Modeling the Electrophoresis of Lysozyme. II. Inclusion of Ion Relaxation

Stuart A. Allison,* Michael Potter,# and J. Andrew McCammon#
*Department of Chemistry, Georgia State University, Atlanta, Georgia 30303, and #Department of Chemistry and Biochemistry, and Department of Pharmacology, University of California at San Diego, La Jolla, California 92093-0365 USA

ABSTRACT In this work, boundary element methods are used to model the electrophoretic mobility of lysozyme over the pH range 2–6. The model treats the protein as a rigid body of arbitrary shape and charge distribution derived from the crystal structure. Extending earlier studies, the present work treats the equilibrium electrostatic potential at the level of the full Poisson-Boltzmann (PB) equation and accounts for ion relaxation. This is achieved by solving simultaneously the Poisson, ion transport, and Navier-Stokes equations by an iterative boundary element procedure. Treating the equilibrium electrostatics at the level of the full rather than the linear PB equation, but leaving relaxation out, does improve agreement between experimental and simulated mobilities. Including ion relaxation improves it even more. The effects of nonlinear electrostatics and ion relaxation are greatest at low pH, where the net charge on lysozyme is greatest. In the absence of relaxation, a linear dependence of mobility and average polyion surface potential, $\langle\Lambda_0\rangle_S$, is observed, and the mobility is well described by the equation

$$\mu \approx \frac{\epsilon_0\langle\Lambda_0\rangle_s}{6\pi\eta}$$

where ϵ_0 is the dielectric constant of the solvent, and η is the solvent viscosity. This breaks down, however, when ion relaxation is included and the mobility is less than predicted by the above equation. Whether or not ion relaxation is included, the mobility is found to be fairly insensitive to the charge distribution within the lysozyme model or the internal dielectric constant.

INTRODUCTION

Electrophoresis and related methods are extensively used in biochemistry, biophysics, and molecular biology. In fact, it has been estimated that over half of all papers in biochemistry utilize electrophoresis to some degree (Vesterberg, 1993). Despite this widespread use, electrophoresis of macroions remains poorly understood at the molecular level, which we attribute to two factors. First of all, it is experimentally difficult to obtain absolute mobilities, and the resulting paucity of mobility data has resulted in a lack of impetus for theortical development. Second, electrophoresis is an intrinsically complex phenomenon involving the polyion, ion atmosphere, solvent flow, and (if present) gel matrix and/or capillary surfaces, and this has made the problem a challenge for theorists. It can be expected that recent developments in both experimental technique as well as numerical methodology will both facilitate the acquisition of mobility data and aid in their interpretation. In the following two paragraphs, both experimental and theoretical issues are briefly reviewed.

The oldest and most straightforward technique of obtaining absolute mobilities involves Tiselius cells (Beychok and Warner, 1959), but the use of these was largely abandoned in the 1960s, when gel electrophoresis came into widespread use. Although it is possible to obtain absolute mobilities from gel electrophoresis measurements by constructing a Ferguson plot and extrapolating to zero gel concentration (Holmes and Stellwagen, 1991), this approach has not been widely used. Electrophoretic light scattering was developed in the 1970s (Ware and Flygare, 1971; Tagaki, 1993) and has been applied to a variety of proteins (Basak and Ladisch, 1995). NMR techniques such as ENMR and MOSY have also been developed to measure mobilities, but to date applications have dealt with relatively small ions (Morris and Johnson, 1993). Capillary electrophoresis (CE) is a promising new technique, but as in the case of NMR methods, application to the problem of absolute mobilities has, for the most part, been restricted to smaller polyions such as short polypeptides (Grossman et al., 1989). A major difficulty with the CE method has been the complicating feature of electroendoosmotic flow, which arises from the small diameter of the capillaries used and the fixed charges that are usually present on the capillary surfaces. Recently, however, this problem was overcome in the measurement of absolute mobilities of DNA by coating the capillaries with acrylamido polymers, which reduces the electroendoosmotic flow to zero (Stellwagen et al., 1997). Finally, electroacoustic methods developed by O'Brien and co-workers over the last 10 years should be mentioned (O'Brien et al., 1995). Although applications to date have been restricted to large particles such as colloids, measurements on proteins and other "smaller" polyions may be

Received for publication 27 January 1997 and in final form 14 April 1997.
Address reprint requests to Dr. Stuart A. Allison, Department of Chemistry, Georgia State University, University Plaza, Atlanta, GA 30303. Tel.: 404-651-1986; Fax: 404-651-3099; E-mail: chesaa@panther.gsu.edu.

0006-3495/97/07/133/08 $2.00

feasible. The electroacoustic measurements have the advantage over other techniques of measuring absolute dynamic mobilities, which provide information about ion atmosphere dynamics. In summary, there is a fairly wide variety of techniques available at this time that either can or have the potential to be used in obtaining absolute electrophoretic mobilities of polyions that are comparable in size to proteins and nucleic acids. These are the polyions of interest in this work.

