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Quantitative Evaluation of Shift of Slipping Plane and Counterion Binding  
to Lysozyme by Electrophoresis Method

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Acknowledgement

This study was financially supported by the KAKENHI (15H04563) from  
Japan Society for the Promotion of Science

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## Abstract

Measurement and analysis of electrophoretic mobility (EPM) are widely used to investigate electric charging properties of proteins. However, the proper way of analysis for EPM of protein has not yet been fully consolidated. In this study, EPM of hen-egg-white lysozyme (LSZ) was measured as a function of pH at different concentrations of KCl solutions. The obtained experimental EPMs are compared to theoretical EPMs which were calculated from charge amount from proton titration. Theoretical EPMs were calculated by a set of models for a small rigid particle including Poisson-Boltzmann model and the effect of double layer relaxation and by that for a soft particle neglecting the relaxation effect. The results of comparisons show that one can analyze EPM of LSZ as a small rigid particle. Nevertheless, all analyses overestimate experimental data. We presume that these discrepancies are caused by the shift of slipping plane from the surface and/or by binding of counterion to LSZ. Therefore, we examined these two effects on the analyses of EPM. Our analyses demonstrate that introducing the 0.5-2 nm shift of slipping plane or the 40-80 % reduction of effective charge generates the quantitative agreement between theoretical EPMs and experimental data. We find the required amount of reduced charge is 4-5 elementary charges per LSZ irrespective of pH and ionic strength below pH 7.

Keywords: Lysozyme, Electrophoretic mobility, Effective charge, Binding of counterion, Coadsorption of counterion, Slipping plane

## 1. Introduction

Electric properties of proteins affect protein-protein interaction, protein-polyelectrolyte interaction [1] and adsorption behavior at solid-solution interface [2,3]. Thus, understanding electric properties of proteins has great importance in protein applications in the field of biomedical, pharmacy, food engineering, environmental function, and so on. Nowadays, electrophoretic mobility (EPM) measurements and analyses are widely used for characterizing the charging behavior of colloid particles including proteins [4–6].

EPM is connected with zeta potential by theoretical models. Because of its simplicity, the classical theories which proposed by Smoluchowski [7], Huckel [8] and Henry [9] are widely used. More rigorous theoretical model which takes account for the surface conductance or double layer relaxation was developed by O'Brien and White [10]. However their model needs complicated numerical computation. Therefore, Ohshima et al. [11] and Ohshima [12] proposed approximate analytical solutions connecting EPM with zeta potential. By applying the Poisson-Boltzmann (PB) equation, one can calculate charge density of a particle from zeta potential. The validities of these models are confirmed by studies

using model particles which are rigid and have known surface charge such as silica particles [13,14] or polystyrene latexes [15–21]. In some cases, quantitative agreement between theoretical mobility and experimental data is achieved by assuming the sub-nanometer or few nanometer shift of slipping plane, where zeta potential is defined, from the particle surface [13–19,21,22].

The charge density obtained from EPM or other electrokinetic techniques is referred as effective or electrokinetic (uncompensated) charge to distinguish from charge density obtained by proton titration. It is well known that effective charge density of some materials such as polymer and protein is less than the original charge density [23–26]. This phenomenon is caused by coadsorption/binding of counterion. When one performs proton titration, one assumes that charge is only derived from protonation and deprotonation of functional groups. Thus, the original proton titration charge does not include binding of counterion and reduction of effective charge from original charge.

Lysozyme (LSZ) is the protein with antimicrobial property for gram-positive bacteria [24] and has high conformational stability. Owing to its useful properties, LSZ has been put to many practical uses and studied widely as model. Measurements and analyses of EPM of LSZ were performed by some researchers [24,25,27,28]. In previous studies, theoretical EPMs calculated from charge amount obtained from proton titration overestimate experiment data. Jachimska et al. [24] calculated the charge amount of LSZ by analyzing EPM with simple electrokinetic formula. They suggested that the effective charge from EPM analysis was 20-30 % of nominal charge which was obtained by theoretical prediction, and they assumed the reduction of effective charge was caused by coadsorption/binding of counterion. In their analysis, however, they do not take account of electric diffuse double layer and ion relaxation effect. Kuehner et al. [29] calculated the amount of coadsorption/binding of counterion to LSZ with the theoretical model. However, the model used by Kuehner cannot distinguish the ion which tightly binds to surface and the ion which loosely associates in diffuse double layer.

In this paper, we report results of EPM measurements of LSZ as a function of pH at different KCl concentrations and analyses for them with some sets of theoretical models. In addition, we suggest the magnitude of the shift of slipping plane from surface and/or the fraction of remaining effective charge. Moreover, we examined the effect of constant charge reduction on EPMs of LSZ.

## **2. Experimental part**

### **2.1. Materials**

LSZ from hen-egg-white (L6876, Sigma Aldrich) was used without further purification. From the literature [2,28], LSZ is oval sphere with  $3 \times 3 \times 4.5$  nm. In this study, we assume LSZ as a sphere with the radius of 1.7 nm from cubic mean radius. The molecular weight of LSZ is 14.3 kDa. KCl (JIS special grade, Wako Pure Chemical Industries) solution was used to control ionic strength. KOH and HCl solutions were used as pH adjuster. Through whole study, deionized water (Elix Millipore) with an

electric conductivity of about 0.07  $\mu\text{S}/\text{cm}$  was used to prepare all solutions.

