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Solubility enhancement of gluten and organic compounds by arginine

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Abstract

Arginine suppresses protein–protein and protein–surface interactions and thus is expected to increase the solubility of the proteins. We have examined here the effects of arginine on the solubility of a highly insoluble protein, gluten, and two organic compounds, octyl-gallate and coumarin, which have low to moderate aqueous solubilities. Arginine significantly increased the solubility of these molecules concentration dependently, while a weak salting-out salt, NaCl, decreased it. The observed ability of arginine to salt-in these compounds can be explained from its binding to aromatic groups and protein surface. Such solubilizing action of arginine may be used to enhance the solubility of poorly soluble organic drug substances.

Keywords: Solubility enhancement; Arginine; Salting-in; Coumarin solubility; Octyl-gallate

1. Introduction

Arginine has been shown to suppress aggregation of proteins (Arakawa and Tsumoto, 2003; Arakawa et al., 2006, 2007a,b,c) and protein adsorption to solid surface (Ejima et al., 2005; Arakawa et al., 2007a,b,c). It also facilitates elution (desorption) of the bound proteins from various chromatographic columns (Tsumoto et al., 2007; Arakawa et al., 2007a,b,c). These results suggest that arginine disrupts protein–protein and protein–surface interactions and a possibility that it may increase the solubility of the proteins. Solubility measurements using commercially available proteins are not an easy task due to their high solubility. The measurements often require extrapolation of the solubility determined in the presence of salting-out agents (Green, 1932; Timasheff and Arakawa, 1988; Jenkins, 1998). Here, we have used a highly insoluble protein, gluten, and two marginally soluble organic compounds, coumarin and octyl-gallate (OG); octyl-gallate has demonstrated a strong virus inactivation and anti-viral activity (Uozaki et al., 2007; Yamasaki et al., 2007). The solubility of these compounds was determined in the absence and presence of a weak salting-out salt, NaCl and arginine as a function of their concentrations. Low solubility of these compounds made the measurements of salting-in effects of arginine and salting-out effects of NaCl possible. The observed increase in solubility of the organic compounds by arginine suggests that this reagent may be useful to enhance the poorly soluble drug substances. We will then attempt to explain the mechanism of the observed salting-in effect of arginine based on the known solution properties of arginine (Arakawa and Tsumoto, 2003; Arakawa et al., 2006, 2007a,b,c).

2. Materials and methods

2.1. Materials

Gluten and guanidine hydrochloride (GdnHCl) were obtained from Sigma. OG was from Wako Chemicals. Arginine hydrochloride (arginine) was a gift from Ajinomoto Co., Inc. All the test solvents for gluten solubility measurements, except $MgCl_2$, were made in 20 mM phosphate, pH 7.0. $MgCl_2$ was made in pure water. All the test solvents for coumarin and OG were prepared in water.

Abbreviations: OG, octyl-gallate; GdnHCl, guanidine hydrochloride.

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Table 1
Gluten solubility

Solvent condition	Solubilization[a] (%)
20 mM phosphate, pH 7.0	23–36
0.2 M Arginine, pH 7.0	46
0.4 M Arginine, pH 7.0	56
0.6 M Arginine, pH 7.0	75
0.8 M Arginine, pH 7.0	82
1 M Arginine, pH 7.0	68
2 M Arginine, pH 7.0	83–92
1 M $MgCl_2$ in water	43
2 M $MgCl_2$ in water	48–51
1 M NaCl, pH 7.0	17
2 M NaCl, pH 7.0	11–15
0.3 M GdnHCl, pH 7.0	43
0.6 M GdnHCl, pH 7.0	65
1.2 M GdnHCl, pH 7.0	81
1.8 M GdnHCl, pH 7.0	85
2 M GdnHCl, pH 7.0	73–87
6 M GdnHCl, pH 7.0	100

[a] The amount of gluten solubilized by 6 M GdnHCl was taken as 100%. Some data are shown in range, as there is not enough data for statistical analysis.

