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# ENHANCEMENT OF DISSOLUTION RATES OF AMORPHOUS SILICA BY INTERACTION WITH BOVINE SERUM ALBUMIN AT DIFFERENT pH CONDITIONS

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**Abstract**—Proteins and protein-like molecules are abundant in various geochemical environments; they form complexes with mineral surfaces and with dissolved organic matter. To evaluate the effect of proteins on rates of dissolution of minerals, experiments on the dissolution of amorphous silica in solutions containing various concentrations of bovine serum albumin (BSA) were performed in this study. The dissolution experiments were carried out by a batch method using solutions of 0.1 mM NaCl with 0.00, 0.05, 0.1, 0.2, 0.5, and 1.0 mg/mL of BSA at three different pH conditions, 6, 5, and 4. The results of the experiments demonstrated that BSA exhibited strong rate-enhancement effects on the dissolution of amorphous silica and were dependent on BSA concentration and the solution pH. At pH 6, the dissolution rates of amorphous silica appeared to increase successively by ~1.6, 2.2, 2.4, 2.5, and 2.9 times with increasing BSA concentrations of 0.05, 0.1, 0.2, 0.5, and 1.0 mg/mL, respectively. The rates of dissolution increased by greater degrees, ~3.1−5.8 and 4.9−13.0 times at pH 5 and 4, respectively. According to the calculated charge distributions of amino acid residues of the BSA molecule, the dissolution rates of amorphous silica were likely to be enhanced by attractive electrostatic interactions of the positively charged side chains of lysine, arginine, and histidine residues with the negatively charged >$SiO^-$ sites on the amorphous silica surface. The negatively charged side chains such as glutamic acid and aspartic acid residues may inhibit the attractive interaction, depending on the degree of deprotonation.

**Key Words**—Amorphous Silica, Bovine Serum Albumin, Dissolution Rates, Protein, Side Chain.

## INTRODUCTION

The interaction of extracellular organic molecules with mineral surfaces is an important catalytic factor affecting the rate of mineral dissolution in natural geochemical environments. Bacteria are ubiquitous on the Earth's surface in both continental and oceanic deep-crust environments with populations typically ranging from $10^5$ to $10^9$ cells/g (Barns and Nierzwicki-Bauer, 1997). The bacteria produce and excrete a variety of low-molecular weight organic acids, polysaccharides, amino acids, and various molecular-sized proteins including polypeptides and enzymes into the surrounding environments. Among these organic molecules, organic acids and polysaccharides are commonly present in soil and various sediments at sufficient concentrations to impact mineral weathering (Barker *et al*., 1997). The effects of these organic molecules on mineral dissolution have been studied extensively, and the molecules have been reported to enhance the rate and extent of mineral dissolution by more than one order of magnitude through complexation of deprotonated carboxyl groups ($COO^-$) with positively charged sites such as Al- and Fe-sites on the mineral surfaces, as well as with dissolved metal ions in solution (Barker *et al*., 1997; Drever and Stillings, 1997; Welch *et al*., 1999; Ullman and Welch, 2002). Amino acids are an essential constituent substance of the organisms, and occur widely in natural geochemical systems including soils, sediments, and various aquatic environments at concentrations of the order of ~μM/g (Dittmar *et al*., 2001; Dittmar and Kattner, 2003; Gupta and Kawahata, 2003; van Hees *et al*., 2005). Experimental studies of the effects of amino acids on mineral dissolution have confirmed that these molecules are also capable of enhancing the mineral dissolution by interaction of their functional groups with the specific sites of a mineral surface, depending on the degree of dissociation. Basic amino acids such as histidine, lysine, and arginine enhance the dissolution rates of amorphous silica by one order of magnitude at pH 6−4. Because basic amino acids are predominantly in cationic species over this pH range, attractive interaction with the negative sites of amorphous silica surfaces is enabled (Kawano and Obokata, 1997). On the other hand, neutral amino acids such as cysteine, asparagine, serine, tryptophan, alanine, threonine, histidine, lysine, and arginine exhibit no significant effect on dissolution rates at pH 6 due to an abundance of neutral or anionic forms, but the dissolution rates increase significantly, ~3−5 times, at pH 4 as a result of an increase in concentration of cationic species (Kawano *et al*., 2009). Proteins, including proteinaceous materials such as polypeptide and various enzymes, are abundant as dissolved and/or adsorbed forms in various geochemical environments such as soils and sediments with concentrations ranging

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from several μg/g to mg/g (Bonmati *et al*., 1998; Murase *et al*., 2003; Weintraub and Schimel, 2005). In freshwater and seawater, the concentration is of the order of ~mg/L (Tanoue *et al*., 1996; Lu *et al*., 2003; Jones *et al*., 2004; Elliott *et al*., 2006; Yamada and Tanoue, 2006). In addition, proteins are major constituents of humic substances in soils and sediments, constituting up to 30% of the total humic material (Lu *et al*., 2003). Extracellular proteins are, therefore, presumed to significantly affect the rate and extent of mineral dissolution in the same manner as other organic ligands do. No experimental study to date has confirmed the effects of such proteins on the dissolution rate of minerals.

