AN ELECTROPHORETIC STUDY OF MIXTURES OF OVALBUMIN AND YEAST NUCLEIC ACID

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INTRODUCTION

In an ideal electrophoresis of a solution of two components the volumes swept through by the rising and descending boundaries due to each component are identical and are proportional to the mobilities of the separate components. Moreover, the area under each “peak” in the electrophoretic pattern is proportional to the stoichiometric concentration of that component in the mixture to which it is due. In this ideal or limiting case the patterns for the two sides of the channel of the Tiselius electrophoresis cell are mirror images of each other.

The two patterns are, however, never exact mirror images. The authors have already discussed¹ the asymmetries, including the δ and ε effects, that are observed in the electrophoretic patterns of a single component and deviations of this type are, of course, to be expected in the electrolysis of mixtures. In the case, however, of some mixtures asymmetries were observed² which appeared to be due to interaction between the constituents. This led to the research, to be described below, in which the effect of interaction of components on electrophoretic patterns has been investigated further, using mixtures of ovalbumin and yeast nucleic acid. It is also the purpose of this paper to indicate a method for the electrophoretic analysis of mixtures in which certain types of interaction occur, and to discuss the manner in which the asymmetries in the electrophoretic patterns arise.

EXPERIMENTAL

The ovalbumin used in this research was prepared by the method of La Rosa³ and was recrystallized three times. The nucleic acid was a sample prepared under the direction of Dr. P. A. Levene of these Laboratories. This material was electrophoretically homogeneous, i.e. gave a single sharp peak,

³ La Rosa, W., Chemist-Analyst, 1927, 16, 3.
other than the δ and ε effects, in the electrophoretic pattern. It was observed, however, that some of the acid was lost on dialysis in cellophane tubing. Hence it was necessary to dialyze for a definite interval and correct for the material lost.

The patterns of Fig. 1 were obtained in the electrophoresis of a mixture of 1.15 per cent ovalbumin, P, and 0.50 per cent nucleic acid, N, in a 0.1 N sodium acetate buffer at pH 5.34. In this buffer both components carry appreciable negative charges, the mobilities having the values \( u_P = -2.8 \times 10^{-6} \) and \( u_N = -13.1 \times 10^{-3} \). Under these conditions a pattern for the mixture is essentially the sum of the patterns for the two components which were obtained separately.

The patterns of Fig. 2 were obtained with a similar mixture, 1.15 per cent P and 0.67 per cent N, but in a 0.1 N sodium acetate buffer at pH 4.63. In this solvent the protein still has a small negative mobility, \( -0.2 \times 10^{-8} \), but the patterns exhibit asymmetries that cannot be explained in terms of the δ and ε effects alone. Thus the displacements of the boundaries \( \beta \) and \( \gamma \), Fig. 2, are proportional to the normal mobilities \( u_P \) and \( u_N \), respectively, but the displacement of the boundary \( \beta \) corresponds to a mobility some eight times greater than the mobility of ovalbumin at this pH, while the displacement of the boundary \( \delta \) corresponds to a mobility appreciably less than that of nucleic acid. Moreover, the area under the boundary at \( \gamma \) corresponds, after correction for the dilution due to the δ effect, to only 0.56 per cent of nucleic acid whereas the actual concentration of that component was 0.67 per cent. Also the area under the boundary at \( \beta \) would correspond to 0.93 per cent if this were due entirely to nucleic acid. The results of this experiment are, as will be shown below, consistent with the assumption that the boundaries \( \gamma \) and \( \beta \) are due to nucleic acid and ovalbumin, respectively, moving with their normal mobilities, but whose concentrations have been modified by interaction. The boundaries \( \beta \) and \( \delta \), on the other hand, be shown to arise from the complexes due to combination of the components. It will be shown that the displacement of these boundaries involves the equilibrium between the components and the complex in addition to the motion of these substances in the existing electric field.

Although the patterns are not reproduced in this paper, experiments have been performed in which each of the four variables, i.e. pH, ionic strength,
protein concentration, and nucleic acid concentration, have been altered systematically. The results, some of which are given in Table I, indicate that

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**Fig. 1**

Electrophoretic patterns of a mixture of ovalbumin, 1.15 per cent, and yeast nucleic acid, 0.50 per cent, in a 0.1 N sodium acetate buffer at pH 5.34. The patterns were obtained after electrophoresis for 5000 seconds at 7.22 volts per cm.

