 M arginine [13], while it showed a ~2.5 °C decrease by 0.2 M GdnHCl [15]. Table 1 shows the changes in melting temperature of RNase at higher arginine concentration. The melting temperature decreases at most 3 °C in 1–2 M arginine. In another case, melting temperature of lysozyme decreased by 13–17 °C in the presence of 1.2 M GdnHCl [16–18], while it changed little in 0–2.0 M arginine (Table 1), consistent with the results of Shiraki et al. [19] and Kudoh et al. [20].

Consistent with little changes in protein stability, a prolonged incubation at 4 °C in 2 M arginine of an enzyme, HsNDK (*Halobacterium salinus* nucleoside diphosphate kinase) used as a model protein, resulted in no apparent decrease in the activity [21]. Namely, the enzyme was incubated in 2 M arginine for 24 h and diluted into the assay buffer containing no arginine, which showed a comparable activity of the sample incubated similarly without arginine. A similar stability of the enzyme activity was observed in 2 M, but not 6 M, GdnHCl at 4 °C. However, the HsNDK stability was compromised in 2 M GdnHCl at elevated temperature. Table 2 shows the activity of HsNDK after incubation at 40 °C for 19 h. While it retains 100% activity in 1 M NaCl, a marginal activity was observed in 1 M GdnHCl. A majority of activity was retained in 1 M arginine.

Table 1
Changes in melting temperature of RNase and lysozyme as a function of arginine concentration[a]

Arginine (M)	ΔMelting temperature	
	RNase	Lysozyme
0.1	0	0
0.2	−1	
0.5	−1	−1
1.0	−3	−1
2.0	−3	0

[a] Data from [18].

Table 2
Stability of HsNDK at 40 °C[a]

Solvent condition	Activity (%)
1 M NaCl	100
1 M arginine	87
1 M GdnHCl	15

[a] HsNDK was incubated at 40 °C for 19 h [21].

Arginine perturbs aromatic environments

As described above, arginine does decrease, although only slightly, the stability of some proteins. Does it affect the structure of the native protein? A few examples exist that the addition of arginine alters the surface properties of proteins as shown below. α-Crystallin, a major lens protein, has been shown to suppress aggregation of proteins, presumably through hydrophobic surface present on the native α-crystallin [22–26]. The ability of α-crystallin to suppress aggregation is enhanced at elevated temperatures or by the addition of 5–200 mM arginine, suggesting that arginine increases exposure of hydrophobic surface on α-crystallin, as does the increased temperature [26]. On the contrary, the intrinsic tryptophan fluorescence of α-crystallin showed little change upon addition of arginine, indicating no apparent changes in the tryptophan environment of α-crystallin. The near UV circular dichroic spectra showed, however, significant changes in the presence of 0.1–0.3 M arginine. Interestingly, the signals above 290 nm (due do tryptophan) and below 270 nm (due to phenylalanine) were little affected. Significant changes occurred between 270 and 290 nm, suggesting that arginine altered environments for tyrosine residues. Arginine resulted in little changes in the secondary structure of α-crystallin. These spectroscopic results indicate that the overall fold of α-crystallin is unchanged in the presence of arginine, but the local structures, specifically surrounding tyrosine residues, are affected. The changes in local structure seem limited, since the fluorescent tryptophan residues are not affected by 0.3 M arginine. However, arginine does induce dissociation of multimeric α-crystallin, perhaps reflecting the effects of arginine on the structure surrounding tyrosine residues.

On the contrary, the following examples indicate the effects of arginine on tryptophan residues. Aminoacylase is a dimeric enzyme containing one Zn^{2+} per subunit [14]. The enzyme aggregates as the protein concentration is increased. At neutral pH, the addition of 0.4 M arginine (data obtained using chloride salt of arginine) suppresses aggregation and interferes with the enzyme activity. There is no dissociation of the dimer in the presence of arginine as determined by native gel electrophoresis [14]. Tryptophan fluorescence gradually changes in both intensity and emission wavelength as the arginine concentration is increased from 0.3 to 2.7 M at neutral pH. The intensity change is ~5% in 2.7 M arginine relative to its absence, while the emission wavelength shifts from 333 nm in the absence to 340 nm in 2.7 M arginine. Gel filtration experiments showed an

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