In the present study, experiments on the dissolution of amorphous silica in solutions containing BSA at pH 6, 5, and 4 were performed in order to evaluate the effects of BSA on the dissolution rates of amorphous silica and to elucidate the rate-enhancement mechanism of proteins. BSA is a well defined protein, comprising 582 amino acid residues with a molecular size of 66 kDa (Peters, 1996). In this high-molecular weight polypeptide chain, the carboxyl and amino groups of each amino acid are linked by peptide bonds; the molecule can carry positive or negative charges, though, because of ionization of side chain functional groups of the specific amino acid residues depending on the degree of dissociation. Thus, the positive charges of BSA may contribute to its attractive electrostatic interaction with the negatively charged $>SiO^-$ site on the amorphous silica surface, possibly resulting in increased dissolution rates of amorphous silica.

## EXPERIMENTAL METHODS

### *Materials*

The amorphous silica used in this study was obtained from Kanto Chemical Co., Inc (Tokyo). The sample was ground in an agate mortar, and grains 5−100 μm in size were separated by dry sieving. The powdered sample was then cleaned ultrasonically to remove adhering ultrafine particles, and was washed at least five times with 0.1 M HCl and deionized distilled water. This sample was then freeze-dried for use in the present study. The surface area of the sample, determined by the BET method, was 294 $m^2/g$. The BSA used in this study was guaranteed chemical reagent grade, purchased from Nacalai Tesque Inc (Kyoto). The BSA is a cigar-shaped ellipsoid with dimensions of 140 Å × 40 Å and an axial ratio of 3.5 with three domains in line (Durchslang and Zipper, 1997; Matulis *et al*., 1999). Its molecular size is ~66 kDa comprising 582 amino acid residues rich in aspartic acid (41), glutamic acid (59), leucine (61), alanine (46), and lysine (59) (Peters, 1996). The isoelectric point (pI) of BSA is 4.7 (Dawson *et al*., 1986). The amino acid sequence and tertiary structure have also been reported (Brown, 1975; Patterson and Geller, 1977; McGillivray *et al*., 1979; Hirayama *et al*., 1990; Peters, 1996).

### *Dissolution experiment and analysis*

Dissolution experiments were conducted using batch reactors consisting of Pyrex® glass flasks containing 0.1 g of amorphous silica grains and 100 mL of 0.1 mM NaCl solution with 0.00, 0.05, 0.1, 0.2, 0.5, and 1.0 mg/mL of BSA. Three sets of reaction systems

Table 1. Experimental conditions for dissolution of amorphous silica in systems A to C.

| System | Run | Solution | pH (Initial) |
|---|---|---|---|
| System A | A0 | 0.1 mM NaCl + 0.00 mg/mL BSA | 6.00 |
| (Total Na = 0.1 mM) | A1 | 0.1 mM NaCl + 0.05 mg/mL BSA | 6.00 |
| | A2 | 0.1 mM NaCl + 0.1 mg/mL BSA | 5.99 |
| | A3 | 0.1 mM NaCl + 0.2 mg/mL BSA | 6.00 |
| | A4 | 0.1 mM NaCl + 0.5 mg/mL BSA | 6.00 |
| | A5 | 0.1 mM NaCl + 1.0 mg/mL BSA | 5.99 |
| System B | B0 | 0.1 mM NaCl + 0.00 mg/mL BSA | 5.01 |
| (Total Na = 0.1 mM) | B1 | 0.1 mM NaCl + 0.05 mg/mL BSA | 5.02 |
| | B2 | 0.1 mM NaCl + 0.1 mg/mL BSA | 5.01 |
| | B3 | 0.1 mM NaCl + 0.2 mg/mL BSA | 5.02 |
| | B4 | 0.1 mM NaCl + 0.5 mg/mL BSA | 5.00 |
| | B5 | 0.1 mM NaCl + 1.0 mg/mL BSA | 5.00 |
| System C | C0 | 0.1 mM NaCl + 0.00 mg/mL BSA | 3.97 |
| (Total Na = 0.1 mM) | C1 | 0.1 mM NaCl + 0.05 mg/mL BSA | 3.96 |
| | C2 | 0.1 mM NaCl + 0.1 mg/mL BSA | 3.96 |
| | C3 | 0.1 mM NaCl + 0.2 mg/mL BSA | 3.96 |
| | C4 | 0.1 mM NaCl + 0.5 mg/mL BSA | 3.95 |
| | C5 | 0.1 mM NaCl + 1.0 mg/mL BSA | 3.96 |

(systems A, B, and C) containing solutions with pH 6, 5, and 4, respectively, were prepared by adding HCl (Table 1). The flasks were sealed with an aerated polyethylene cap to prevent bacterial contamination and incubated at 25ºC for 10 days without shaking or stirring.