**Fig. 2**

Electrophoretic patterns of a mixture of ovalbumin, 1.15 per cent, and yeast nucleic acid, 0.67 per cent, in a 0.1 N sodium acetate buffer at pH 4.63. The patterns were obtained after electrophoresis for 7000 seconds at 6.97 volts per cm.

Pattern asymmetries of the type described above are enhanced by decreasing the ionic strength or by increasing the concentrations of the protein or the nucleic acid or both. The patterns of Figs. 1 and 2 illustrate the important
role that pH plays in such experiments. Experiments at pH values below 4.6, the isoelectric point of ovalbumin, are complicated by the partial precipitation of the components. For electrophoretic study, removal of the precipitate that forms on dialysis of the mixture is not sufficient since additional precipitation frequently occurs, during an experiment, that interferes with observation of the boundaries. Thus in an experiment at pH 4.45, with conditions otherwise similar to those represented by Fig. 2, only a slight precipitate formed on dialysis and this was removed before electrolysis but during the latter procedure an optically opaque stratum of precipitate formed in the channel between the δ and the β boundaries on the anode side. This precipitation was probably due to the decreased ionic strength in this region of the cell arising from the δ effect and suggests that the solubility of the ovalbumin-nucleic acid complex is very sensitive to the ionic strength as well as to the pH.

Electrophoretic Analysis of Ovalbumin-Nucleic Acid Mixtures

The distribution of the components in the cell before and after electrophoresis is shown in Figs. 3 and 4. The boundaries between the buffer solution and solution (in the buffer) of ovalbumin at an initial or total concentration $p_i$ and nucleic acid at a total concentration $n_i$, were present in the planes $a$ and $g$ of Fig. 3 at the time the potential was applied. After passage of a current the boundaries in the anode side of the channel (Fig. 4) had swept through the volumes $V_a$ and $V_g$, while on the cathode sides the volumes were $V_b$ and $V_c$.

The material recovered from the channel between the boundaries at β and γ was found by direct experiment to be pure nucleic acid at a "separated" concentration $n$, whereas that between the boundaries at $b$ and $c$ was pure ovalbumin at a corresponding separated concentration $p$. Since the passage of an electric current does not produce changes of composition in the body of a

The precipitate formed in a 15 ml. sample of a 1.15 per cent P-0.58 per cent N mixture in a 0.1 N sodium acetate buffer at pH 4.34 was separated, dissolved in 15 ml. of a 0.1 μ sodium phosphate buffer at pH 6.80, dialyzed against this buffer, and analyzed electrophoretically. The pattern indicated no interaction at this pH and corresponded to a mixture of 0.40 per cent P and 0.13 per cent N. Moreover the ovalbumin thus separated electrophoretically from the nucleic acid was still native and showed the same relative amounts of the $A_1$ and $A_2$ modifications (cf. footnote 2) as in the original stock solution of the protein. It thus appears that precipitation under the conditions outlined here does not denature the protein and that the complex with nucleic acid is reversibly dissociated at pH values sufficiently above the isoelectric point of the ovalbumin.

Our observations concerning the influence of pH and ionic strength upon the solubility of the ovalbumin-yeast nucleic acid complex are in qualitative accord with those of Hammarsten and Hammarsten (Hammarsten, E., and Hammarsten, G., Acta med. Scand., 1928, 68, 199) on the complex formed by the related material, thymus nucleic acid, and ovalbumin.
homogeneous solution the protein solution in the bottom section of the channel, and the buffer solution in the two sides of the top section, remain unchanged. Moreover, ovalbumin and nucleic acid neither enter nor leave the cell. Consequently if the pattern for one side of the channel indicates a loss by electrophoretic migration of one of these components the pattern for the other side should indicate a corresponding gain of that component.