2.2. Gluten solubility

Gluten solubility (or dispersion) was determined by the absorbance measurement at 280 nm. Gluten powder was homogenized at 20 mg/ml in 1 mM HCl. A 0.1 ml aliquot (2 mg gluten) was suspended into 10 ml of test solvents listed in Table 1. The suspension was gently mixed at room temperature (~25 °C) for 2–3 days and then spun with microfuge. The supernatant was mixed with one volume of 6 M GdnHCl to minimize light scattering. The gluten concentration of this mixture was obtained from the absorbance measurement at 280 nm.

2.3. Coumarin solubility

Coumarin powder was suspended at 20 mg/ml in water at room temperature (~25 °C), resulting in white suspension. Tubes containing this suspension were heated in boiling water, resulting in phase separation into the upper aqueous layer and the lower oil-like layer. The upper layer contained soluble coumarin and the lower layer primarily contained coumarin as an oil. The upper aqueous layer was mixed with one volume of water or test solvents containing arginine or NaCl at different concentrations. A similar experiment was done in PBS. The resultant mixture was incubated at room temperature (~25 °C) with frequent mixing for 2–3 days. The suspension was then microfuged to obtain clear supernatant. The supernatant was diluted 500-fold with water to make absorbance measurement possible. The concentration of coumarin was determined by absorbance measurement at 278 nm. The solubility of coumarin was expressed as the ratio of absorbance for the test solvent to that in water. When the test solvents were prepared in PBS, the ratio was determined against the solubility in PBS. The pH of coumarin solution in water or in aqueous arginine and NaCl solution ranged from 5.3 to 6.0, not much different from the pH of PBS-containing samples (pH 7).

Coumarin solubility was also determined by dissolving coumarin in arginine solution. Coumarin was dispersed at 30 mg/ml in 1 M arginine and heated in boiling water. This also caused phase separation, consisting of the upper layer of aqueous arginine solution and the lower oil-like coumarin liquid. The upper solution was mixed with water at a ratio of 1:0 (1 M arginine), 0.8:0.2 (0.8 M), 0.6:0.4 (0.6 M), 0.5:0.5 (0.5 M), 0.4:0.6 (0.4 M) and 0.2:0.8 (0.2 M). The mixtures were incubated at room temperature (~25 °C) for 2–3 days with frequent mixing. The concentration of coumarin in the supernatant was similarly determined.

2.4. Octyl-gallate solubility

OG was suspended at 10 mg/ml in water and heated in boiling water, which resulted in phase separation. The upper phase was diluted 2-fold or 10-fold into the test solvents for final arginine or NaCl concentrations of 0, 0.5 and 1 M. The resulting suspension was incubated at room temperature for 2–3 days with frequent mixing. The insoluble materials were spun down with microfuge and the supernatant was diluted 5-fold with water to make absorbance measurements possible. The absorbance at 272 nm was used to determine the concentration of OG.

3. Results

3.1. Gluten solubility

Gluten showed no apparent precipitation and turbidity in 6 M guanidine hydrochloride (GdnHCl), a strong protein solubilizing additive and hence the absorbance at this condition was taken as 100%, which corresponds to 0.2 mg/ml gluten per ml. The amount of soluble protein in 20 mM phosphate was 23–36% of that in 6 M GdnHCl (Table 1). Although what constitutes the soluble protein is not clear, the solubility of gluten in 20 mM phosphate is thus ~0.05–0.07 mg/ml. As shown in Table 1, the solubility of gluten in 0.2 M arginine is 46%, significantly greater than that in the absence of arginine. The solubility is plotted in Fig. 1 as a function of arginine concentration and reaches a level of 80–90% in 2 M arginine that is achieved by 6 M GdnHCl.