## 2.2. Electrophoresis experiments

Electrophoretic mobility of LSZ was measured as a function of pH in solutions of different KCl concentrations. EPM measurements were carried out with Zeta Sizer Nano (Malvern) at the LSZ concentration of 5 mg/mL. LSZ solution, KCl solution, and HCl or KOH solution were mixed right before each measurement. The mixture was injected into a measurement cell and mobility measurement was started. The pH of the mixture was measured with a combination electrode (6.0234.100, Metrohm). Measurements were performed three times for each sample. All experiments were carried out at 20 °C.

It should be noted that we added appropriate amount of HCl or KOH as pH adjuster without relation to KCl concentration. Since LSZ solution takes pH around 4 without any pH adjuster, the total ionic strength of high pH sample is slightly different from KCl concentration. The maximum deviation of ionic strength from KCl concentration is 10 mM in KCl 50 mM solution at pH 11.8.

## 3. Theoretical analysis part

Theoretical electrophoretic mobility of LSZ was calculated by a set of equations from charge amount obtained from proton titration by Tan et al. [30]. By dividing the amount of charge by surface area ( $3.8 \times 10^{-17} \text{ m}^2$  for oval sphere), we estimate surface charge density  $\sigma$ . Then, theoretical values are compared with experimental data. To demonstrate effects of electric diffuse double layer and relaxation effect, we used some sets of equations which include these effects or not.

### 3.1. Lorenz-Stokes equation

Neglecting the electric diffuse double layer and relaxation effect, we can calculate electrophoretic mobility  $\mu$  of a small rigid particle from charge amount by using the Lorentz-Stokes equation [24,31].

$$\mu = \frac{q}{6\pi a\eta} \quad (1)$$

where  $q$  is a charge amount of LSZ,  $a$  is radius of particle and  $\eta$  is viscosity of solvent.

### 3.2. Including the diffuse double layer and relaxation effect

The Poisson-Boltzmann (PB) equation is used to include the effect of diffuse double layer. By solving the PB equation for a small particle in  $z:z$  electrolyte solution, one can obtain the relationship between charge density  $\sigma$  and surface potential  $\psi_0$  as [32]

$$\sigma = \frac{2\epsilon_r\epsilon_0\kappa k_B T}{ze} \sinh\left(\frac{ze\psi_0}{2k_B T}\right) \times \left[ 1 + \frac{1}{\kappa a} \frac{2}{\cosh^2\left(\frac{ze\psi_0}{4k_B T}\right)} + \frac{1}{(\kappa a)^2} \frac{8 \ln\left[\cosh\left(\frac{ze\psi_0}{4k_B T}\right)\right]}{\sinh^2\left(\frac{ze\psi_0}{2k_B T}\right)} \right]^{\frac{1}{2}} \quad (2)$$

with

$$\kappa = \sqrt{\frac{2e^2 z^2 n}{\epsilon_r \epsilon_0 k_B T}} \quad (3)$$

where  $\epsilon_r$  is the relative permittivity of the liquid,  $\epsilon_0$  is the permittivity of a vacuum,  $k_B$  is the Boltzmann constant,  $T$  is the absolute temperature,  $e$  is the elementary electronic charge, and  $n$  is the concentration of  $z:z$  electrolyte.  $1/\kappa$  is the Debye length in a  $z:z$  electrolyte solution. Here,  $z = 1$  in this study.

The potential distribution around a small particle is also given from PB equation by [33]

$$\psi(r) = \frac{2k_B T}{ze} \ln \left[ \frac{(1 + Bs) \left(1 + \frac{Bs}{2\kappa a + 1}\right)}{(1 - Bs) \left(1 - \frac{Bs}{2\kappa a + 1}\right)} \right] \quad (4)$$

with

$$B = \frac{\left(1 + \frac{\kappa a}{\kappa a + 1}\right) \tanh\left(\frac{y_0}{4}\right)}{1 + \left\{1 - \frac{2\kappa a + 1}{(\kappa a + 1)^2} \tanh^2\left(\frac{y_0}{4}\right)\right\}^{\frac{1}{2}}} \quad (5)$$

$$s = \frac{a}{r} e^{-\kappa(r-a)} \quad (6)$$

$$y_0 = \frac{ze\psi_0}{k_B T} \quad (7)$$

where  $r$  is the distance from the center of particle. In this case,  $r - a$  means the distance from particle surface. Zeta potential  $\zeta$  is defined as the potential at slipping plane where the relative velocity of solvent to particle is zero. Thus, zeta potential does not equal to surface potential  $\psi_0$  when the position of slipping plane is away from the surface (see Fig. 1). In this case, one can convert surface potential to zeta potential by changing the value of  $r - a$  in eq. (6).

EPM can be calculated from zeta potential. Neglecting the relaxation effect, one can use Ohshima's approximate Henry equation [34]

$$\mu = \frac{2\epsilon_r \epsilon_0 \zeta}{3\eta} \left( 1 + \frac{1}{2 \left[ 1 + \frac{2.5}{\kappa a (1 + 2e^{-\kappa a})} \right]^3} \right) \quad (8)$$

Note that, in the present condition ( $\kappa a = 1.2$  at 50 mM KCl), the Ohshima's approximate Henry equation

is not significantly different from the Huckel equation.