Referring to Fig. 4, the quantity of protein initially present in the volume $V_s$ was $V_s \phi_t$, while that present in the same volume after electrolysis was $(V_s - V_b) \phi_t$. Thus the loss of this component from the cathode side was

$V_o \phi_t - (V_s - V_b) \phi_t$ and this should equal the gain on the anode side, namely, $V_o \phi_t \rho_P$ in which $\rho_P$ is the dilution factor of the protein at the $\delta$ boundary. Therefore,

$$V_o \phi_t - (V_s - V_b) \phi_t = V_o \phi_t \rho_P \tag{1}$$

and similarly for the nucleic acid,

$$V_o n_t = V_o n_t \rho_N + (V_s - V_b) n_t \tag{2}$$

The buffer electrolytes, on the other hand, move through the cell but the quantity of these materials present at any instant remains constant, except for negligibly small effects due to volume changes accompanying electrophoretic separation of the components if the partial volumes of the latter are not additive.
in which $\rho_N$ is the corresponding dilution factor for the nucleic acid. All of the terms in equation 1, for example, can be obtained from the electrophoretic patterns of the rising and descending boundaries, with the exception of $\rho_i$, if the specific refractive increment of the protein is known. Thus from the two patterns the total concentration of protein, $p_t$, may be computed whether interaction takes place or not. This is obviously also true of equation 2, and $n_i$. Since the composition of the initial solution, and therefore $p_i$ and $n_i$, were known a comparison of the observed and computed concentrations affords a test of our interpretation of the phenomena occurring during electrophoresis.

In making computations with the aid of equations 1 and 2 it will be assumed that the dilution factors are given by the relation

$$\rho_p = \rho_N = \frac{(A_i - A_k)}{A_i}$$

in which $A_i$ is the total area of the electrophoretic pattern and $A_k$ that due to the $\delta$ boundary. The assumption that $\rho_p = \rho_N$ is in accord with the theory of Henry and Brittain who showed, for a somewhat simpler system, that at the $\delta$ boundary "the advancing column will hold its constituent ions in the same relative proportion as in the original sol." The additional assumption contained in equation 3; namely, that $\rho = \frac{(A_i - A_k)}{A_t}$, represents an approximation based upon the observation that the gradients of buffer salts in the $\delta$ boundary, being similar to those in the $\epsilon$ boundary, are small in comparison with the gradients of $P$ and $N$.

Equation 1 may be rearranged to give

$$p_i = \frac{V_c - V_{\beta}}{V_c - V_{\beta p}} \rho_i = \frac{V_c - V_{\beta}}{V_c - V_{\beta p}} k_p A_b$$

in which $A_b$ is the area under the boundary at $b$ and $k_p$ is a factor converting this area into protein concentration. This factor, whose value is 0.003776, depends only upon constants of the apparatus and the specific refractive increment of ovalbumin, taken as 0.00184 for the Hg blue and violet lines used in the present research. Similarly, equation 2 may be rearranged to

$$n_i = \frac{V_{\gamma} - V_{\beta}}{V_c - V_{\beta p}} n_i = \frac{V_{\gamma} - V_{\beta}}{V_c - V_{\beta p}} k_N A_{\gamma}$$

in which $k_N = 0.005185$, the specific refractive increment for nucleic acid being taken as 0.0013.


10 This value is taken from the work of Seibert and Watson (Seibert, F. B., and Watson, D. W., *J. Biol. Chem.*, 1941, 140, 55). It may be noted in this connection that the conclusions of our paper are actually independent of the values of the specific refractive increments since the concentrations of our stock solutions of both ovalbumin and nucleic acid were determined refractometrically.
The data necessary for computations with the aid of the foregoing relations, as obtained in five experiments in which the relative proportions of ovalbumin and nucleic acid were varied, are given in Table I. The data obtained from the patterns of Fig. 2 are given in the fourth column of this table. The other patterns were qualitatively similar. The areas (lines 1 to 5) under the peaks of these patterns are in arbitrary planimeter units whereas the displacement volumes (lines 6 to 9) are in milliliters per second per unit potential gradient. Line 10 of Table I contains the concentration of ovalbumin used in each experiment whereas line 11 contains the value of the concentration of albumin computed with the aid of equation 1'. Lines 12 and 13 contain the corresponding values for nucleic acid. The average difference between the observed and computed values is 2.4 per cent for the ovalbumin and 3.5 per cent for the nucleic acid. It is of interest that if interaction were neglected and the area $A_\theta$ interpreted as due entirely to ovalbumin the average difference between the observed and computed values of $p_\theta$ would be 6.9 per cent whereas if the area $A_\gamma$ were interpreted as due entirely to nucleic acid the average difference between the observed and computed values of $n_\gamma$ would be 45 per cent.

