free f, as well as the f originally bound in the f–P complex, would appear in the clot. The definition of α in eq. 11 is thus equivalent to that of eq. 4 and independent of the equilibrium constant of reaction 10.

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Effect of Binding of Ions and other Small Molecules on Protein Structure. IV. Two Electrophoretically Distinguishable Types of Interaction of Bovine Serum Albumin with Acidic Media[1]

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Bovine serum albumin undergoes two electrophoretically distinguishable types of interaction with acidic media. The first type of interaction is observed only in acetate-containing media at relatively high protein concentration, and is interpretable in terms of reversible complexing of the protein with undissociated buffer acid. The other type of interaction is best studied at low protein concentration, although with proper choice of electrolyte composition it can also be observed at high concentration. This type of interaction has been interpreted by Aoki and Foster in terms of a *p*H dependent protein transformation.

Introduction

A new effect of acetate buffer (NaAc–HAc) on the electrophoretic patterns of bovine serum albumin (BSA) and other proteins in acidic media has been described previously.[2–4] The patterns of 1% BSA at *p*H 4.7–4.0 show multiple moving boundaries, whose proportions and velocities depend upon the concentration of NaAc–HAc in the supporting medium and are very non-enantiographic even at ionic strengths as high as 0.3. These observations were interpreted in terms of reversible complexing of the protein with undissociated buffer acid.

Aoki and Foster[5–9] also have reported a new electrophoretic behavior of BSA. Their patterns of 0.2% BSA in NaCl–HCl solutions of ionic strength as low as 0.02, are fairly enantiographic and show two moving boundaries whose proportions depend upon *p*H. These observations have been interpreted in terms of a reversible, *p*H dependent protein transformation. These workers[6] also found that the proportions of the two boundaries are shifted by substitution of NaAc–HAc for NaCl–HCl. Unfortunately, this observation has led to some confusion since it would seem to suggest that the effect of NaAc–HAc on the patterns of BSA at high protein concentration is simply an effect of acetate on the same transformation postulated by Aoki and Foster to explain the electrophoretic behavior at low protein concentration. That this is not the case is demonstrated by the present experiments, which show that BSA actually undergoes two distinct processes in acid solution, one of which is observed only in NaAc–HAc at high protein concentration and has been described by us previously.[2–4] The other process is the one studied by Aoki and Foster[5–9] at low protein concentration.

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(2) R. A. Phelps and J. R. Cann, THIS JOURNAL, **78**, 3539 (1956).
(3) J. R. Cann and R. A. Phelps, *ibid.*, **79**, 4672 (1957).
(4) R. A. Phelps and J. R. Cann, *ibid.*, **79**, 4677 (1957).
(5) K. Aoki and J. F. Foster, *ibid.*, 3538 (1956).
(6) K. Aoki and J. F. Foster, *ibid.*, 3385 (1957).
(7) K. Aoki and J. F. Foster, *ibid.*, 3393 (1957).
(8) J. F. Foster and K. Aoki, *J. Phys. Chem.*, **61**, 1369 (1957).
(9) J. F. Foster and K. Aoki, THIS JOURNAL, **80**, 1117 (1958).

Experimental

Electrophoretic analyses were carried out in the usual manner, using the Perkin–Elmer Tiselius apparatus, fitted with a current-regulating power supply and a potentiometer to measure the voltage drop across a standard resistance placed in series with the cell. Glass hypodermic needles were used to fill the electrophoresis cell in order to avoid contaminating the solution with the corrosion products of metal needles. Boundary velocities (10^5 x.v.) are shown above or beside the corresponding peaks in the electrophoretic patterns presented in Figs. 1 and 2.

The bovine serum albumin was Armour crystallized bovine plasma albumin.

Results and Discussion

Two kinds of electrophoretic experiments have been carried out on BSA. The results of the first kind of experiment are presented in Fig. 1. Electrophoretic analyses were carried out at different protein concentrations in an acetate–chloride buffer, ionic strength 0.02 and *p*H 4.0. (Essentially the same results were obtained in 0.01 and 0.02 *M* acetate buffers.) At 1% protein the rising pattern shows two moving boundaries: a diffuse, slow moving boundary and a sharp fast one which is actually composed of two poorly resolved peaks. As the protein concentration is decreased, the proportion of the slow boundary decreases until it disappears (or nearly so) at 0.2% protein. At this concentration the patterns are essentially the same as those described by Aoki and Foster, and the two rising boundaries are closely related to the two peaks constituting the fast boundary at 1% protein.