Throughout the dissolution experiments, 0.5 mL of each solution was collected every 2 days and filtered using 0.2 μm Minisart membranes in order to measure the Si and BSA concentrations. The measurement was made using the post-column pH buffer HPLC method which is a modified procedure proposed by Li and Chen (2000). The HPLC instrument used for this method was an Hitachi LaChrom Elite system equipped with an electrical conductivity (EC) detector and an ion-exclusive column of TSKgel OApak-A. To separate $H_4SiO_4$ ions, a 1.0 mM $H_2SO_4$ solution was used for the mobile phase, with a flow rate of 1.0 mL/min. Subsequently, 0.1% diethylaminoethanol was mixed with the mobile phase at a flow rate of 0.75 mL/min to increase the solution pH to ~10, thereby enabling detection of Si ions as $H_3SiO_4^-$ anions using an EC detector. The relative error in this method is 5%. In addition, the pH and BSA concentrations of each solution were measured every 2 days using a glass electrode and a UV-vis spectrophotometer (Shimadzu UV-1650 PC) at 280 nm wavelength. The dissolution rates of amorphous silica were calculated using Si concentrations in the initial linear stages for 2−10 days.

## RESULTS AND DISCUSSION

The dissolution experiments confirmed that the Si concentrations of all systems increased linearly with time, and that the solution pH remained almost constant throughout the reactions (Figure 1). The increasing rates of Si concentration in system A appeared to increase with increasing BSA concentration, suggesting that BSA exhibits a rate-enhancement effect on amorphous silica dissolution. Although the increasing rates tended to decrease as solution pH decreased from 6 to 4, a similar rate-enhancement effect of BSA can also be observed in systems B and C. The rates of dissolution of amorphous silica in these systems plotted against the solution pH, together with previously published data in 0.1, 1.0, and 10.0 mM NaCl solutions with no BSA are shown in Figure 2 (Kawano and Obokata, 2007). The dissolution rates in solutions containing no BSA are consistent with those of amorphous silica in a 0.1 mM NaCl solution as plotted on the dotted line (Figure 2). However, the dissolution rates increased progressively with increasing BSA concentrations, *e.g.* dissolution rates in system A increased by up to 2.9 times with increasing BSA concentrations compared with BSA-free controls. Systems B and C showed much greater enhancement effects by maximum factors of ~5.8 and 13.0, respectively (Table 2). The results confirmed that BSA exhibits significant rate-enhancement effects on amorphous silica dissolution at pH 6−4, and that rate-enhancement effects increased progressively with decreasing solution pH.

During the dissolution experiments, BSA tended to adsorb on the surface of amorphous silica in varying amounts, depending on the solution pH. The BSA adsorption in systems A, B, and C demonstrated that the maximum adsorption occurred at a pH of ~5 (system B), which is close to the pI value of BSA (Figure 3). The BSA adsorption in system B reached ~85, 81, 62, 37, and 26% in solutions containing 0.05−1.0 mg/mL BSA.

Table 2. Dissolution rates of amorphous silica in systems A to C.

| System | Run | BSA (mg/mL) | pH (Average) | Log rate (mol $s^{-1}$ $m^{-2}$) | Enhancement rate |
|---|---|---|---|---|---|
| System A | A0 | 0.00 | 6.05 | −11.81 | – |
| (Total Na = 0.1 mM) | A1 | 0.05 | 5.95 | −11.62 | 1.6 |
| | A2 | 0.1 | 6.05 | −11.48 | 2.2 |
| | A3 | 0.2 | 6.03 | −11.44 | 2.4 |
| | A4 | 0.5 | 6.01 | −11.42 | 2.5 |
| | A5 | 1.0 | 6.02 | −11.36 | 2.9 |
| System B | B0 | 0.00 | 4.94 | −12.21 | – |
| (Total Na = 0.1 mM) | B1 | 0.05 | 4.98 | −11.73 | 3.1 |
| | B2 | 0.1 | 5.06 | −11.55 | 4.6 |
| | B3 | 0.2 | 5.06 | −11.49 | 5.3 |
| | B4 | 0.5 | 4.93 | −11.46 | 5.7 |
| | B5 | 1.0 | 4.98 | −11.45 | 5.8 |
| System C | C0 | 0.00 | 3.89 | −12.71 | – |
| (Total Na = 0.1 mM) | C1 | 0.05 | 3.84 | −12.03 | 4.9 |
| | C2 | 0.1 | 3.88 | −11.82 | 7.9 |
| | C3 | 0.2 | 3.93 | −11.67 | 11.0 |
| | C4 | 0.5 | 3.89 | −11.63 | 12.2 |
| | C5 | 1.0 | 3.88 | −11.60 | 13.0 |