To take account of relaxation effect, one can use Ohshima's approximate electrokinetic formula for a rigid particle [12].

$$\begin{aligned} \mu = & \frac{2\epsilon_r\epsilon_0\zeta}{3\eta} \left( 1 + \frac{1}{2 \left[ 1 + \frac{2.5}{\kappa a(1 + 2e^{-\kappa a})} \right]^3} \right) \\ & - \frac{2\epsilon_r\epsilon_0\zeta}{3\eta} \left( \frac{ze\zeta}{k_B T} \right)^2 \left[ \frac{\kappa a \{ \kappa a + 1.3 \exp(-0.18\kappa a) + 2.5 \}}{2 \{ \kappa a + 1.2 \exp(-7.4\kappa a) + 4.8 \}^3} \right. \\ & \left. + \left( \frac{m_+ + m_-}{2} \right) \frac{9\kappa a \{ \kappa a + 5.2 \exp(-3.9\kappa a) + 5.6 \}}{8 \{ \kappa a - 1.55 \exp(-0.32\kappa a) + 6.02 \}^3} \right] \end{aligned} \quad (9)$$

with dimensionless ionic drag coefficient  $m_{\pm}$  defined by

$$m_{\pm} = \frac{2\epsilon_r\epsilon_0 k_B T}{3\eta z^2 e^2} \lambda_{\pm} \quad (10)$$

Where  $\lambda_{\pm}$  is the drag coefficient of cations and anions defined by  $\lambda_{\pm} = N_A e^2 z / \Lambda_{\pm}^0$  where  $N_A$  is Avogadro's number and  $\Lambda_{\pm}^0$  is limiting conductance of ions. Note that the first term of eq. (9) is the same as eq. (8) and the second and the third terms denote the relaxation effect.

### 3.3. Hermans-Fujita formula for spherical polyelectrolyte

The Hermans-Fujita formula [35] is used to calculate the EPM of spherical polyelectrolyte. In this theoretical model, an ion-penetrable region which has uniform charge density  $N$  with ionized group of valence  $Z$  is assumed.

$$\begin{aligned} \mu = & \frac{ZeN}{\eta\lambda^2} \left[ 1 + \frac{1}{3} \left( \frac{\lambda}{\kappa} \right)^2 \left( 1 + e^{-2\kappa a} - \frac{1 - e^{-2\kappa a}}{\kappa a} \right) \right. \\ & \left. + \frac{1}{3} \left( \frac{\lambda}{\kappa} \right)^2 \frac{1 + \frac{1}{\kappa a}}{\left( \frac{\lambda}{\kappa} \right)^2 - 1} \left\{ \left( \frac{\lambda}{\kappa} \right) \frac{1 + e^{-2\kappa a} - \frac{1 - e^{-2\kappa a}}{\kappa a}}{\frac{1 + e^{-2\lambda a}}{1 - e^{-2\lambda a}} - \frac{1}{\lambda a}} - (1 - e^{-2\kappa a}) \right\} \right] \end{aligned} \quad (11)$$

where  $\lambda$  is electrophoretic softness which is completely adjustable parameter. In many situations,  $ZeN$  is also treated as adjustable parameter. However, in this study, we calculate  $ZeN$  by dividing charge amount by the volume of LSZ ( $2.1 \times 10^{-26} \text{ m}^3$  for oval sphere).

## 4. Result & Discussion

Results of EPM measurements of LSZ are presented in Fig. 2. Symbols denote the average of three measurements for each sample and error bars represent standard deviation. LSZ exhibits positive

mobility at low pH region and the magnitude of the mobility decreases with increasing pH as expected from the proton titration [30]. EPM goes to zero around pH 10 which consists with isoelectric point (iep). LSZ shows negative value of EPM at pH higher than iep. The absolute value of the mobility decreases with increasing ionic strength. These tendencies are also found elsewhere [24,27,28]. Note that iep is getting smaller with increasing ionic strength. This strong dependence of iep (better to say point of zero charge for proton titration) on ionic strength is not observed for proton titration. It is also worth noting that we measured EPMs around pH 4 at various KCl concentrations. The EPMs hardly change over 150 mM KCl, and do not approach zero even when ionic strength is very high. Similar trends with this result can be found for the mobility of soft particle.

Figure 2 shows theoretical mobilities in 10 mM KCl solution calculated by the Hermans-Fujita equation for several values of adjustable parameter  $1/\lambda$  nm which represents softness and water permeability of a spherical polyelectrolyte. Theoretical EPMs decrease with decreasing value of  $1/\lambda$ . Below  $1/\lambda = 0.001$  nm, theoretical EPMs do not change dramatically and collapse onto a single curve. We can see that mobility for the smallest  $1/\lambda$  is the closest to but is still larger than experiment data. Again,  $1/\lambda$  nm represents softness and the water permeability of a spherical polyelectrolyte. Therefore, our result of  $1/\lambda$  adjustment means that LSZ has very low or almost no softness and water permeability.