In the second kind of experiment, all electrophoretic analyses were carried out on 1% BSA but the composition of the supporting medium was varied. The results of this experiment are shown in Fig. 2. The patterns obtained in 0.01*M* NaCl–HCl are very similar to those of Aoki and Foster

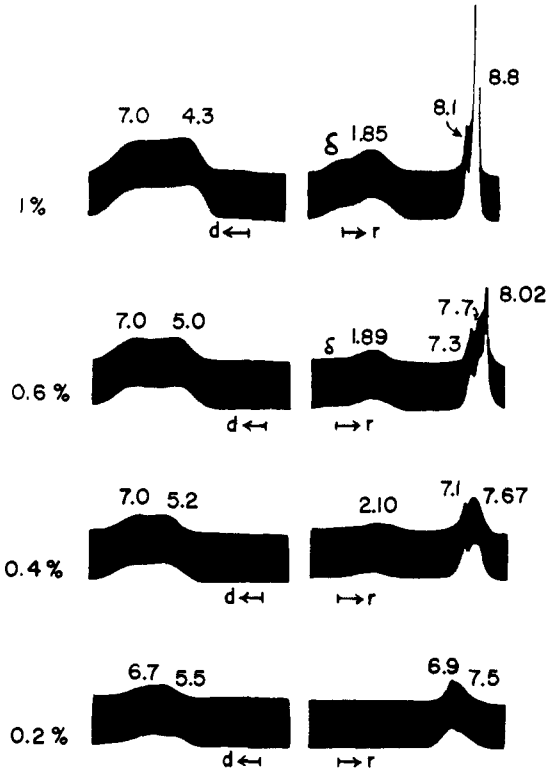

Fig. 1.—Electrophoretic patterns of BSA at various protein concentrations in 0.01 *M* NaAc–0.05 *M* HAc–0.01 *M* NaCl, *p*H 4.0.

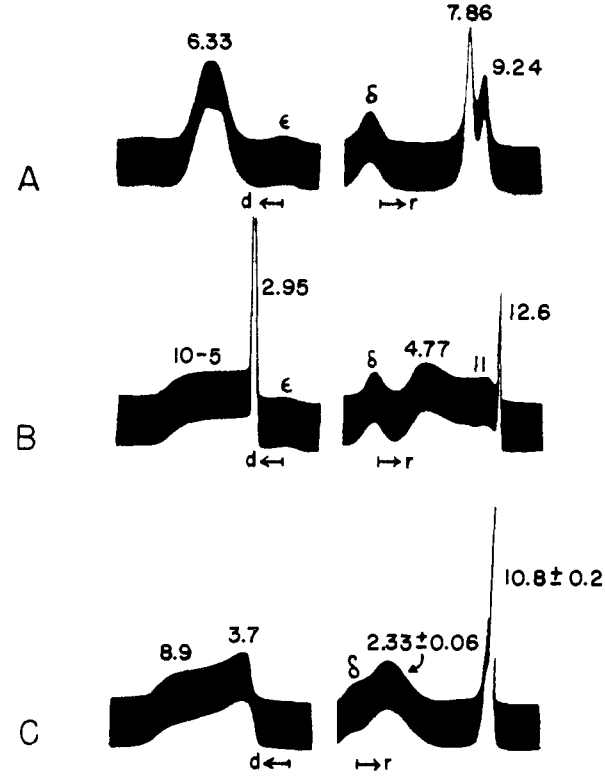

Fig. 2.—Electrophoretic patterns of 1% BSA in various supporting media at *p*H 4.0: A, 0.01 *M* NaCl–HCl; B. 0.002 *M* NaAc–0.01 *M* HAc–0.008 *M* NaCl; C, 0.01 *M* NaAc–0.05 *M* HAc.

for 0.2% protein except for the very poor resolution of the descending boundaries. Substitution of NaAc–HAc for NaCl–HCl at constant ionic strength and *p*H results in striking and progressive changes in the electrophoretic patterns. At low concentration of NaAc–HAc the descending pattern shows a large and very sharp, slow moving boundary and a diffuse, more rapidly moving one; whereas the rising pattern shows two or three diffuse and poorly resolved peaks and a small, faster moving, sharp peak. Increasing the acetate concentration results in an increase in the area under the faster moving boundaries at the expense of slower moving ones. Thus, at the highest concentration of NaAc–HAc, the descending pattern now shows two diffuse and poorly resolved boundaries, while the rising pattern shows a large and very sharp, rapidly moving boundary and a diffuse, slower-moving one. In addition to these changes in electrophoretic behavior, the boundary velocities vary in a rather complex fashion with the concentration of NaAc–HAc. This experiment shows that the extremely non-enantiographic nature of the patterns in acetate-containing media is a reflection of interaction between the protein and NaAc–HAc and is not due to the high protein concentration *per se*.[10]

(10) The possibility that the observed effect is actually due to decreasing chloride concentration already has been eliminated.[8]

These experiments demonstrate that BSA undergoes two electrophoretically distinguishable types of interactions with acidic media. The first type of interaction is observed only in acetate-containing media at relatively high protein concentration and has been described in previous papers of this series.[2-4] The dependence of the electrophoretic patterns of 1% BSA upon the concentration of NaAc–HAc in the supporting medium has been interpreted in terms of reversible complexing of the protein with undissociated buffer acid.

The second type of interaction is the one studied by Aoki and Foster[5-9] at low protein concentration, although we find that with proper choice of electrolyte composition it can also be observed at high protein concentration. Thus, the two rising boundaries of Aoki and Foster are closely related to the peaks which constitute the faster rising boundary at high protein concentration. This type of interaction has been interpreted by Aoki and Foster in terms of a reversible, *p*H dependent transformation of BSA between two states in which the protein molecule has different electrophoretic mobilities. However, the situation may be more complex, as evidenced by the complexity of the electrophoretic patterns shown by 0.6% BSA, Fig. 1. At this protein concentration, the fast-rising boundary is resolved into three rather than two peaks.

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