There exists an extensive literature on the theory of electrophoresis, which is reviewed in the text by Dukhin and Shilov (1974) or in review articles such as that of Anderson (1989). A recent text by Schmitz (1993) provides a good overall view of current polyion theory and experiment. In the present work, we shall narrow the field substantially by focusing only on those theories of polyions that model them as a rigid structure of arbitrary shape and charge distribution that are not small compared to the thickness of the ion atmosphere. The ion atmosphere is approximately κ^{-1} in thickness, where κ is the Debye-Huckel screening parameter. For a 0.1 M NaCl solution, κ^{-1} is ~1 nm, which is roughly the size of a protein or "hydration radius" of DNA. Two years ago, boundary element (BE) procedures were developed and applied to calculate the mobility of rigid polyions such as proteins (Chae and Lenhoff; Allison and Tran, 1995). The application to lysozyme yielded only fair agreement between theory and experiment, and it was suggested that a Stern layer of fluid and ions moving with the protein as a rigid body could reconcile the two (Allison and Tran, 1995). There were, however, approximations made in these BE studies that can be removed. The first approximation was to treat the electrostatics at the level of the linear Poisson-Boltzmann equation. At high salt and/or electrostatic potential, this approximation breaks down. The second approximation was to neglect "ion relaxation," which refers to the perturbation of the ion atmosphere in the vicinity of the polyion in response to the imposed electric and/or flow field. From theories on spheres containing centrosymmetrical charge distributions, it has been known for a long time that ion relaxation has a significant effect on mobility when the polyion is highly charged or, more precisely, when the electrostatic potential at the polyion surface is large (Wiersema et al., 1966). Within the last year, the BE method has been generalized to account for both factors and applied to spherical model polyions (Allison, 1996). The solvent and mobile ion distributions are treated at the continuum level. Following a long-established protocol (Wiersema et al., 1966; O'Brien and White, 1978; Fixman and Jagannathan, 1983), the Poisson, Navier-Stokes, and ion transport equations are solved simultaneously. What the BE approach makes possible is the application of this protocol to polyions of arbitrary size and charge distribution, such as detailed models of lysozyme. The objective of the present work is to reconsider lysozyme, but to use a more realistic model that accounts more accurately for electrostatics and includes ion relaxation. As shown below, these considerations result in a substantial improvement in the model results relative to experiment.

Because the BE procedure, in both the absence (Allison and Tran, 1995) and presence (Allison, 1996) of ion relaxation, is described in detail elsewhere, only an outline of the methodology is presented here in the next four subsections. The methodology is then applied to lysozyme to assess the importance of ion relaxation and a more accurate treatment of electrostatics to the calculated mobilities.

OVERVIEW OF THE PROBLEM

An important quantity required in the mobility calculation is the external force per unit volume, $\mathbf{s}(\mathbf{r})$, exerted on the fluid at position $\mathbf{r}$. It shall be assumed that $\mathbf{s}$ arises from the various electrostatic interactions that are present. If Λ is the total electrostatic potential and ρ is the charge density that arises as a result of a local imbalance between co- and counterions, then

$$\underset{\sim}{s}(\underset{\sim}{r}) = -\rho(\underset{\sim}{r})\underset{\sim}{\nabla}\Lambda(\underset{\sim}{r}) \tag{1}$$

The ion atmosphere is treated as a continuum, and ρ and Λ are related through Poisson's equation,

$$\underset{\sim}{\nabla}\cdot(\epsilon(\underset{\sim}{r})\underset{\sim}{\nabla}\Lambda(\underset{\sim}{r})) = -4\pi\rho(\underset{\sim}{r}) \tag{2}$$

where ϵ is the local dielectric constant, assumed to be equal to ϵ_i inside the polyion and ϵ_o outside. Outside the polyion, ρ represents the charge density due to mobile salt ions:

$$\rho(\underset{\sim}{r}) = q\sum_{\alpha} z_\alpha n_\alpha(\underset{\sim}{r}) \tag{3}$$

where q is the protonic charge, α is an index over the mobile ion species present, z_α is the valence of ion α, and $n_\alpha(\mathbf{r})$ is the local concentration. Inside the polyion, ρ represents the discrete fixed charge distribution relevant to some model of interest. In the present case, this corresponds to the charged residues of lysozyme derived from the crystal structure. It is convenient to break the problem down into the potential of a stationary polyion in the absence of an external electric or flow field, Λ_0, and a perturbation potential, ψ, defined through

$$\Lambda = \Lambda_0 + \psi - \underset{\sim}{e}\cdot\underset{\sim}{r} \tag{4}$$

where $\mathbf{e}$ is an external electric field assumed to be constant (or zero) in this work. In general, the local ion densities cannot be determined by the potentials Λ_0 and ψ alone. Additional potentials, Φ_α, are also introduced (O'Brien and White, 1978), which represent the departure of n_α from its equilibrium value, $n_{\alpha 0}$:

$$n_\alpha(\underset{\sim}{r}) = n_{\alpha 0}(\underset{\sim}{r})e^{-\beta z_\alpha q(\psi(\mathbf{r})+\Phi_\alpha(\mathbf{r}))} \tag{5}$$

$$n_{\alpha 0}(\underset{\sim}{r}) = c_{\alpha 0}e^{-\beta z_\alpha q\Lambda_0(\mathbf{r})} \tag{6}$$

where $\beta = 1/k_BT$, and $c_{\alpha 0}$ is the ambient concentration of ion α. To obtain Φ_α, we must solve the ion transport equation, which can be cast in the form (Allison, 1996)

$$\nabla^2\Phi_\alpha(\underset{\sim}{r}) = f_\alpha(\underset{\sim}{r}) \tag{7}$$

$$f_\alpha(\underset{\sim}{r}) = \left[\frac{1}{D_\alpha}\underset{\sim}{v}(\underset{\sim}{r}) + \beta z_\alpha q(\underset{\sim}{\nabla}\Phi_\alpha(\underset{\sim}{r}) + \underset{\sim}{e})\right]\cdot\underset{\sim}{\nabla}\Lambda_0(\underset{\sim}{r}) \tag{8}$$

where D_α is the (scalar) diffusion constant of ion α, and $\mathbf{v}(\mathbf{r})$ is the local fluid velocity. To obtain Φ_α, it is clear that we also need $\mathbf{v}$. The fluid is treated as an incompressible continuum, and determination of the fluid velocity requires solution of the Navier-Stokes (NS) and solvent incompressibility equations,

$$\eta\nabla^2\underset{\sim}{v}(\underset{\sim}{r}) - \underset{\sim}{\nabla}p(\underset{\sim}{r}) = -\underset{\sim}{s}(\underset{\sim}{r}) \tag{9}$$

$$\underset{\sim}{\nabla}\cdot\underset{\sim}{v}(\underset{\sim}{r}) = 0 \tag{10}$$

where η is the solvent viscosity and p is the pressure. Equation 9, however, requires $\mathbf{s}(\mathbf{r})$, given by Eq. 1.