Theoretical EPMs in 10 mM KCl solution calculated from some sets of equations are compared with experimental data in Fig. 4. “Lorenz-Stokes” is based on the Lorenz-Stokes equation without the PB model. “Hermans-Fujita” is calculated by the Hermans-Fujita formula for spherical polyelectrolyte with  $1/\lambda = 0.000001$  nm. “PB & Henry” is including PB model and Ohshima’s approximate Henry equation which does not take account of the effect of double layer relaxation. “PB & relaxation” is based on Ohshima’s approximate electrokinetic formula for a small rigid particle including PB model as well as the effect of double layer relaxation. In Fig. 4, we assume that the slipping plane is at the surface and the effective charge is the same as the charge amount from proton titration. The difference between “Lorenz-Stokes” and “PB & Henry” is caused by taking account of PB model or not, and the values by “Lorenz-Stokes” are much larger than the experimental value. The difference shows that one needs to take account of PB model when we calculate mobility of LSZ. “Hermans-Fujita” values are close to “PB & Henry”. This result shows that we can analyze the mobility of LSZ with theoretical models for a rigid particle. The difference between “PB & relaxation” and “PB & Henry” is caused by taking account of the effect of double layer relaxation or not. We can see that relaxation effect appears at low pH region. The appearance of relaxation is because high charge density at low pH region generates large zeta potential enough to cause double layer relaxation. We calculated theoretical EPMs at three different KCl concentrations (5, 10, and 50 mM). We can find trends which we state above at all KCl concentrations. While LSZ is not a perfect sphere, we think that the shape of LSZ does not significantly affect EPM. This is because Ohshima [36] calculated the Henry function of cylindrical particle and showed that the difference between Henry function of spherical particle and that of cylindrical particle is insignificant.

Thus, EPM of LSZ, which is oval sphere, can be analyzed by models for a sphere. It is also worth noting that Kim et al. [27] calculated the EPM of LSZ by the model taking account of charge distribution and showed that the effect of charge distribution is insignificant. Therefore, our analysis here is reasonable.

While “PB & relaxation” takes the closest value to experimental data, it is still larger than experimental values as reported elsewhere [27,28]. We presume that these discrepancies are caused by the shift of slipping plane from the surface and/or by the reduction of effective charge by counterion ( $\text{Cl}^-$  in this study) binding to LSZ. Therefore, we examine these two effects on the EPM in the set of equations for a small rigid particle with the PB model and relaxation effect (“PB & relaxation”).

We evaluated the distance between the slipping plane and the surface by fitting the theoretical EPM to experimental data by changing value of  $r - a$  in eq. (6). Figure 5 shows the result of fit in 10 mM KCl solution. Our analyses demonstrate that introducing 0.5-1.4 nm shift of slipping plane generates the quantitative agreement between theoretical EPMs and experimental data at 10 mM KCl. This result implies that the distance to the slipping plane from LSZ surface in 10 mM KCl solution is 0.5-1.4 nm. Table 1 shows the evaluated results of the distance to slipping plane for various ionic strengths (see support information). We find insignificant dependence of the distance between slipping plane and surface on ionic strength. At all analysis conditions, at 5, 10, and 50 mM KCl concentrations, the range of shift of slipping plane is 0.5-2.0 nm. By using model colloid particles such as silica and latex, some studies reported that the distance to the slipping plane from the surface is zero to sub-nanometer [13,15–19,23], and other studies reported a few nanometers shift [14,21]. The distance to the slipping plane from the surface is understood as the thickness of the immobile fluid layer near the surface. Since the sub-nanometer is comparable to the size of hydrated ion, it is not unrealistic value of shift of slipping plane. Besides, a few nanometers are comparable to the size of several hydrated ions. Then, we presume that a few nanometers shift has less possibility than sub-nanometer shift. Therefore we presume that our results, 0.5-2 nm shift, are rather large. It is also worth noting that, in previous studies with model colloid particle, the shift of slipping plane is independent of pH. Nevertheless, our results show that the distance to slipping plane from LSZ surface is strongly dependent on pH. From above discussions, other factors such as binding of counterion should be taken into account.

We also presume that the effective charge is smaller than the original charge obtained from proton titration because of binding of counterion. We estimated the fraction of remaining effective charge from the original proton charge by fitting the theoretical EPMs to experimental data. Note that, by including the PB model in analyses, we can distinguish ions in the electric diffuse double layer from ions binding to LSZ surface. Figure 6 shows the results of mobility fit in 10 mM KCl solution. Our analyses demonstrate that theoretical mobilities are in quantitative agreement with experimental data when the effective charge is 30-60% of original charge from proton titration. In other words, 40-70 % of original charges are cancelled by the binding of counterions. Table 1 shows the results of remaining effective charge from mobility fitting for various ionic strengths. We find the insignificant dependence of the



fraction of remaining effective charge on ionic strength.

The fractions of remaining charge dramatically change at pH lower than 7 (see Fig. 6). While 60 % of the original charge remains as effective charge at pH 4, only 30 % of original charge remains at pH higher than 7. This trend may be caused by the reduction of constant charge irrespective of pH. At high pH region, LSZ has lower charge amount than at low pH region. Then, the reduction of same amount of charge number generates low fraction of remaining charge at high pH region. Starting with this assumption, we examined the effect of constant charge reduction in the “PB & relaxation” and calculated the EPM of LSZ. From the result of proton titration, proton binding on LSZ is 0.7 mmol/g at pH 4 in 10 mM KCl. By using this value, we can estimate the charge number per LSZ which is canceled by  $0.7 \text{ mmol/g} \times 14.3 \text{ kg/mol} \times 0.4 = 4$ . In the same manner, we obtained the number of charge reduction at pH 3 in 5 mM and 50 mM KCl solution as 5 and 7 respectively. Theoretical EPMs of LSZ at (a) 5 mM KCl, (b) 10 mM KCl and (c) 50 mM KCl with constant charge reduction are compared with experimental data in Fig. 7. In all analyses, reduction of 4 or 5 elementary charges per LSZ from titration charge generates quantitative agreement between theoretical and experimental EPM of LSZ at pH lower than 8. This result implies that, at pH lower than 8, some functional groups have high affinity for  $\text{Cl}^-$  and their charges are always canceled by binding of  $\text{Cl}^-$  without significant relation to pH and KCl concentration.