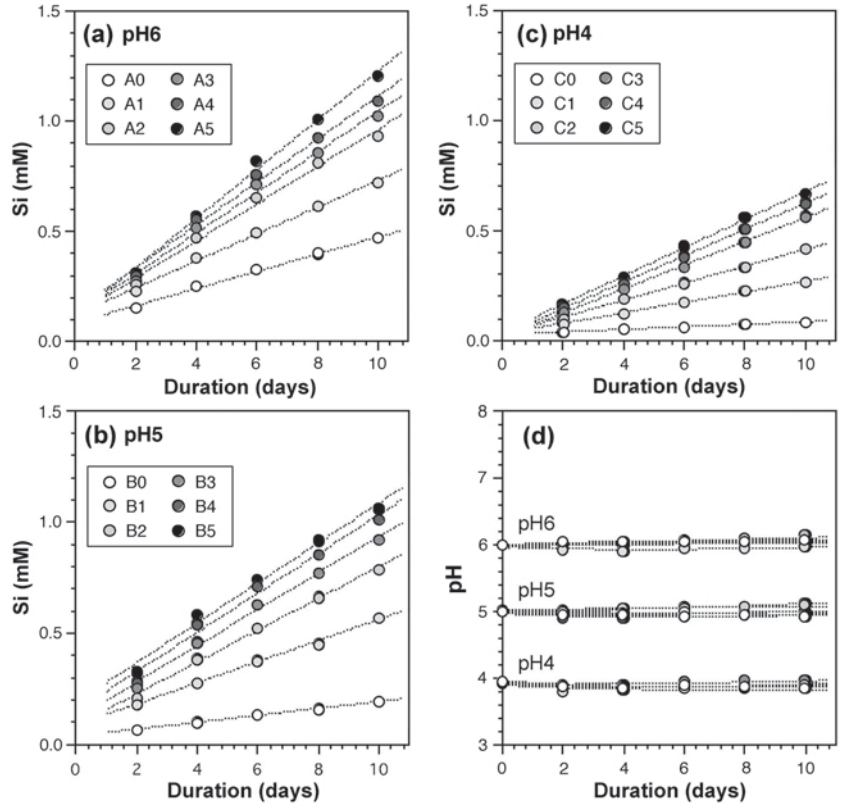


Figure 1. Concentrations of Si during dissolution of amorphous silica in systems A, B, and C at 25ºC, and variation of their solution pH. Systems A, B, and C signify experimental systems at pH 6, 5, and 4, respectively.

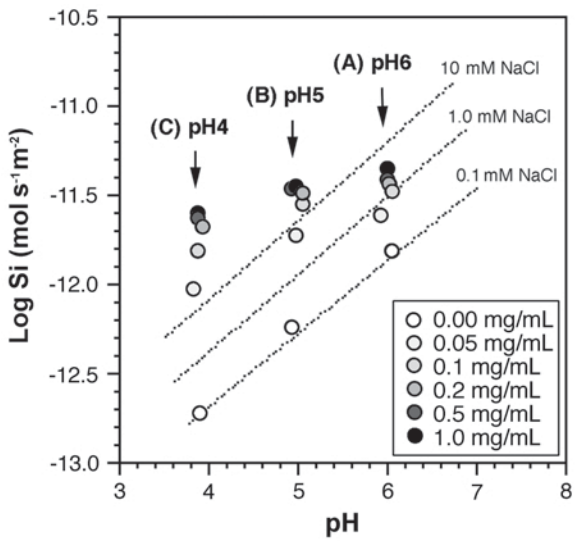


Figure 2. Dissolution rates of amorphous silica in systems A, B, and C at 25ºC, plotted as a function of average solution pH. The dotted lines indicate dissolution rates of amorphous silica in solution containing 0.1, 1.0, and 10 mM NaCl (Kawano and Obokata, 1997).

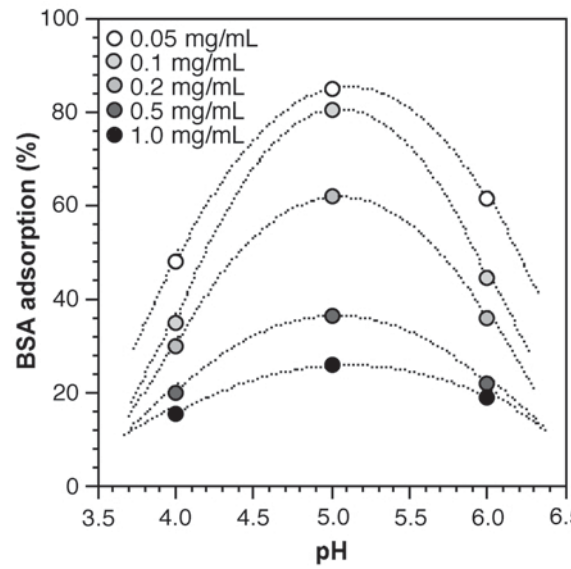


Figure 3. Amounts of BSA adsorption on the surface of BSA as a function of average solution pH in systems A, B, and C at 25ºC.