In the general case, it can be seen that determination of $\mathbf{s}$ requires simultaneous solution of the Poisson, Navier-Stokes, and ion transport equations and that these equations are inextricably coupled. In the absence of an electric or flow field, the problem is greatly simplified, and that case shall be considered next, followed by a discussion of the general solution.

CALCULATION OF Λ_0

Before the external field is turned on or before the polyion is translated with uniform velocity through the fluid, Λ reduces to Λ_0 (ψ goes to 0), and Eqs. 2, 3, and 6 reduce to the nonlinear Poisson-Boltzmann equation. This, in turn, is uncoupled from both the ion transport and NS equations. As described elsewhere (Allison, 1996), Λ_0 is solved by a nonlinear boundary element procedure similar to that of Zhou (1994). The polyion surface is subdivided into N triangular plates, and the assumption is made that the electrostatic potential and normal derivative are constant over a given plate. The space around the polyion is also divided into J (typically 50) shells or "onion skins" that conform closely to the polyion surface. Each shell, in turn, is divided into N volume elements. In the present work, the shells are not of uniform thickness. Shell thickness increases as the distance from the polyion surface increases. Because potentials enter as nonlinear source terms in the BE formulation, it is necessary to iterate the potentials until they converge (typically 5–10 iterations are sufficient).

INCLUSION OF ION RELAXATION

Equation 2 for ψ and Eq. 7 for Φ_α can be put into the general form

$$\nabla^2\phi(\underset{\sim}{r}) = f(\underset{\sim}{r}) \tag{11}$$

which is amenable to BE procedures similar to those applied to Λ_0. Because the source terms, f, contain unknown quantities that we are seeking to obtain, an iterative procedure is adopted in which previous extimates of ψ, Φ_α, (and $\mathbf{v}$) are used in $f(\mathbf{r})$ to obtain updated ψ and Φ_α. To begin with, ion relaxation is ignored ($n_\alpha = n_{\alpha 0}$), and ψ can be solved directly. Physically, $\psi - \mathbf{e}\cdot\mathbf{r}$ is the potential of the uncharged, (low) dielectric polyion placed in a constant external field, $\mathbf{e}$. In those cases in which the polyion is translated in the absence of an electric field, ψ is simply set to zero to begin with. From Eq. 5, we can also set our initial estimate of Φ_α equal to $-\psi$. Our initial estimate of $\mathbf{s}$ can be written

$$\underset{\sim}{s}_0(\underset{\sim}{r}) = -\rho_0(\underset{\sim}{r})\underset{\sim}{\nabla}(\Lambda_0(\underset{\sim}{r}) + \psi(\underset{\sim}{r}) - \underset{\sim}{e}\cdot\underset{\sim}{r}) \tag{12}$$

The Λ_0 term on the right side of Eq. 12 actually does not contribute to the net force on the polyion and can be ignored.

As in the case of the Poisson and ion transport equations, a BE method is employed in the solution of the NS equation. Given a rigid polyion translating with velocity $\mathbf{u}$ through an incompressible fluid at rest far from the polyion, we can write

$$\underset{\sim}{v}(\underset{\sim}{r}) = -\int_S \underset{\approx}{U}(\underset{\sim}{x},\underset{\sim}{r})\cdot\underset{\sim}{w}(\underset{\sim}{x})\mathrm{d}S_x - \int_V \underset{\approx}{U}(\underset{\sim}{x},\underset{\sim}{r})\cdot\underset{\sim}{s}(\underset{\sim}{x})\mathrm{d}V_x \tag{13}$$

where S represents the polyion surface, V is the volume external to the polyion, $\mathbf{s}$ is the external force on the fluid per unit volume (Eq. 1), $\mathbf{w}$ represents the hydrodynamic stress force per unit area on the polyion, and

$$\underset{\approx}{U}(\underset{\sim}{x},\underset{\sim}{r}) = -\frac{1}{8\pi\eta y}[\underset{\approx}{I} + \underset{\approx}{R}(\underset{\sim}{x},\underset{\sim}{r})] \tag{14}$$

$$(\underset{\approx}{R}(\underset{\sim}{x},\underset{\sim}{r}))_{ij} = \frac{y_i y_j}{y^2} \tag{15}$$

where $\mathbf{y} = \mathbf{r} - \mathbf{x}$, $y = |\mathbf{y}|$, and $\mathbf{I}$ is the 3×3 identity matrix. As discussed previously, the surface is discretized into N platelets and the volume into $M = J \times N$ volume elements. The assumption is made that $\mathbf{w}$ (or $\mathbf{s}$) is constant over a surface (or volume) element. It shall be assumed we have an estimate for $\mathbf{s}$ and that stick boundary conditions hold, which allows us to set $\mathbf{v}(\mathbf{r}) = \mathbf{u}$ for points on the polyion surface. At the polyion surface, the only unknowns are the $\mathbf{w}$'s, which can be computed by inverting a $3N \times 3N$ matrix.

Once the **w**'s are known, a discretized version of Eq. 13 can be used to compute **v**(**r**) at the M volume elements.

With initial estimates of ψ, Φ_α, and **v**, Eq. 11 is used to obtain better estimates of Φ_α and ψ, which then allows us to reestimate **s**. These can then be used in Eq. 13 to recompute **w** and **v**. The whole procedure is iterated until all quantities converge.