## 5. Conclusion

Electrophoretic mobility (EPM) of hen-egg-white lysozyme (LSZ) is measured as a function of pH at different concentrations of KCl solutions. The obtained experimental EPMs are analyzed by a set of equations for a small rigid particle including the effect of double layer relaxation and by that for a soft particle neglecting the relaxation effect. Comparison of theoretical EPMs and experimental data shows that one can analyze EPM of LSZ with a set of equations for a small rigid particle including effect of double layer relaxation. Nevertheless, all analyses overestimate the experimental data. We presume that these discrepancies are caused by the shift of slipping plane from the surface and/or by binding of counterion to LSZ. Our analyses demonstrate that introducing the 0.5-2 nm shift of slipping plane or the 40-80 % reduction of effective charge generates the quantitative agreement between theoretical EPMs and experimental data. At pH below 7, the reduction of 4-5 elementary charges per LSZ from proton charge provides good fit with experimental data irrespective of pH and ionic strength.

## Acknowledgement

This study was financially supported by the JSPS KAKENHI (15H04563).

## Conflict of interest

The authors declare that they have no conflict of interest associated with this article.

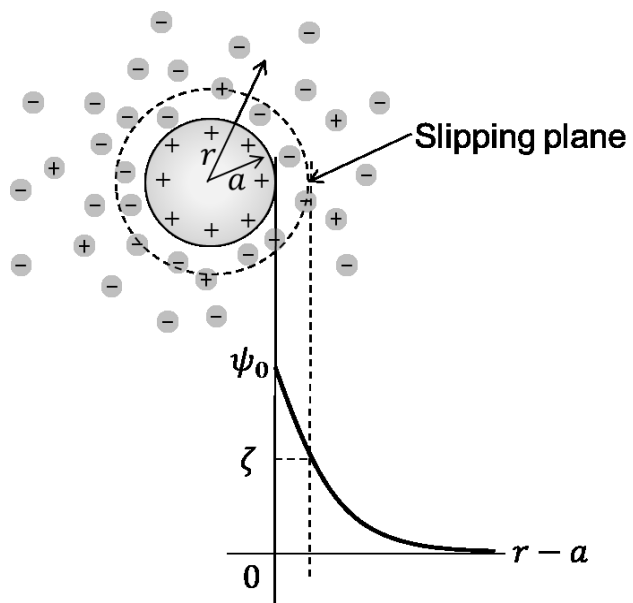
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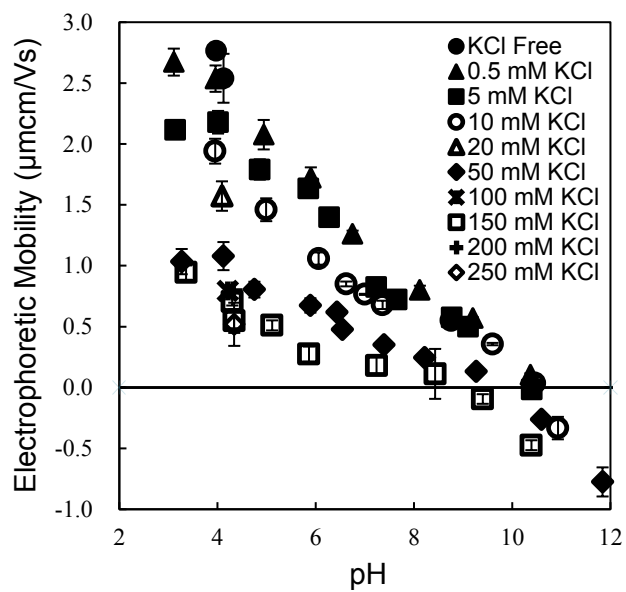
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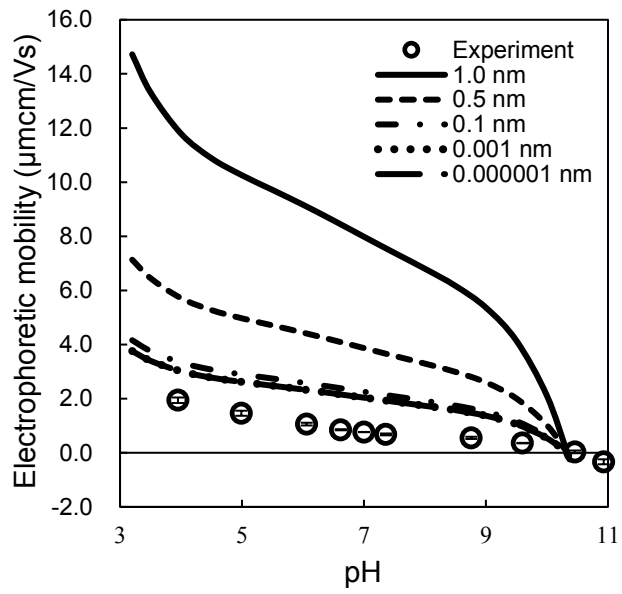
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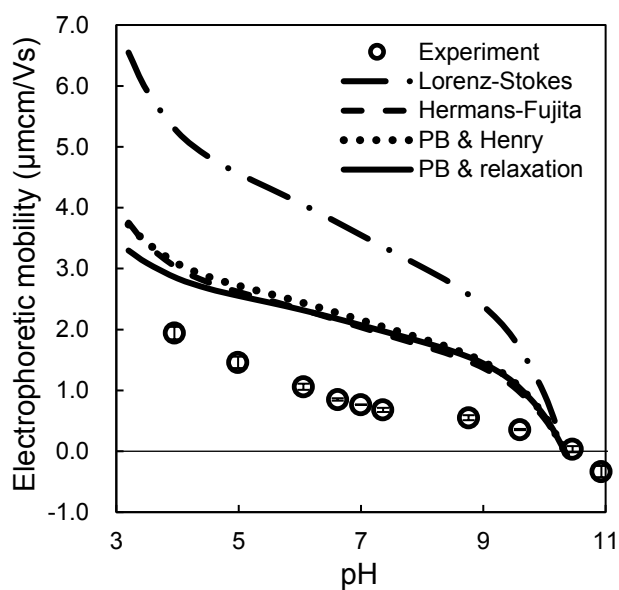
**Fig. 1** Schematic representation of slipping plane