The Interaction between Ovalbumin and Yeast Nucleic Acid

In considering the probable nature of the interaction between ovalbumin and nucleic acid it will be assumed that they combine reversibly to form a complex $X = PN_\gamma$

$$P + vN \xrightleftharpoons[k_2]{k_1} PN_\gamma$$

(4)
in which the velocity constant for the forward reaction is \( k_1 \) and for the reverse reaction \( k_2 \), and the equilibrium constant is \( K = \frac{k_2}{k_1} \). The available evidence indicates that the complex has a mobility, \( u_x \), intermediate between \( u_p \) and \( u_N \). For the purpose of this discussion no restriction is placed on \( v \). It doubtless varies with both \( pH \) and ionic strength and may vary, as in the precipitin reaction,\(^{11}\) with the concentrations of \( P \) and \( N \). In the electrophoresis of such a mixture the equilibrium shown in equation 4 is not disturbed in the body of the solution by the migration of the constituents \( P \), \( N \), and \( X \). At the boundaries, however, the tendency of the components to separate, due to their mobility differences, is accompanied by a disturbance of the equilibrium. The extent to which the equilibrium will shift to compensate for the altered conditions produced by electrophoretic separation depends upon the magnitudes of the velocity constants in comparison with the rate of separation. The following cases may be distinguished.

1. If \( k_1 \) is small and \( k_2 \) large, \( K \) will also be large. Under these conditions the complex is essentially completely dissociated and the mixture will behave as a normal mixture of \( P \) and \( N \).

2. If \( k_1 \) is large and \( k_2 \) small then \( K \) will be small. This system behaves like a mixture of the complex and either \( P \) or \( N \), depending upon which is in excess. If neither is in excess a single boundary, due to \( X \) alone, will be present in each side of the channel.

3. If \( k_1 \) and \( k_2 \) are both small and of similar magnitudes then \( K \) will be near unity. In this case finite concentrations of \( P \), \( N \), and \( X \) will exist at equilibrium. The mixture will behave like a normal mixture of the three components since the adjustment of the equilibrium is slow in comparison with the rate of electrophoretic separation.

4. If the rate of adjustment of the equilibrium is comparable with the rate of

\(^{11}\) See, for instance, Kendall, F. E., Ann. N. Y. Acad. Sc., in press.

\(^{12}\) From the description by Stenhagen and Teorell (Stenhagen, E., and Teorell, T., Tr. Faraday Soc., 1939, 35, 743) of their electrophoresis experiments on mixtures of serum albumin and thymus nucleic acid it appears possible that this system corresponds to case 2. Thus only two boundaries were present in each side of the channel and the patterns appeared to be symmetrical. Their analyses showed both nucleic acid and protein to be present in the region between the two boundaries on the anode side but essentially pure protein in the corresponding region on the cathode side, thus indicating protein to be present in excess.

Seibert (Seibert, F. B., J. Biol. Chem., 1940, 133, 593) has observed somewhat similar phenomena in her electrophoretic studies of the naturally occurring mixture of protein and nucleic acid from the tubercle bacillus. It is of considerable interest that her separation of these components, by salt precipitation, was much more effective at alkaline reactions, where both components are negatively charged, than at acid reactions.
electrophoretic separation, the behavior will be difficult to predict although
one would expect the pattern to depend upon the rate of separation of the
constituents.

5. If $k_1$ and $k_2$ are both large and of the same order of magnitude the equilib-
rium is adjusted as rapidly as required by the electrophoretic separation of the
components. Consequently only two boundaries, aside from the $\delta$ and $\epsilon$
effects, appear in each side of the channel but the patterns are quite different
from those of a normal mixture of two components. $^{13}$ The patterns shown in
Fig. 2 and those for which data are given in Table I appear to be compatible
with the conditions postulated in this last case. It is of interest to visualize
how these patterns may arise.

The initial conditions in the electrophoresis cell may be represented by Fig. 3
in which the concentrations of combined, free, and total protein in the mixture
are $p_c$, $p_f$, and $p_t$, respectively, and the corresponding concentrations of nucleic
acid are $n_c$, $n_f$, and $n_t$. The conditions in the cell after electrophoresis are
indicated diagrammatically in Fig. 4 in which, as has been stated, pure $N$
is present at a concentration $n_c$ in the region between the two leading boundaries
on the anode side and pure $P$ at a concentration $p_t$ in the region between the
two boundaries on the cathode side. The sequence of events leading from the
initial to the final state is as follows.