ELECTROPHORETIC MOBILITY

Consider a polyion translating with velocity **u** through a fluid that is at rest far from the polyion. The jth component of the total force is computed using a Teubner relation (Teubner, 1982):

$$z_j = \underset{\sim}{u} \cdot \underset{\sim}{z}_{h0}^{(j)} + \int_V [\underset{\sim}{v}^{(j)}(\underset{\sim}{x}) - \underset{\sim}{i}_j] \cdot \underset{\sim}{s}(\underset{\sim}{x}) dV_x \qquad (16)$$

where $z_{h0}^{(j)}$ is the hydrodynamic force on an identical but uncharged model polyion translating with unit velocity in direction j, $\mathbf{v}^{(j)}(\mathbf{x})$ is the fluid velocity at **x** that is due to the uncharged polyion (translating with unit velocity in direction j), $\mathbf{i}_j$ is a unit vector in direction j, and **s** is the external force per unit volume (Eq. 1). It is straightforward to calculate $\mathbf{v}^{(j)}$ by solving Eq. 13 subject to the boundary condition $\mathbf{v}^{(j)} = \mathbf{i}_j$ at the polyion surface and setting the volume integral to zero (because **s** equals 0 in this case). If we let $\mathbf{w}_0^{(j)}$ represent the corresponding hydrodynamic stress force per unit area on the uncharged polyion, we can also identify

$$\underset{\sim}{z}_{h0}^{(j)} = -\int_S \underset{\sim}{w}_0^{(j)}(\underset{\sim}{x}) dS_x \approx -\sum_{k=1}^{N} \underset{\sim}{w}_{0k}^{(j)} A_k \qquad (17)$$

where A_k is the area of platelet k.

Following O'Brien and White (1978), it is useful to consider two transport cases and then combine them by superposition to yield the mobility. In case 1, no external electric field, **e**, is present, but the polyion is translated with velocity **u** through an unbounded fluid that is at rest far from the polyion. The total force, **z**(1), can be written

$$\underset{\sim}{z}(1) = -\eta \underset{\approx}{K} \cdot \underset{\sim}{u} \qquad (18)$$

where **K** is a friction tensor. The components of **K** are readily obtained by translating the polyion along three orthogonal directions and computing **z**(1) for each. In case 2, the polyion is held stationary in a constant **e**-field, and the total force can be written

$$\underset{\sim}{z}(2) = \eta \underset{\approx}{Q} \cdot \underset{\sim}{e} \qquad (19)$$

As before, the components of **Q** can be determined by placing a stationary particle in constant external fields along three orthogonal directions. A total of six complete calculations (three of **z**(1) and three of **z**(2)) are required to completely determine **K** and **Q**. From this, an electrophoretic mobility tensor, **M**, is readily obtained:

$$\underset{\approx}{M} = \underset{\approx}{K}^{-1} \cdot \underset{\approx}{Q} \qquad (20)$$

where -1 denotes matrix inversion. Averaging over all orientations (which is valid, provided the external field is sufficiently weak not to significantly orient the polyion) gives the"low field" electrophoretic mobility

$$\mu = \frac{1}{3} \mathrm{Tr}(\underset{\approx}{M}) \qquad (21)$$

where Tr denotes trace. For the cases considered in this work, we have found that an accurate approximation for μ is given by

$$\mu \approx \frac{1}{3} \sum_{i=1}^{3} \frac{Q_{ii}}{K_{ii}} \qquad (22)$$

This corresponds to simply ignoring the off-diagonal terms in the **K** and **Q** matrices.

LYSOZYME STUDIES

The mobility studies on lysozyme reported here extend earlier work (Allison and Tran, 1995) by 1) treating Λ_0 at the level of the full Poisson Boltzmann (PB) rather than the linear PB equation, and 2) inclusion of ion relaxation. As in previous work, we have the coordinates of the nonhydrogen atoms of hen eggwhite lysozyme from the Brookhaven Protein Databank (6lyz.brk). Associated with each nonhydrogen atom is an atom "exclusion radius," σ, which is assumed to be constant for all atoms. Following a procedure described in detail previously (Allison and Tran, 1995), surfaces made up of 128 triangular platelets are constructed to approximate the actual surface of the protein. Basically, each vertex vector associated with each platelet (three vectors per platelet) is placed just far enough from the center of mass of the protein to ensure that no "exclusion sphere" lies beyond the vertex vector. For the $N = 128$ platelet structure, σ is set to 0.45 nm. This choice gives a translational diffusion constant of 10.6×10^{-7} cm^2/s at $T = 20$°C and $\eta = 1$ cp, in good agreement with the low pH diffusion constant measured experimentally (Dubin et al., 1971). We also need the charge state of lysozyme as a function of pH, and for this purpose we initially use the pK_a values of Kuramitsu and Hamaguchi (1980), which were also employed in our earlier study (Allison and Tran, 1995). With these pKa's, the total lysozyme charge (in protonic units) equals 16.79, 13.96, 12.80, 10.23, and 8.98 at pH = 2, 3, 4, 5, and 6, respectively. We shall focus on the mobilities at low pH as before, because of the reported dimerization of lysozyme above a pH of ~6 (Sophianopoulos and Van Holde, 1964). To compare computed mobilities with experimental values of Beychok and Warner (1959), the calculations are carried out

at 0°C and 0.15 M monovalent salt. For the D_α's, a hydrodynamic ion radius of 0.132 nm is employed, which corresponds fairly well to the ionic conductivities of Na^+ and Cl^- (Allison, 1996).