**Fig. 2** Experimental electrophoretic mobilities of LSZ vs. pH at different KCl concentrations. Symbols represent the experimental values

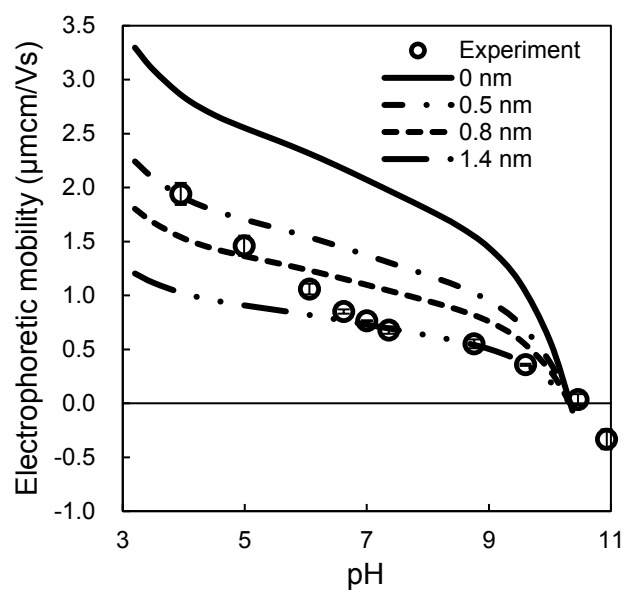


**Fig. 3** The electrophoretic mobility of LSZ in 10 mM KCl solution as a function of pH for several values of  $1/\lambda$  nm. Symbols denote experimental values. Lines are theoretical values calculated by the Hermans-Fujita formula (eq. (11))

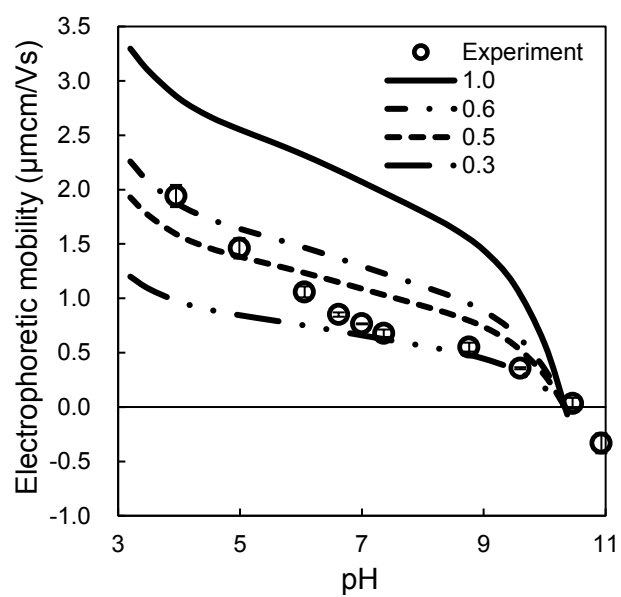


**Fig. 4** Comparison of theoretical and experimental electrophoretic mobilities of LSZ in 10 mM KCl solution as a function of pH. Symbols denote experimental values. Lines are theoretical calculation. “Lorenz-Stokes” is based on the Lorenz-Stokes equation without the PB model. “Hermans-Fujita” is calculated by the Hermans-Fujita formula for spherical polyelectrolyte for  $1/\lambda = 0.000001$  nm. “PB & Henry” is including PB model and Ohshima’s approximate Henry’s equation. “PB & relaxation” is based on Ohshima’s approximate electrokinetic equations for a small rigid particle including PB model and the effect of double layer relaxation