In the anode side of the channel the free $N$ in the mixture escapes and moves
upward with its normal mobility to a position $\gamma$. The complex $X$ also moves
upward through the plane $\alpha$ but in so doing tends to leave the slower moving
$P$ behind. Consequently a portion of the complex $X$ dissociates in order to
maintain the equilibrium indicated in equation 4. The $N$ resulting from this
dissociation moves ahead and fills the volume between the boundaries $\beta$ and $\gamma$.
The resulting concentration, $n_e$, of the separated nucleic acid is thus the sum
of the concentration, $n_f$, due to the free acid in the body of the mixture and
that arising from the decomposition of the complex. The factor $p_t$ it will be
recalled, corrects for the dilution in the $\delta$ boundary. The protein $P$ resulting
from the partial dissociation of the complex, $X$, accumulates in the volume $V_{\beta}$

$^{13}$ It may be noted that in all of the cases considered here, with the possible excep-
tion of the fourth case, a qualitative symmetry is retained by the patterns insofar as
the same number of boundaries, exclusive of the $\delta$ and $\epsilon$ effects, is present in both
channels. In some mixtures, however, even this type of symmetry is not observed.
Chem., 1941, 139, 383), in their electrophoretic studies of serum albumin-heparin
mixtures, frequently observed three boundaries in one channel and two in the other.
We have made similar observations with mixtures of ovomucoid and nucleic acid.
Asymmetries of this type have not been satisfactorily explained but are, possibly,
a reflection of the known complexity of whole serum albumin (McMeekin, T. L.,
$J$. Am. Chem. Soc., 1940, 62, 3393) on the one hand and ovomucoid on the other
(cf. footnote 2).
at the concentration $P_P$. Due to the dissociation occurring in the boundary at $\beta$, the displacement, $V_\beta$, of this boundary is proportional to neither $u_X$ nor $u_P$ but has an intermediate value.

In the cathode side of the channel the nucleic acid, $N$, instead of escaping from the mixture, migrates into it. The complex thus tends to find itself in a region devoid of $N$ and dissociates in the boundary at $c$, maintaining the equilibrium. The displacement, $V_c$, of this boundary is again proportional to neither $u_N$ nor $u_X$ but to an intermediate value. Pure $P$ is left behind in the region between $b$ and $c$ at a concentration $p_b$ which is greater than $p_f$ but less than $p_i$. This material migrates with the normal mobility of $P$ and hence the displacement $V_b$ is proportional to $u_P$.

Much effort has been expended in an attempt to compute the mass action constant, $K$, of equation 4. For this computation from the electrophoretic data it is necessary to find a value of $u_X$; i.e., the mobility of the complex. However, as indicated above, the motion of the boundaries involved also includes an effect due to the decomposition of this complex, making direct determination of this quantity impossible.

**SUMMARY**

Electrophoretic patterns of mixtures of ovalbumin and yeast nucleic acid indicate that the constituents migrate independently of each other in buffer solutions of 0.1 ionic strength and at pH values somewhat higher than the isoelectric point of the protein. In the isoelectric region, however, the patterns from the two sides of the channel exhibit asymmetries that can be explained by assuming the existence in the mixture of appreciable concentrations of a reversibly dissociable complex between the components. Formation of this complex is favored by increasing concentrations of the components and decreasing ionic strength. At pH values below the isoelectric point partial precipitation of the complex occurs.

The patterns obtained from each side of the channel in the electrophoresis of a mixture of two components, which form a dissociable complex, indicate only two boundaries, aside from the $\delta$ and $\epsilon$ effects. One of these is a normal boundary whose displacement is proportional to the mobility of a component that has separated from the mixture. In the other boundary, however, dissociation of the complex occurs and consequently the displacement of this boundary corresponds to the mobility of neither component nor to that of the complex. Moreover, the areas under the refractive index gradient curves are not proportional to the stoichiometric concentrations of the components. However, equations are developed with the aid of which an electrophoretic analysis of the mixture is possible. This analysis requires the use of data from the patterns of both channels.