Once the model surface and charge states are defined, the next step is to calculate Λ_0 by the BE procedure. Shown in Fig. 1 is the average surface potential of lysozyme at pH 3 as a function of iteration number. Unless otherwise stated, $\epsilon_i = 2$, and $\epsilon_o = 78$ in this and subsequent cases, and charges are placed at the appropriate positions based on the crystal structure. The first potential represents the linearized PB value. As the figure illustrates, the surface potential converges in about eight iterations. As the pH increases and the magnitude of the net charge of the protein decreases, nonlinear terms contribute progressively less to the potential. Shown in Fig. 2 is the behavior of the mobility of lysozyme at pH 3 as a function of iteration number. The first entry represents the mobility (using Λ_0 computed at the level of the full PB equation) in the absence of ion relaxation. At the start of the second iteration, new Φ_α and ψ are computed using previous estimates of Φ_α, ψ, and **v** in the "source terms," $f(\mathbf{r})$, in Eq. 11. Then **s** (Eq. 1) and then **v** (Eq. 13) are updated, followed by calculation of net forces (Eq. 16) and, finally, mobilities (Eqs. 18–22). Successive iterations recompute Φ_α, ψ, and **v** by the same procedure. From Fig. 2 it is seen that the mobility converges in about eight iterations.

The pH-mobility studies for lysozyme are summarized in the next three figures. Ion relaxation is ignored in Fig. 3, but the effect of going from the use of linear PB to full PB Λ_0's is demonstrated. On going from linear to full, the mobilities decrease in the direction of experimental values. The effect is seen to be greatest at low pH, which is what one would expect, given the variation in net charge on lysozyme with pH. In Fig. 4, ion relaxation is included, and calculated mobilities are reduced more, which leads to still better agreement between calculated and experimental mobilities. Except at the lowest pH studied, agreement between the two

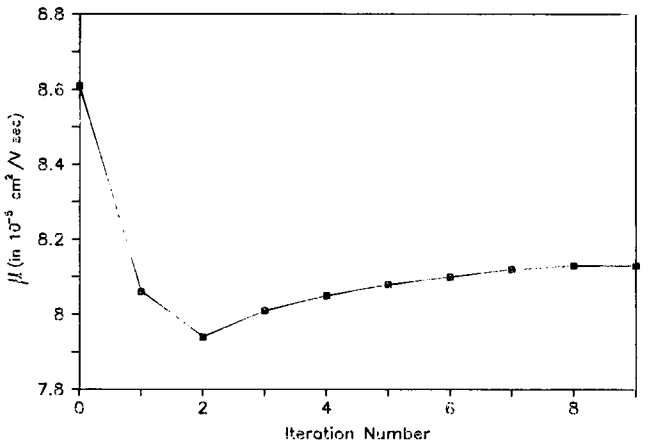

FIGURE 2 Mobility versus iteration number. Lysozyme at pH 3, N = 128, salt = 0.15 M, T = 0°C, $\epsilon_i = 2$, $\epsilon_o = 78$.

is seen to be quite good. Furthermore, inclusion of ion relaxation is seen to make the biggest difference when the protein is most highly charged. This is qualitatively consistent with the findings of Wiersema et al. (1966) on spheres.

The assumed charge distribution on lysozyme merits additional consideration. In the studies reported so far, the pK_a's summarized by Kuramitsu and Hamaguchi (1980) (KH values) have been used. Quite recently (Bartik et al., 1994), these have been reexamined, and the revised pKa's are called the B values. Although the two sets are, for the most part, in good agreement, some significant differences do exist. These include the following (residue, pKa(KH), pKa(B)): Asp^{18}, 2.0, 2.7; Asp^{48}, 4.3, 1.6; Asp^{52}, 3.4, 3.7; Asp^{66}, 1.6, 0.9; Asp^{119}, 2.5, 3.2; and C-term, 3.1, 2.8. At most pH's, the net charge on lysozyme is very similar for the two sets. At pH 2, for example, the net charge is estimated to be 16.79 (KH) or 16.42 (B), so using the B pKa values is expected to yield a slightly lower mobility. The greatest difference is around a pH of 4.0, where the net charge is estimated to be 12.80 (KH) and 11.41 (B). Be-

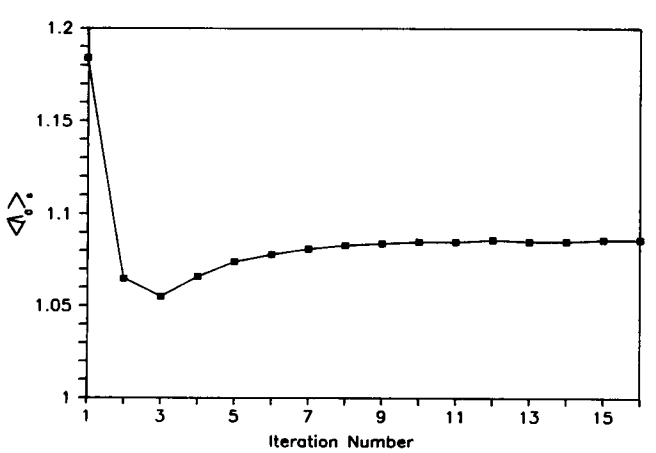

FIGURE 1 Average surface potential, Λ_0, versus iteration number. Lysozyme at pH 3, N = 128, salt = 0.15 M, $\epsilon_i = 2$, $\epsilon_o = 78$. The surface potential is in 10^{-4} erg/esu.

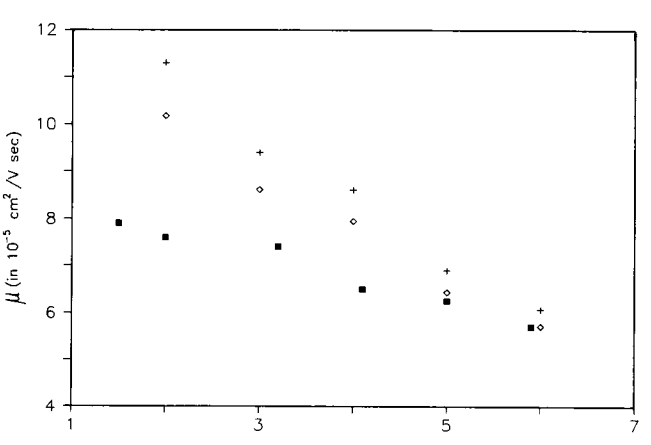

FIGURE 3 Mobility of lysozyme at 0.15 M versus pH. +, Linear PB, no relaxation; ◇, full PB, no relaxation; ■, experiment.