These adsorption values were 1.5−2 times greater than those observed for systems A and C. Fundamentally, the adsorption of proteins on the surfaces of amorphous silica is considered to result from electrostatic interaction between the negatively charged >$SiO^-$ sites on the amorphous silica surface and the positively charged side chains of amino acid residues such as arginine, lysine, and histidine (Rezwan *et al*., 2005). Furthermore, the amounts of proteins, including BSA, adsorbed on the surface of oxide minerals reach their maximum value at a pH near the pI values as observed in this study (Fukuzaki *et al*., 1996; Li and Li, 2007; Demanèche *et al*., 2009). Several possible mechanisms including symmetric and asymmetric modes have been proposed to explain these adsorption phenomena (Quiquampoix and Ratcliffe, 1992; Lee *et al*., 2002). However, the mechanism of such characteristic adsorption behavior of proteins is still being debated, due to insufficient experimental evidence permitting quantitative interpretation of effects of various factors involving adsorption phenomenon, such as the three-dimensional structure of adsorbed protein and surface coverage area on the oxide mineral surface (Quiquampoix *et al*., 2002).

As shown in Figure 2, dissolution rates of amorphous silica appeared to be significantly enhanced by interaction with BSA, and this effect of BSA increased successively with decreasing solution pH. In previous studies of dissolution of amorphous silica by interaction with amino acids in solution with pH values 6 and 4 (Kawano *et al*., 2009), basic amino acids such as histidine, lysine, and arginine were found to enhance the dissolution rate by about one order of magnitude at both pH conditions. However, neutral amino acids such as cysteine, asparagine, serine, tryptophan, alanine, and threonine showed no significant rate enhancement at pH 6, but the dissolution rate was enhanced by a factor of 3−5 at pH 4. In these pH conditions, the basic amino acids are present mainly as cationic species, which are capable of interacting electrostatically with the negatively charged surface sites of amorphous silica. On the other hand, the neutral amino acids exist mainly as neutral forms at pH 6, whereas concentrations of cationic species increase progressively in solution with lower pH values. Therefore, neutral amino acids are much more effective at increasing the rate of dissolution of amorphous silica at pH 4 as compared to pH 6.

BSA comprises 582 amino acid residues combined in a specific sequence by peptide bonds, with the amino acids remaining neutral unless the side chain is charged. The amino acids containing a chargeable side chain are aspartic acid, glutamic acid, cysteine, tyrosine, histidine, lysine, and arginine, in which aspartic acid, glutamic acid, cysteine, and tyrosine can be charged negatively by the deprotonation of COOH, HS, and OH groups, whereas histidine, lysine, and arginine are able to carry a positive charge by protonation of imidazaole, $NH_2$, and guanidino groups, respectively (Table 3). The concentrations of charged side chains of amino acid residues with solution pH were calculated using *ChemEQL* by Müller (1996), using the pK values of monomer amino acids listed in Table 3. The calculated charge distributions confirmed that lysine, arginine, and histidine were positively charged by full protonation of the side chains' functional groups, and that the charge remained constant over a wide range of pH values from acid to weak alkali regions (Figure 4). In contrast, the negative charge of glutamic acid and aspartic acid appeared to increase with increasing solution pH from 3 to 6, and then the charge remained constant in the higher pH range. The amino acid residues of cysteine and tyrosine did not carry any charge in acid to neutral regions, whereas they tended to be charged negatively in alkali regions. At pH 6−4, therefore, the positively charged side chains of basic amino acid residues, lysine, arginine, and histidine, are capable of interacting with the negatively charged surface sites of amorphous silica. These electrostatic interactions are likely to be an important factor in enhancing the dissolution rates of amorphous silica at this pH range, due to the same rate-enhancing mechanism of free basic amino acids (Kawano *et al*., 2009). However, the negatively charged side chains of acidic amino acid residues such as glutamic acid and aspartic acid may affect the attractive interaction depending on the degree of dissociation. At pH 6, these side chains are fully deprotonated resulting in a maximum negative charge, which produces electrostatic repulsive forces between BSA molecules and the amorphous silica surface. At pH 4, however, these repulsive forces are weaker than those at pH 6, because the degree of deprotonation decreases successively with decreasing pH values. Consequently, BSA can enhance the rates of

