SHORT COMMUNICATIONS

Acta Cryst. (1997). D53, 456–457

Electrophoretic mobility and zeta potential of lysozyme crystals

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(Received 4 September 1996; accepted 2 January 1997)

Abstract

Using free-solution capillary electrophoresis, the electrophoretic mobility of μm-sized lysozyme crystals in their growth solution at 283 K, 1.5%(*w*/*v*) NaCl, and over a range of pH values between 3.59 and 5.70 has been measured. Under these conditions, the mobility is independent of crystal size, while the calculated zeta potential increases from +8 to +24 mV as the pH decreases. Since the pH dependence of the zeta potential mirrors the pH dependence of charge on the free molecule, as determined by acid titration, it is concluded that the charge on the crystal is a result of H^+ adsorption from solution.

1. Introduction

Many proteins have been crystallized from pH-buffered aqueous solutions of strong electrolytes (McPherson, 1982). A prime example is lysozyme, which readily crystallizes when in solution with any number of salts, of which the most common is NaCl (Guilloteau, Riès-Kautt & Ducruix, 1992). By titration, Roxby & Tanford (1971) have shown that lysozyme molecules are positively charged macro-ions at the pH values where crystallization is ordinarily carried out. The crystal nuclei, themselves, may also be charged, although the charge is probably not directly observable. On the other hand, when the crystals have developed edges a few μm in length, so that they can be seen with an optical microscope, any charge which they carry, can be determined by the time-of-flight method of free-solution capillary electrophoresis. Below, we report measurements of the electrophoretic mobility and zeta potential of tetragonal lysozyme crystals. On the basis of these measurements, we propose a hypothesis to explain the effects of pH and ionic strength on the rate of crystallization of lysozyme.

2. Experimental

All experiments were carried out at 283 K. Hen egg-white lysozyme, obtained from Sigma Pharmaceuticals, was used without further purification. Precipitation of tetragonal lysozyme crystals was carried out in aqueous 1.5%(*w*/*v*) NaCl in 0.05 *M* aqueous sodium acetate to which glacial acetic acid was added dropwise to establish the desired pH (Howard, Twigg, Meehan & Baird, 1988). Electrophoretic measurements were made using a Rank Brothers Mark I electrophoresis apparatus with Ag|AgCl reversible electrodes and a 1 mm inside diameter uncoated capillary. The electric field, E, down the axis of the capillary was calculated from measurements of the d.c. conductivity (James, 1979) carried out with a Radiometer (Copenhagen) CDM 83 conductivity meter. The viscosity of the centrifuged growth solution was determined with an Ostwald viscometer (Hardy, 1962). The mark I apparatus was calibrated by measuring the magnitude of the electrophoretic velocity, $v_p = uE$, of standardized red blood cells having a mobility, u which was accurately known.

Micrometre-sized crystals of lysozyme that failed to sediment upon standing for a few days were transferred along with their growth solution to the capillary. Using the microscope eyepiece, a crystal was located at the radius of zero electro-osmotic flow (James, 1979). At this position, the time, t, required for the crystal to traverse the distance, L, between two graticule marks within the eyepiece was recorded. The electrophoretic velocity was computed from the equation, $v_p = L/t$. A number of trials with the same crystal were used to determine an average value of v_p. Also by measuring the time, Δt, for a crystal to make a complete traverse of a single graticule mark, its dimension, d, in the direction of motion was calculated using $d = v_p \Delta t = L\Delta t/t$. The value of L was 26 μm, while t ranged between 10 and 20 s depending upon the particle mobility, and Δt ranged between 3 and 13 s depending upon the particle mobility and size. Our measured values of v_p and our reported values of u have a precision of ±5%.

The sodium chloride, sodium acetate and acetic acid in the growth solution combined to produce a background ionic strength of $I = 0.3\,M$. The thickness, κ^{-1}, of the Debye layer surrounding the crystal was calculated from the Debye–Hückel formula,

$$\kappa^{-1} = \left(\frac{\varepsilon\varepsilon_0 k_B T}{2000\, e^2 N_A}\right)^{1/2} I^{-1/2}, \qquad (1)$$

where e is the electron charge, k_B is Boltzmann's constant, N_A is Avogadro's number, ε_0 is the dielectric constant of free space, $T = 283$ K is the absolute temperature, $\varepsilon = 84.11$ is the relative dielectric constant of water at this temperature (Weast, 1967–1968), and I is the ionic strength in mol l^{-1} (Berry, Rice & Ross, 1980). Upon substitution of $I = 0.3\,M$ into (1), one finds the thickness, $\kappa^{-1} = 0.6$ nm. As the size of our particles was much greater than this thickness, the zeta potential (electrostatic potential of the particle with respect to the solution) could be calculated from the mobility using the Smoluchowski formula,

$$\zeta = \eta u/\varepsilon\varepsilon_0, \qquad (2)$$

where η is the viscosity of the solution (James, 1979).