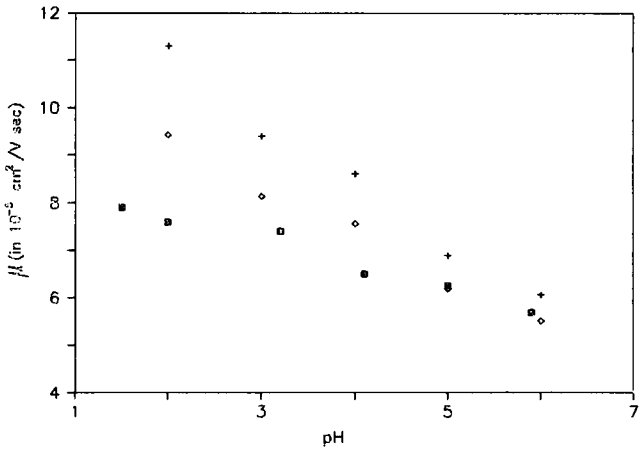

FIGURE 4 Mobility of lysozyme at 0.15 M versus pH. +, Linear PB, no relaxation; ◇, full PB, ion relaxation included; ■, experiment.

cause of these differences, we felt it important to also study the mobility versus pH using the B pKa's. The results (full PB, ion relaxation included) for both the KH (*diamonds*) and B (+) pKa sets are shown in Fig. 5, along with experimental mobilities. The calculated mobilities are about the same for the two cases, except at pH 4, where the mobility calculated using the B pKa's is significantly lower and much closer to the experimental value. Thus excellent agreement between calculated and experimental mobilities has been achieved in the pH range of 4–6, although differences remain at lower pH.

In colloid science, there is a long history of relating the average polyion surface potential (zeta-potential) to electrophoretic mobilities. Consider the relation

$$\mu = \left(\frac{\epsilon_0 k_B T}{4\pi\eta q}\right) yc \tag{23}$$

where

$$y = \frac{q\langle\Lambda_0\rangle_s}{k_B T} \tag{24}$$

is a reduced surface potential ($\langle\rangle_S$ denotes the average over the polyion surface) and c is a dimensionless quantity. In the theory of electrophoresis of thin double layers ($\kappa a \gg 1$), and a constant surface potential, c equals 1 (Smoluchowski, 1921). Fair and Anderson (1989) also considered thin double layers in the absence of ion relaxation, but with a surface potential that was not necessarily constant over the polyion surface. They derived an equation equivalent to Eq. 23 with $c = 1$. In the theory of electrophoresis of spheres (centrosymmetrical charge, radius = a) in the absence of ion relaxation (Henry, 1931), c is a complicated sigmoidal function of κa that approaches 2/3 in the limit $\kappa a \to 0$ ("thick" double layer limit) and approaches 1 in the limit of thin double layers. Similar behavior is also predicted for spheroidal particles (Yoon and Kim, 1989) in the absence of ion relaxation. For the size of polyions ($a \approx 2$ nm) and monovalent salt concentration (less than ~0.15 M) of interest in this work, c should be ~2/3 on the basis of the Henry, Yoon, and Kim models. On the basis of electrophoresis theory, we would expect a strong correlation between mobility and average surface potential. Plotted in Fig. 6 is the mobility versus y for the lysozyme model studies (salt = 0.15 M) described above, using the KH pK_a values. Note that both the linear and full PB mobility results in the absence of relaxation fall, to a good approximation, on a straight line. In fact, the c values extracted from these mobilities lie close to 2/3, which is expected from the Henry or Yoon and Kim model-thick double layer limit. When ion relaxation is included, mobilities fall below the no-relaxation curve, and the descrepancy increases with increased surface potential.

To explore the dependence of mobility on surface potential further, additional detailed models of lysozyme are

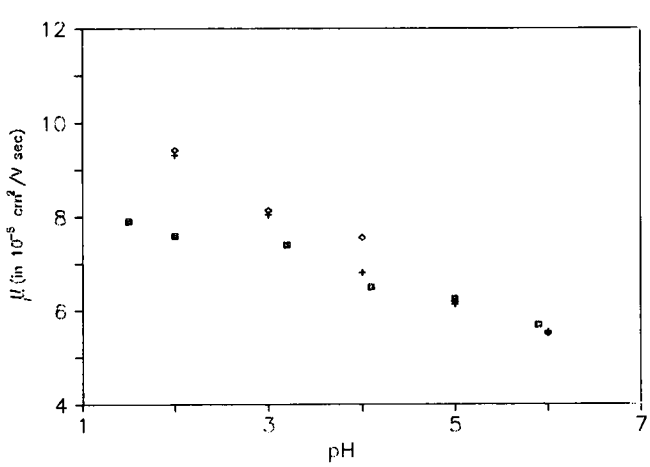

FIGURE 5 Mobility of lysozyme versus pH for Kuramitsu and Hamaguchi (KH) and Bartic (B) pK_a) data sets. In the calcuated mobilities, salt equals 0.15 M, electrostatics are calculated at the full PB level, and ion relaxation is included. +, B data set; ◇, KH data set; ■, experiment.