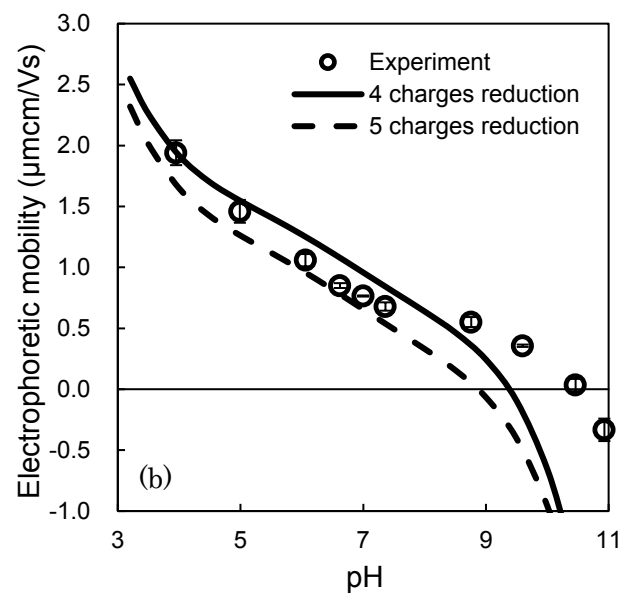
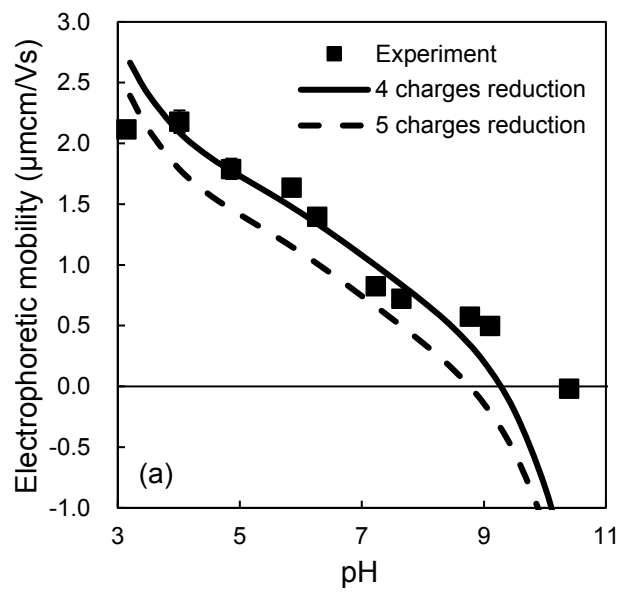


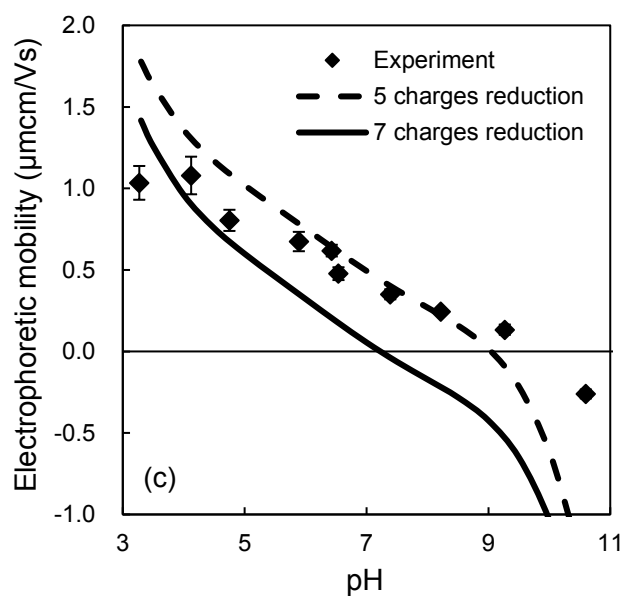


**Fig. 5** Electrophoretic mobility of LSZ in 10 mM KCl solution vs. pH for several values of shifts of slipping plane from the surface. Symbols denote experimental values. Lines are theoretical values calculated by the same set as “PB & relaxation” for various values of  $r - a$  in eq. (6)



**Fig. 6** Electrophoretic mobility of LSZ in 10 mM KCl solution vs. pH for different fractions of remaining effective charge. Symbols denote experimental values. Lines are theoretical values calculated by the same set as “PB & relaxation” for different fractions of remaining effective charge





**Fig. 7** Comparisons between theoretical electrophoretic mobilities of LSZ with constant charge reduction and experimental data. Symbols denote experimental values. Lines are theoretical values calculated by a same set as “PB & relaxation” with (a) 4 and 5 elementary charges reduction at 5 mM KCl, (b) 4 and 5 $e$  reduction at 10 mM KCl, and (c) 5 and 7 $e$  reduction at 50 mM KCl

**Table 1** The distance between slipping plane and the surface and the fraction of remaining effective charge from proton titration in different KCl concentration solutions

KCl concentration	5 mM	10 mM	50 mM
Shift of slipping plane (nm)	0.6-2.0	0.5-1.4	0.6-1.5
Remaining charge ratio (%)	30-60	30-60	20-40

Support Information

Quantitative Evaluation of Shift of Slipping Plane and Counterion Binding  
to Lysozyme by Electrophoresis Method