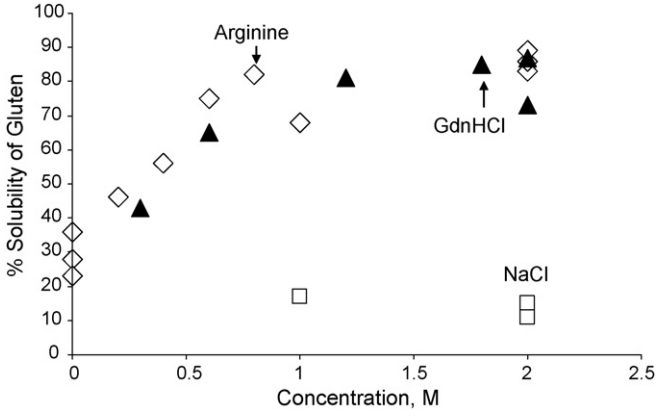

Fig. 1. Solubility of gluten. The solubility in 6 M GdnHCl was taken 100%, from which the solubility of gluten in buffer was estimated to be 0.05–0.07 mg/ml. (Diamond) arginine; (solid triangle) GdnHCl; (square) NaCl.

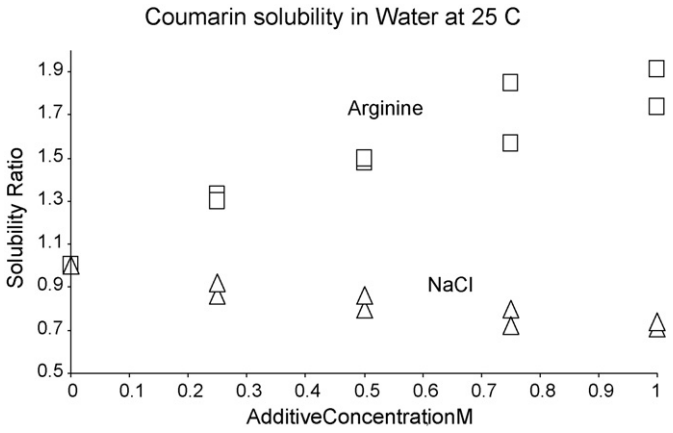

Fig. 2. Solubility of coumarin in aqueous arginine and NaCl. The solubility of coumarin in water is ~6 mg/ml.

The solubility curve for arginine is similar to that for GdnHCl, indicating that arginine and GdnHCl are equally effective in solubilizing (dispersing) gluten protein. As expected, NaCl, a weak salting-out salt (Arakawa and Timasheff, 1982, 1984), decreased gluten solubility and $MgCl_2$, a weak salting-in salt (Arakawa and Timasheff, 1984; Arakawa et al., 1990; Ishibashi et al., 2003), increased it (Table 1 and Fig. 1).

3.2. Coumarin solubility

The solubility of coumarin in water was determined to be ~6 mg/ml under the condition used here. Fig. 2 plots the ratio of coumarin solubility in aqueous arginine solution to that in water. The solubility increases with arginine concentration, leading to ~90% increase at 1 M (square). The observed increase in coumarin solubility resembles the trend observed for gluten solubility. Addition of NaCl caused decrease, although slightly, in coumarin solubility (triangle), again reminiscent of gluten solubility in NaCl. The results are essentially identical in PBS, solubility increasing ~75% in 1 M arginine and decreasing ~20% in 1 M NaCl (data not shown).

Solubility measurements were also carried out by first suspending coumarin in 1 M arginine solution. At 30 mg/ml and heating in boiling water, insoluble fraction of coumarin phase-separated into an oily liquid. The upper aqueous phase was used for solubility measurements. Since coumarin is already in 1 M arginine, the solubility in pure water could not be determined. The lowest arginine concentration tested was hence 0.2 M. The absorbance and solubility of coumarin in 0.2 M arginine was used to calculate solubility change at higher arginine concentration. The results, summarized in Table 2, are consistent with the solubility data described above using a stock coumarin suspension in water. For example, the solubility in 1 M arginine was 1.7 times that in 0.2 M arginine. This ratio is close to the ratio of the solubility in the same solvent system shown in Fig. 2.

3.3. OG

First the solubility of OG in water was determined as ~0.072 mg/ml under the condition used here. Fig. 3 shows the ratio of absorbance and hence solubility of OG in the presence of arginine or NaCl to the solubility in their absence. The solubility increased by about 1.6-fold and 2.2-fold by the addition of 0.5 and 1 M arginine; this magnitude is similar to that observed for coumarin. On the contrary, NaCl decreased the solubility by ~30 and 50% at 0.5 and 1 M; the effect of NaCl appears to be greater than the effect observed for coumarin.