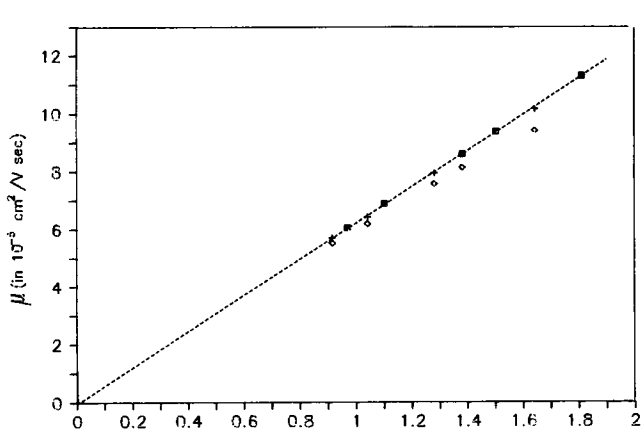

FIGURE 6 Mobility of lysozyme at 0.15 M versus reduced surface potential. ■, Linear PB, no relaxation; +, full PB, no relaxation; ◇, full PB, ion relaxation included.

considered in which 1) the actual charge distribution is replaced with a single net charge placed at the "center of mass"; 2) a range of monovalent salt concentrations is considered; 3) the internal dielectric constant, ϵ_i, is varied. The results of these studies are summarized in Table 1. Mobility results are given in terms of the reduced variable c defined by Eq. 23. Keep in mind that y is obtained from the calculation of Λ_0 that precedes the actual mobility calculation. A number of conclusions can be arrived at on the basis of these results. In the absence of relaxation, we can conclude that Eq. 23, with $c \approx 2/3$, gives a very good estimate of the electrophoretic mobility. On the basis of the Henry (1931) or Yoon and Kim (1989) models, it would be expected that c would actually be larger than 2/3 and approach 1 in the limit of high salt and/or large polyion size. From the salt = 0.6 M case in Table 1 (where $\kappa a = 5.4$ if one adopts a hydrodynamic radius (from the diffusion constant) of 2.025 nm for lysozyme), it does appear that c is indeed larger than 2/3 in the absence of relaxation, but not by very much. Furthermore, the details of the charge distribution and internal dielectric constant have little effect on the mobility whether or not ion relaxation is included or not. Ion relaxation becomes progressively important as y increases, which is consistent with the findings of Wiersema et al. (1966) on spherical polyions containing a centrosymmetrical charge distribution.

DISCUSSION

In earlier modeling studies (Allison and Tran, 1995) mobilities were calculated at the level of the linear PB equation in the absence of ion relaxation; the resulting mobilities are given by + in Figs. 3 and 4. At the time, it was proposed that a possible explanation for the discrepancy between calculated and experimental mobilities was the existence of a Stern layer of fluid and ions adjacent to the protein surface that moved with the protein as a rigid body. The present results, however, indicate that much of the discrepancy can be removed by treating the equilibrium electrostatics more accurately and by including ion relaxation.

As expected on the basis of electrophoresis theory, a strong correlation between mobility and average surface potential was shown to exist for lysozyme by considering a range of pH, salt, and charge distributions. In the absence of relaxation, a good estimate of the electrophoretic mobility is given by

$$\mu \approx \frac{\epsilon_0 \langle \Lambda_0 \rangle_s}{6\pi\eta} \tag{25}$$

The advantage of this approximate expression is that it only requires knowledge of the equilibrium potential on the polyion surface rather than a full mobility calculation. Equation 25 was arrived at by examining an array of models for lysozyme, and we anticipate that it is valid for small globular polyions in general, provided κa (a is the hydrodynamic radius of the polyion) is less than ~5. Ion relaxation reduces the mobility and the degree of reduction increases as the average surface potential increases. From Table 1, however, it appears that mobilities are not sensitive to charge distribution and the internal dielectric constant, even when ion relaxation is present.

In the present study, lysozyme was represented as a structure made up of 128 plates. In previous work on lysozyme in the absence of ion relaxation (Allison and Tran, 1995), as well as spherical polyions containing a variety of charge distributions where ion relaxation was included (Allison, 1996), it was found that mobilities obtained from the 128-platelet structures were comparable to those obtained from more detailed 512-platelet structures. Furthermore, the present mobility results on lysozyme are nearly independent of charge distribution. On this basis, we have decided to forego model calculations on 512-platelet models, because they are computationally time consuming and, in all probability, would not change the results or conclusions of this work. When ion relaxation is included and the electrostatics are treated at the level of the full PB equation, calculated and experimental mobilities are in very good agreement above a pH of ~3, where the absolute charge on lysozyme is relatively small. At lower pH, experimental mobilities fall below calculated values. This could be due to the adsorption of counterions to the polyion surface, resulting in the formation of "contact pairs." The contact pairs, in turn, would not be expected to contribute to the mobility (Fuoss, 1978). This point merits further study.

We expect that the conclusions arrived at in this work regarding lysozyme can be extended to other globular proteins, and future work will address that issue. A major difficulty in the modeling of proteins comes in assigning the appropriate charge to the residues. Fortunately, recent advances in the computation of ionization states of proteins (Antosiewicz et al., 1996) are expected to be of considerable value in that regard.

TABLE 1 Mobility c coefficients for various lysozyme models

pH	Salt (M)	Charge model*	ϵ_i	y	c(nr)#	c(r)
2	0.01	d	2	3.247	0.676	0.579
2	0.05	d	2	2.235	0.682	0.610
2	0.15	d	2	1.635	0.685	0.635
2	0.15	d	78	1.655	0.640	0.659
2	0.15	p	2	1.646	0.666	0.619
2	0.60	d	2	1.043	0.699	0.662
3	0.15	d	2	1.382	0.686	0.648
4	0.15	d	2	1.279	0.684	0.651
4	0.15	p	2	1.302	0.664	0.636
5	0.15	d	2	1.035	0.684	0.659
6	0.15	d	2	0.914	0.687	0.664
6	0.15	p	2	0.942	0.665	0.647

*d/p refers to detailed/point charge distributions.
#nr/r refers to no ion relaxation/ion relaxation included.