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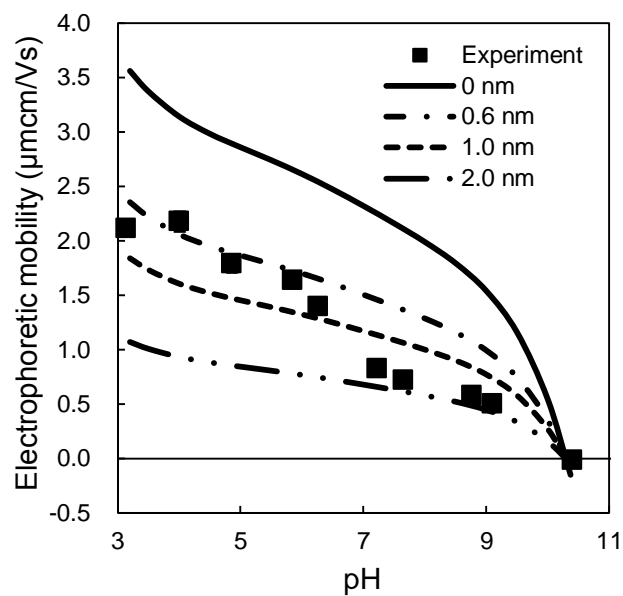
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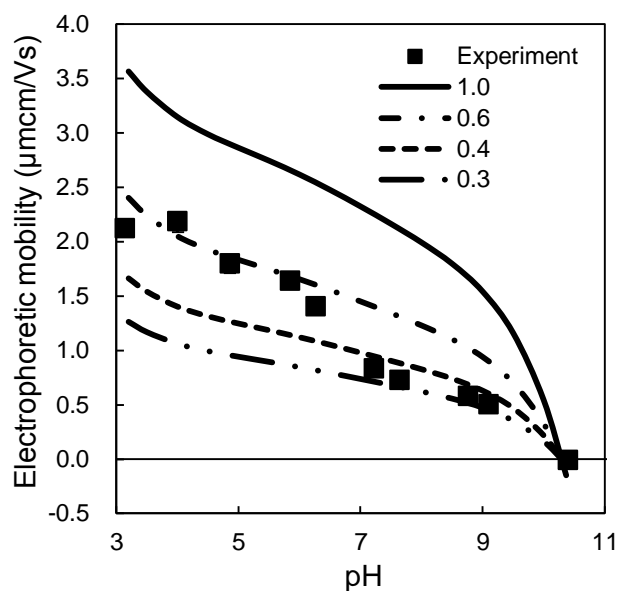
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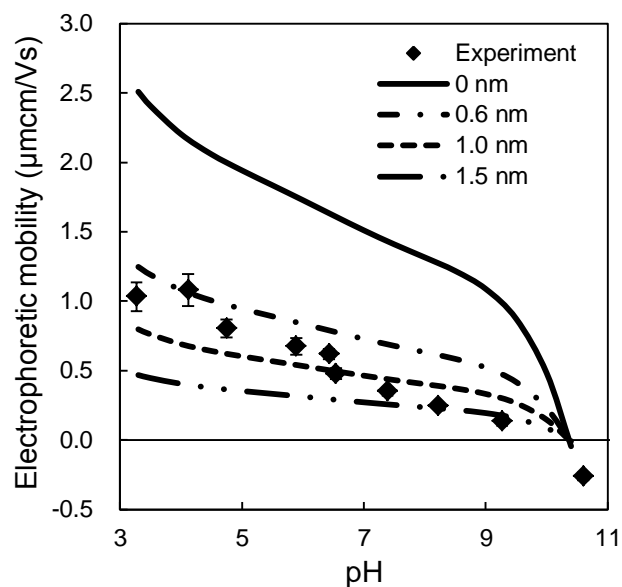
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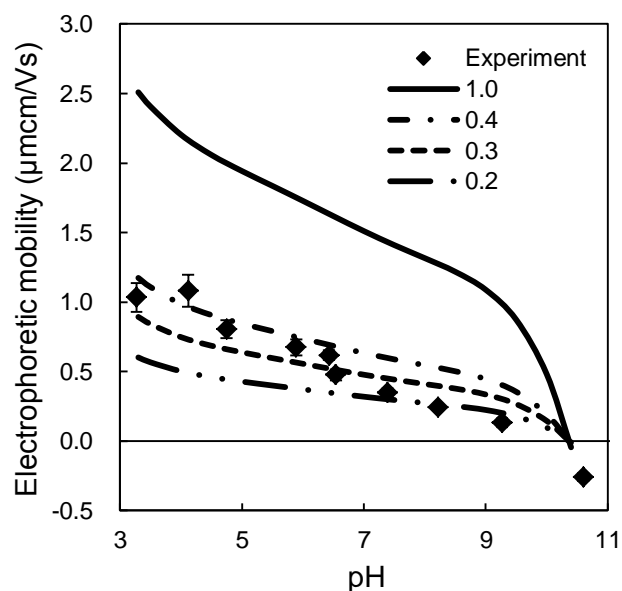
**Fig. S1** Electrophoretic mobility of lysozyme in 5 mM KCl solution vs. pH for several values of shifts of slipping plane from the surface. Symbols denote experimental values. Lines are theoretical values calculated by the same set as “PB & relaxation” for various values of  $r - a$  in eq. (6)



**Fig. S2** Electrophoretic mobility of lysozyme in 5 mM KCl solution vs. pH for different fractions of remaining effective charge. Symbols denote experimental values. Lines are theoretical values calculated by the same set as “PB & relaxation” for different fractions of remaining effective charge



**Fig. S3** Electrophoretic mobility of lysozyme in 50 mM KCl solution vs. pH for several values of shifts of slipping plane from the surface. Symbols denote experimental values. Lines are theoretical values calculated by the same set as “PB & relaxation” for various values of  $r - a$  in eq. (6)



**Fig. S4** Electrophoretic mobility of lysozyme in 50 mM KCl solution vs. pH for different fractions of remaining effective charge. Symbols denote experimental values. Lines are theoretical values calculated by the same set as “PB & relaxation” for different fractions of remaining effective charge