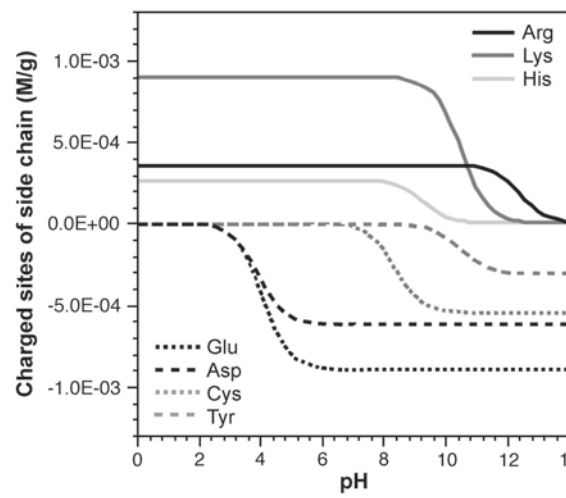


Figure 4. Concentrations of charged side chains of amino acid residues of BSA as a function of solution pH calculated with the geochemical program *ChemEQL* using amino acid compositions and their dissociation data listed in Table 3.

Table 3. Amino acid compositions and their functional BSA characteristics.

| Amino acid | pI[1] | Amino acid composition[2] | Side chain group | Side chain pK[3] |
|---|---|---|---|---|
| Aspartic acid | 2.98 | 41 | COOH | 3.90 |
| Glutamic acid | 3.22 | 59 | COOH | 4.07 |
| Cysteine | 5.02 | 35 | HS | 8.37 |
| Asparagine | 5.43 | 13 | $NH_2$ | – |
| Phenylalanine | 5.48 | 27 | Non-polar | – |
| Glutamine | 5.65 | 20 | $NH_2$ | – |
| Tyrosine | 5.64 | 19 | OH | 10.46 |
| Threonine | 5.64 | 34 | OH | – |
| Serine | 5.68 | 28 | OH | – |
| Methionine | 5.74 | 4 | Non-polar | – |
| Tryptophan | 5.89 | 2 | Non-polar | – |
| Leucine | 5.89 | 61 | Non-polar | – |
| Isoleucine | 5.94 | 14 | Non-polar | – |
| Valine | 5.96 | 36 | Non-polar | – |
| Glycine | 5.97 | 16 | Non-polar | – |
| Alanine | 6.11 | 46 | Non-polar | – |
| Proline | 6.30 | 28 | Non-polar | – |
| Histidine | 7.47 | 17 | Imidazole | 9.33 |
| Lysine | 9.59 | 59 | $NH_2$ | 10.54 |
| Arginine | 10.76 | 23 | Guanidino | 12.48 |

[1] Liu *et al*. (2004), [2] Peters (1996), [3] Dawson *et al*. (1986).

dissolution of amorphous silica in solution with a pH range of 6 to 4, and the rate-enhancement effects were more pronounced at lower pH.

In natural environments, proteins and protein-like molecules including peptides and enzymes are abundant in soils and terrestrial and marine sediments, and in various aquatic environments, at concentrations of μg/g to mg/g (Mayer *et al*., 1986; Tanoue *et al*., 1996; Mayer *et al*., 1999; Murase *et al*., 2003; Schulze, 2005; Weintraub and Schimel, 2005; Elliott *et al*., 2006). The proteinaceous materials are continuously secreted by microorganisms and plants living in these environments, and are also released by cell lysis after their death. Although microorganisms tend to decompose these extracellular proteins into peptide-size fragments, significant amounts of proteinaceous materials remain due to complexation with mineral surfaces and also with dissolved organic matter (Pantoja and Lee, 1999). Such proteinaceous materials exhibit a strong adsorption property in relation to the mineral surfaces as the result of electrostatic interaction (Ding and Henrichs, 2002), whereas their effects on mineral dissolution have not yet been reported and remain uncertain. The results of this study suggest that these proteinaceous materials may also contribute significantly to the rate enhancement of mineral dissolution by acting as a biological catalytic factor in natural environments.

## CONCLUSIONS

Dissolution experiments of amorphous silica in BSA-containing solutions with pH 6, 5, and 4 confirmed that BSA can greatly enhance the rate of dissolution of amorphous silica in a manner that is dependent on the solution pH. At pH 6, the dissolution rate increased by 1.6 to 2.9 times in solutions ranging from 0.05 to 1.0 mg/mL BSA concentrations, respectively. The rates of dissolution increased much more dramatically by ~4.9 to 13.0 times in the same BSA range of solutions with pH 4. The dissolution rates of amorphous silica are likely to be enhanced by competitive interaction of both positively and negatively charged side chains of amino-acid residues with the amorphous silica surface. The positively charged side chains of basic amino acid (lysine, arginine, and histidine) residues can interact with the negative $>SiO^-$ site on the amorphous silica surface, contributing to the enhancement of dissolution rates. However, the rate-enhancement effect may be inhibited by the repulsive interaction of the negatively charged side chains of acidic amino acid (glutamic acid and aspartic acid) residues depending on the degree of deprotonation.