This work was supported in part by a grant from the National Institutes of Health to JAM. MP is a Howard Hughes Predoctoral Fellow.

REFERENCES

Allison, S. A. 1996. Modeling the electrophoresis of rigid polyions. Inclusion of ion relaxation. *Macromolecules.* 29:7391–7401.

Allison, S. A., and V. Tran. 1995. Modeling the electrophoresis of rigid polyions: application to lysozyme. *Biophys. J.* 68:2261–2270.

Anderson, J. L. 1989. Colloid transport by interfacial forces. *Annu. Rev. Fluid Mech.* 21:61–99.

Antosiewicz, J., J. M. Briggs, A. H. Elcock, M. K. Gilson, and J. A. McCammon. 1996. Computing ionization states of proteins with a detailed charge model. *J. Comp. Chem.* 17:1633–1644.

Bartik, K., C. Redfield, and C. M. Dobson. 1994. Measurement of the individual pKa values of acidic residues of hen and turkey lysozymes by two-dimensional ^{1}H NMR. *Biophys. J.* 66:1180–1184.

Basak, S. K., and M. R. Ladisch. 1995. Correlation of electrophoretic mobilities of proteins and peptides with their physicochemical properties. *Anal. Biochem.* 226:51–58.

Beychok, S., and R. C. Warner. 1959. Denaturation and electrophoretic behavior of lysozyme. *J. Am. Chem. Soc.* 81:1892–1897.

Chae, K. S., and A. M. Lenhoff. 1995. Computation of the electrophoretic mobility of proteins. *Biophys. J.* 68:1120–1127.

Dubin, S. B., N. A. Clark, and G. B. Benedek. 1971. Measurement of rotational diffusion coefficient of lysozyme by depolarized light scattering: configuration of lysozyme in solution. *J. Chem. Phys.* 54: 5158–5164.

Dukhin, S. S., and V. N. Shilov. 1974. Dielectric Phenomenon and the Double Layer in Disperse Systems and Polyelectrolytes. John Wiley and Sons, New York.

Fair, M. C., and J. L. Anderson. 1989. Electrophoresis of nonuniformly charged ellipsoidal particles. *J. Colloid Interface Sci.* 127:388–400.

Fixman, M., and S. Jagannathan. 1983. Spherical macroions in strong fields. *Macromolecules.* 16:685–699.

Fuoss, R. M. 1978. Conductance-concentration function for the paired ion model. *J. Phys. Chem.* 82:2427–2440.

Grossman, P. D., J. C. Colburn, and H. H. Lauer. 1989. A semiempirical model for the electrophoretic mobilities of peptides in free-solution capillary electrophoresis. *Anal. Biochem.* 179:28–33.

Henry, D. C. 1931. The cataphoresis of suspended particles. Part I. The equation of cataphoresis. *Proc. R. Soc. Lond. Ser. A.* 133:106–129.

Holmes, D. L., and N. C. Stellwagen. 1991. Estimation of polyacrylamide gel pore size from Ferguson plots of normal and anomolously migrating DNA fragments. *Electrophoresis.* 12:253–263.

Kuramitsu, S., and K. Hamaguchi. 1980. Analysis of the acid-base titration curve of hen lysozyme. *J. Biochem. (Tokyo).* 87:1215–1219.

Morris, K. F., and C. S. Johnson, Jr. 1993. Mobility-ordered 2D NMR spectroscopy for the analysis of ionic mixtures, *J. Magn. Res. Ser. A.* 100:67–73.

O'Brien, R. W., D. W. Cannon, and W. N. Rowlands. 1995. Electroacoustic determination of particle size and zeta potential. *J. Colloid Interface Sci.* 173:406–418.

O'Brien, R. W., and L. R. White. 1978. Electrophoretic mobility of a spherical colloidal particle. *J. Chem Soc. Faraday II.* 74:1607–1626.

Schmitz, K. S. 1993. Macroions in Solution and Colloidal Suspension. VCH Publishers, New York.

Smoluchowski, M. 1921. Electro-osmosis and current flow. *In* Handbuch der Electrizitat und des Magnetismus, Vol. 2. L. Graetz, editor. Barth, Leipzig, Germany.

Sophianopoulos, A. J., and K. E. Van Holde. 1964. Physical studies of muramidase (lysozyme). *J. Biol. Chem.* 239:2516–2524.

Stellwagen, N. C., C. Gelfi, and P. G. Righetti. 1997. The free solution mobility of DNA. *Biophys. J.* 72:A323.

Takagi, T. 1993. Electrophoretic light scattering. *Electrophoresis.* 14: 1255–1256.

Teubner, M. 1982. The motion of charged colloidal particles in electric fields. *J. Chem. Phys.* 76:5564–5573.

Vesterberg, O. 1993. A short history of electrophoretic methods. *Electrophoresis.* 14:1243–1249.

Ware, B. R., and W. H. Flygare. 1971. The simultaneous measurement of the electrophoretic mobility and diffusion coefficient in bovine serum albumin solutions by light scattering. *Chem. Phys. Lett.* 12:81–85.

Wiersema, P. H., A. L. Loeb, and J. Th. G. Overbeek. 1966. Calculation of the electrophoretic mobility of a spherical colloid particle. *J. Colloid Interface Sci.* 22:78–99.

Yoon, B. J., and S. Kim 1989. Electrophoresis of spheroidal particles. *J. Colloid Interface Sci.* 128:275–288.

Zhou, H.-X. 1994. Macromolecular electrostatic energy within the nonlinear Poisson Boltzmann equation. *J. Chem. Phys.* 100:3152–3162.