Table 2
Coumarin solubility

Arginine concentration (M)	Solubility ratio
0.2	1
0.4	1.4
0.5	1.5
0.6	1.6
0.8	1.7
1	1.7

Stock coumarin suspension in 1 M arginine was diluted with water to generate arginine concentration indicated.

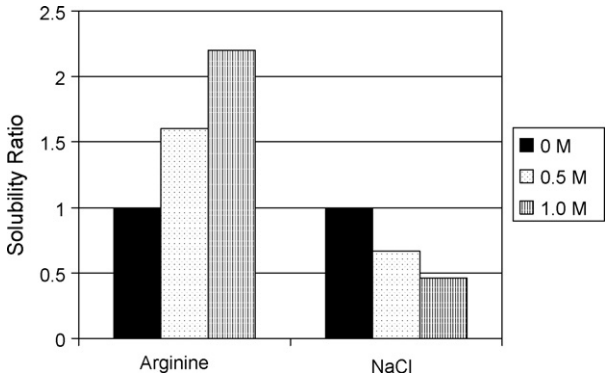

Fig. 3. Solubility of octyl-gallate in aqueous arginine and NaCl. The solubility of octyl-gallate in water is ~0.072 mg/ml.

4. Discussion

The solubility measurements of gluten, coumarin and OG clearly demonstrated that arginine increases their solubility and NaCl decreases it. The effect of NaCl is expected from its known weak salting-out effect on proteins (Arakawa and Timasheff, 1982, 1984). The preferential interaction study demonstrated that NaCl is weakly excluded from the protein surface, creating an energetically unfavorable state (Arakawa and Timasheff, 1982, 1984). This unfavorable free energy will be released upon protein association, causing a decrease in protein solubility. The salting-in effects of arginine may be explained from its ability to bind to the proteins (Kita et al., 1994; Lin and Timasheff, 1996; Tsumoto et al., 2005; Arakawa et al., 2006, 2007a,b,c). The preferential interaction analysis for arginine indicated its weak binding to the protein surface (Kita et al., 1994; Lin and Timasheff, 1996). Although the mechanism of such affinity of arginine for protein surface is not entirely clear, arginine has shown binding to aromatic groups through π electron–cation interaction (Crowley and Golovin, 2005; Woods, 2004). As coumarin and OG both contain aromatic rings, arginine may

bind to these compounds through this mechanism and hence stabilize the monomeric state, which should bind more arginine molecules.

We have shown before that arginine can solubilize proteins from loose inclusion bodies, but not from hard ones (Tsumoto et al., 2003; Umetsu et al., 2005). Baynes and Trout, 2004 and Baynes et al., 2005 proposed "gap theory", in which weakly binding additive, such as arginine, does not bind to the gaps in protein complexes. Small water molecules can penetrate the gaps, while a relatively large excluded volume of arginine precludes such penetration. It is thus possible that arginine affect the solubility of gluten, coumarin and OG, kinetically or thermodynamically by destabilizing such loosely associated state.

Lastly OG has shown virus inactivation and anti-viral activity (Uozaki et al., 2007; Yamasaki et al., 2007). However, it is poorly soluble in water and in fact has been studied by dissolving it initially in dimethyl sulfoxide (Uozaki et al., 2007; Yamasaki et al., 2007). Arginine should be a much safer solvent for biological systems. Here, we have observed that 1 M arginine increases the solubility of OG by about two-fold. As arginine is less toxic, this reagent may find wider use in not only suppressing aggregation of proteins, but also increasing the solubility of poorly soluble drug substances, as the solubility is a critical parameter for these compounds (Al-Maaieh and Flanagan, 2002). The same argument applies for a pharmacological agent, coumarin as well. The observed trend of increased solubility of gluten by arginine may also find some application. Gluten plays a key role in determining the quality of wheat flour products, including bread. Gluten forms a network during dough development and hence the observed ability of arginine to increase the solubility of gluten suggests that arginine may modulate the property of flour products.

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