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## REFERENCES

Barker, W.W., Welch, S.A., and Banfield, J.F. (1997) Biogeochemical weathering of silicate minerals. Pp. 391–428 in: *Geomicrobiology: Interactions Between Microbes and Minerals* (J.F. Banfield and K.H. Nealson, editors). Reviews in Mineralogy, Vol. **35**. Mineralogical

Society of America, Washington D.C.

Barns, S.M. and Nierzwicki-Bauer, S.A. (1997) Microbial diversity in ocean, surface and subsurface environments. Pp. 35–79 in: *Geomicrobiology: Interactions Between Microbes and Minerals* (J.F. Banfield and K.H. Nealson, editors). Reviews in Mineralogy, Vol. **35**. Mineralogical Society of America, Washington D.C.

Bonmatia, M., Ceccantib, B., and Nannipieric, P. (1998) Protease extraction from soil by sodium pyrophosphate and chemical characterization of the extracts. *Soil Biology and Biochemistry*, **30**, 2113–2125.

Brown, J.R. (1975) Structure of Bovine serum albumin. *Federation Proceedings*, **34**, 591–591.

Dawson, R.C., Elliott, D.C., Elliott, W.H., and Jones, K.M. (1986) *Data for Biochemical Research. Third Edition*. Clarendon Press, Oxford, UK, 580 pp.

Demanèche, S., Chapel, J., Monrozier, L.J., and Quiquampoix, H. (2009) Dissimilar pH-dependent adsorption features of bovine serum albumin and α-chymotrypsin on mica probed by AFM. *Colloids and Surfaces B*, **70**, 226–231.

Ding, X. and Henrichs, S.M. (2002) Adsorption and desorption of proteins and polyamino acids by clay minerals and marine sediments. *Marine Chemistry*, **77**, 225–237.

Dittmar, T. and Kattner, G. (2003) The biogeochemistry of the river and shelf ecosystem of the Arctic Ocean: a review. *Marine Chemistry*, **83**, 103–120.

Dittmar, T., Fitznar, H.P., and Kattner, G. (2001) Origin and biogeochemical cycling of organic nitrogen in the eastern Arctic Ocean as evident from D- and L-amino acids. *Geochimica et Cosmochimica Acta*, **65**, 4103–4114.

Drever, J.I. and Stillings, L.L. (1997) The role of organic acids in mineral weathering. *Colloids and Surfaces A*, **120**, 167–181.

Durchslang, H. and Zipper, P. (1997) Calculation of hydrodynamic parameters of biopolymers from scattering data using whole body approaches. *Progress in Colloid and Polymer Science*, **107**, 43–47.

Elliott, S., Lead, J.R., and Baker, A. (2006) Characterisation of the fluorescence from freshwater, planktonic bacteria. *Water Research*, **40**, 2075–2083.

Fukuzaki, S., Urano, H., and Nagata, K. (1996) Adsorption of bovine serum albumin onto metal oxide surface. *Journal of Fermentation and Bioenginering*, **81**, 163–167.

Gupta, L.P. and Kawahata, H. (2003) Amino acids and hexosamines in the Hess Rise core during the past 220,000 years. *Quaternary Research*, **60**, 394–403.

Hirayama, K., Akashi, S., Furuya, M., and Fukuhara, K. (1990) Rapid confirmation and revision of the primary structure of bovine serum albumin by ESIMS and Frit-FAB LC/MS. *Biochemical and Biophysical Research Communications*, **173**, 639–646.

Jones, V., Ruddell, C.J., Wainwright, G., Rees, H.H., Jaffé, R., and Wolff, G.A. (2004) One-dimensional and two-dimensional polyacrylamide gel electrophoresis: a tool for protein characterization in aquatic samples. *Marine Chemistry*, **85**, 63–73.

Kawano, M. and Obokata, S. (2007) The effect of amino acids on the dissolution rates of amorphous silica in near-neutral solution. *Clays and Clay Minerals*, **55**, 361–368.

Kawano, M., Hatta, T., and Hwang, J. (2009) Enhancement of dissolution rates of amorphous silica by interaction with amino acids in solution at pH4. *Clays and Clay Minerals*, **57**, 161–167.

Lee, W., Ko, J., and Kim, H. (2002) Effect of electrostatic interaction on the adsorption of globular proteins on octacalcium phosphate crystal film. *Journal of Colloid and Interface Science*, **246,** 70–77

Li, H. and Chen, F. (2000) Determination of silicate in water by ion exclusion chromatography with conductivity detection. *Journal of Chromatography A*, **874**, 143–147.

Li, W. and Li, S. (2007) A study on the adsorption of bovine serum albumin onto electrostatic microspheres: Role of surface groups. *Colloids and Surfaces A*, **295**, 159–164.