3. Results and discussion

At all pH values between 3.59 and 5.70, where we observed tetragonal lysozyme crystals to grow, the electrophoretic mobility of the crystals was found to be positive and largely

independent of the radius, $\frac{1}{2}d$, as is shown in Fig. 1. The mobility of the crystal increased with decreasing pH, forming an s-shaped curve (Fig. 2). The s-shape is reminiscent of the pH dependence of the mobility of the free lysozyme macro-ion (Beychok & Warner, 1959) and also the pH dependence of the average charge of the macro-ion as determined by the acid–base titration curve (Roxby & Tanford, 1971). The connection with the titration curve indicates that both the macro-ions and the crystals have adsorbed hydrogen ions from solution. As summarized in Table 1, we converted the mobility to zeta-potential using (2) and our measured values of the viscosities of the growth solutions, which increased from 0.017 to 0.02 g cm^{-1} s^{-1} with decreasing pH. Like the mobilities, the zeta potentials in Table 1 increase with falling pH.

According to the Bronsted–Bjerrum theory of the primary salt effect, the rate of reaction between ions having charges of the same sign is accelerated by an inert electrolyte in the solution due to electrostatic Debye–Hückel screening of the charges (Amis, 1966). Let $(1)^{Z_1}$ be a lysozyme macro-ion in solution with a pH-dependent valence, Z_1, which is determined from the titration curve. Let $(n)^{Z_n}$ be a crystallite of charge, Z_n, formed by agglomeration of n lysozyme macro-ions. [If the crystal adjusts its charge as it grows by incorporation of Cl^-, as reported by Elgersma, Ataka & Katsura (1992), we should expect $0 < Z_n < nZ_1$.] In any case, the binary rate constant, k,

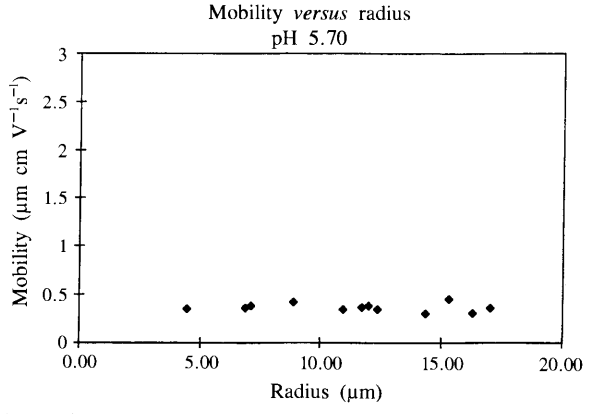

Fig. 1. Electrophoretic mobilities of lysozyme crystals as a function of radius. Solution conditions were temperature, 283 K, pH = 5.7 while the background ionic strength produced by 1.5%(*w/v*) NaCl, 0.05 *M* sodium acetate, and acetic acid was 0.3 *M*.

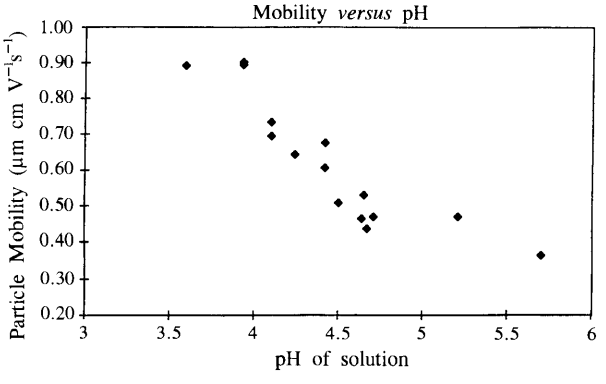

Fig. 2. Electrophoretic mobility of lysozyme crystals as a function of pH. Other solution conditions were the same as in Fig. 1.

Table 1. *Zeta potential as a function of pH*

The temperature was 283 K, while the background ionic strength contributed by 1.5%(*w/v*) NaCl, 0.05 *M* sodium acetate, and acetic acid was 0.3 *M*.

pH	ζ (mV)
3.59	24
3.93	20
4.10	17
4.25	15
4.64	12
5.21	12
5.70	8

for incorporation of $(1)^{Z_1}$ into $(n)^{Z_n}$ to form a crystallite of the next largest size is given by (Amis, 1966),

$$k = k_0 \exp[2Z_1 Z_n A(I)^{1/2}], \quad (3)$$

where $A = 0.92\,M^{-1/2}$ is the Debye–Hückel constant, and k_0 is the value of k when the ionic strength, $I = 0$. As $Z_1 Z_n > 0$, the argument of the exponential is positive, and k increases with increasing values of I. According to (3), substances which contribute to I serve as 'crystallizing agents' in the sense that they speed up the rate of agglomerization.

We would like to thank Dr J. M. Van Alstine of our department for the loan of his Rank Brothers electrophoresis apparatus and Dr R. J. Knox of Emerald Diagnostics, Inc., Eugene, OR, USA, for providing us with red blood cells of known electrophoretic mobility. We also thank Dr G. Bunich of the Oak Ridge National Laboratory, Oak Ridge, TN, for introducing us to the Wal Mart product, Rain-X, which prevents lysozyme crystals from sticking to the walls of glassware. This research was supported by grant 1R15 GM51018 from the National Institute of General Medical Sciences, National Institutes of Health, and by the Naval Research Laboratory through grant N00014-94-1-G016 from the Office of Naval Research.

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