Liu, H.X., Zhang, R.S., Yao, X.J., Liu, M.C., Hu, Z.D., and Fan, B.T. (2004) Prediction of the isoelectric point of an amino acid based on GA-PLS and SVMs. *Journal of Chemical Information and Computer Sciences*, **44**, 161–167.

Lu, X.Q., Maie, N., Hanna, J.V., Childers, D.L., and Jaffé, R. (2003) Molecular characterization of dissolved organic matter in freshwater wetlands of the Florida Everglades. *Water Resarch*, **37**, 2599–2606.

Matulis, D., Baumann, C.G., Bloomfield, V.A., and Lovrien, R.E. (1999) 1-Anilino-8-naphthalene sulfonate as a protein conformational tightening agent. *Biopolymers*, **49**, 451–488.

Mayer, L.M., Schick, L.L., and Setchekk, F.W. (1986) Measurement of protein in nearshore marine sediments. *Marine Ecology Progress Series*, **30**, 159–165.

Mayer, L.M., Schick, L.L., and Loder III, T.C. (1999) Dissolved protein fluorescence in two Maine estuaries. *Marine Chemistry*, **64**, 171–179.

McGillivray, R.T.A., Chung, D.W., and Davie, E.W. (1979) Biosynthesis of bovine plasma proteins in a cell-free system. Amino-terminal sequence of preproalbumin. *European Journal of Biochemistry*, **98**, 477–485.

Müller, B. (1996) *ChemEQL V.2.0. A Program to Calculate Chemical Speciation and Chemical Equilibria*. Eidgenössische Anstalt für Wasserversorgung, Dübendorf, Switzerland.

Murase, A., Yoneda, M., Ueno, Y., and Yobebayashi, K. (2003) Isolation of extracellular protein from greenhouse soil. *Soil Biology and Biochemistry*, **35**, 733–736

Pantoja, S. and Lee, C. (1999) Molecular weight distribution of proteinaceous material in Long Island Sound sediments. *Limnology and Oceanography*, **44**, 1323–1330

Patterson, J.E. and Geller, D.M. (1977) Bovine microsomal albumin: Amino terminal sequence of bovine proalbumin. *Biochemical and Biophysical Research Communications*, **74**, 1220–1226.

Peters, T.J. (1996) *All About Albumin: Biochemistry, Genetics and Medical Applications*. Academic Press, San Diego, California, 580 pp.

Quiquampoix, H. and Ratcliffe, R.G. (1992) A $^{31}$P NMR study of the adsorption of bovine serum albumin on montmorillonite using phosphate and the paramagnetic cation $Mn^{2+}$: modification of conformation with pH. *Journal of Colloid and Interface Science*, **148**, 343–352.

Quiquampoix, H., Servagent-Noinville, S., and Baron, M. (2002) Enzyme adsorption on soil mineral surfaces and consequences for the catalytic activity. Pp. 285–306 in: *Enzymes in the Environment, Activity, Ecology, and Applications* (R.G. Burns and R.P. Dick, editors). Taylor and Francis, London, UK.

Rezwan, K., Meier, L.P., and Gauckler, L.J. (2005) Lysozyme and bovine serum albumin adsorption on uncoated silica and AlOOH-coated silica particles: the influence of positively and negatively charged oxide surface coatings. *Biomaterials*, **26**, 4351–4357.

Schulze, W.X. (2005) Protein analysis in dissolved organic matter: What proteins from organic debris, soil leachate and surface water can tell us – a perspective. *Biogeosciences*, **2**, 75–83.

Tanoue, E., Ishii, M., and Midorikawa, T. (1996) Discrete dissolved and particulate proteins in oceanic waters. *Limnology and Oceanography*, **41**, 1334–1343.

Ullman, W.J. and Welch, S.A. (2002) Organic ligands and feldspar dissolution. Pp. 3–35 in: *Water-Rock Interactions,*

*Ore Deposits, and Environmental Geochemistry: A Tribute to David A. Crearar* (R. Hellmann and S.A. Wood, editors). Special Publication. No. **7**, Geochemical Society, St. Louis, Missouri.

van Hees, P.A.W., Jones, D.L., Finlay, R., Godbold, D.L., and Lundström, U.S. (2005) The carbon we do not see – the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: A review. *Soil Biology and Biochemistry*, **37**, 1–13.

Weintraub, M.N. and Schimel, J.P. (2005) Seasonal protein dynamics in Alaskan arctic tundra soils. *Soil Biology and Biochemistry*, **37**, 1469–1475.

Welch, S.A., Barker, W.W., and Banfield, J.F. (1999) Microbial extracellular polysaccharides and plagioclase dissolution. *Geochimica et Cosmochimica Acta*, **63**, 1405–1419.

Yamada, M. and Tanoue, E. (2006) The inventory and chemical characterization of dissolved proteins in oceanic waters. *Progress in Oceanography*, **69**, 1–18.

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