tity, *i.e.*, vitamin produced per unit weight of mycelium.

IV. Antibacterial Tests.—The antimetabolite, 2-amino-2-carboxyethanesulfonamide (I), did not affect the growth, *in vitro*, of a variety of bacteria and fungi, including *Staph. aureus*, *E. coli*, *C. albicans*, *N. asteroides*, in concentrations as high as 1% in some cases. *M. tuberculosis* strain H37Rv grown on Proskauer–Beck[1] or Kirchner medium was not inhibited by compound I.

Mice infected with *M. tuberculosis* strain H37Rv were treated for 21 days with daily doses of 10 mg./mouse given subcutaneously; the treated animals did not differ from the control group.

For the sake of completeness, we add that the material was not cytotoxic to chick fibroblasts at 200 γ/ml., nor did it affect vaccinia virus in eggs.

Discussion

The results demonstrate that the asparagine analog, 2-amino-2-carboxyethanesulfonamide, possesses definite ability to compete with asparagine. The action of serum asparaginase was inhibited by 40% when assayed in the presence of six molecules of the antimetabolite for each molecule of natural substrate. The data obtained with the amount of material available for enzyme kinetic studies are insufficient for analysis according to Lineweaver and Burk[16]; in the absence of direct evidence to the contrary, one may reasonably assume this inhibition to be of the competitive type.

The compound is toxic for an asparagine-requiring strain of *Neurospora crassa* when eight to forty molecules of sulfonamide are present for every molecule of asparagine. The growth inhibition caused by a given concentration of I is reversed by addition of asparagine; this holds true for levels of I ranging from 20 γ/ml. to 1000 γ/ml. Since the parent and mutant strains do not differ in asparaginase content,[12] the inhibition of this enzyme is not a likely mechanism for the growth depression of mutant cultures by compound I. Possibly, the sulfonamide competes with asparagine transport across the cell wall.

Compound I was not toxic for the parent, wild strain of *N. crassa*, growth of which does not depend on an exogenous supply of asparagine. Though asparagine could be demonstrated in the filtrates from such cultures, we have no information about the intracellular concentrations of asparagine and of compound I. Thus, we cannot rule out the simplest explanation for the lack of effect of I on the wild strain, *viz.*, that effective ratios of I to asparagine were not reached.

In the case of *E. ashbyii*, Table IV shows that the sulfonamide I suppresses growth at a concentration between 133 to 660 γ/ml. when asparagine is absent. In the presence of 940 γ/ml. of asparagine, the toxicity of 660 γ/ml. of the sulfonamide (molar ratio approximately 2:1) appears less pronounced, since half-maximal growth occurred, indicating the possibility that the effect may be due to antimetabolic competition. However, when the organisms were exposed to a higher concentration of the sulfonamide (1320 γ/ml.), the toxicity could not be reversed by increasing the asparagine concentration up to the same molar ratio of 2:1. Moreover, since *E. ashbyii* responds favorably to several amino acids other than asparagine, the effect noted here may well be one of general toxicity rather than of antimetabolic competition.

SUMMIT, NEW JERSEY

(16) H. Lineweaver and D. Burk, THIS JOURNAL, **56**, 658 (1934).

[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, STATE UNIVERSITY OF IOWA]

Electrophoresis of Adsorbed Protein

BY DIPTI K. CHATTORAJ[1] AND HENRY B. BULL

RECEIVED FEBRUARY 18, 1959

The electrophoretic mobilities of bovine serum albumin and of egg albumin adsorbed on inert particles exhibit over a substantial *p*H range two maxima as a function of protein concentration which are related to structural changes in the adsorbed protein monolayers. Comparison of the electrophoretic mobilities of dissolved and of adsorbed bovine serum albumin is consistent with the view that the electrical double layer associated with the adsorbed protein molecules can be treated as a plane plate condenser, although the nature of the underlying surface influences the electrophoretic mobility of adsorbed protein in important ways.

Attention has been called recently to the fact that the effective electrophoretic radii of protein molecules adsorbed on microscopically visible glass particles are very much larger than their solution radii.[2] There are a number of additional and interesting problems associated with the electrophoresis of adsorbed proteins and some of these problems we have now considered. We have studied the electrophoretic mobilities of Pyrex glass particles as well as of particles of liquid (Nujol) and of solid paraffin particles as functions of protein concentration and of *p*H at constant ionic strength and are able to present certain conclusions from these investigations.

Experimental

The crystalline bovine serum albumin (B.S.A.) was obtained from Armour and Co. The egg albumin (E.A.) was prepared from fresh hens' eggs by the method of Kekwick and Cannan.[3] Both proteins were exhaustively dialyzed against water and the concentrations determined by dry weight.

The microelectrophoretic measurements were made in a flat cell oriented laterally, each experimental point being the

(1) Chemistry Department, Jadavpore University, Calcutta, India.

(2) H. B. Bull, THIS JOURNAL, **80**, 190 (1958).

(3) R. A. Kekwick and R. K. Cannan, *Biochem. J.*, **30**, 227 (1936).

average of 10 measurements of the mobility. The ionic strength has been maintained at 0.05 and sodium acetate buffers have been used except in the more acidic region in which solutions of hydrochloric acid and sodium chloride have been employed. The mobilities were adjusted to 25° by a viscosity correction and where significant, the viscosity of the protein solution has been considered and the mobilities corrected to those in water. The moving boundary measurements were conducted at 25° in a Perkin–Elmer Model 39 which had been somewhat modified to accommodate our needs.

Nujol was vigorously shaken with 40% sodium hydroxide and then washed exhaustively with water until the washings were neutral. The solid paraffin wax was from Fisher Scientific Co. and had a melting point of 68–70°. It was melted in the presence of 40% sodium hydroxide, vigorously shaken and washed in the melted condition with water until the washings were neutral.

The emulsions were manually prepared and solid paraffin was melted and emulsified at an elevated temperature. Suitable amounts of diluted protein solutions were added after the emulsions had been formed. The particles were in the neighborhood of 5 μ in diameter which at an ionic strength of 0.05 gives a κr value of about 1,000 (κ is the reciprocal Debye–Huckel distance and r is the radius of the particle). This value of κr permits the treatment of the electrical double layer at the surface of the particles as a plane plate condenser.

The powdered Pyrex glass was from the same lot as previously used[4] and was ground to a suitable size for electrophoretic measurements in an agate mortar.

Results and Discussion

Figures 1, 2, 4 and 5 show plots of the electrophoretic mobilities of Pyrex glass particles as functions of the square roots of the protein concentrations expressed in per cent. at various pH values in sodium acetate buffers at an ionic strength of 0.05

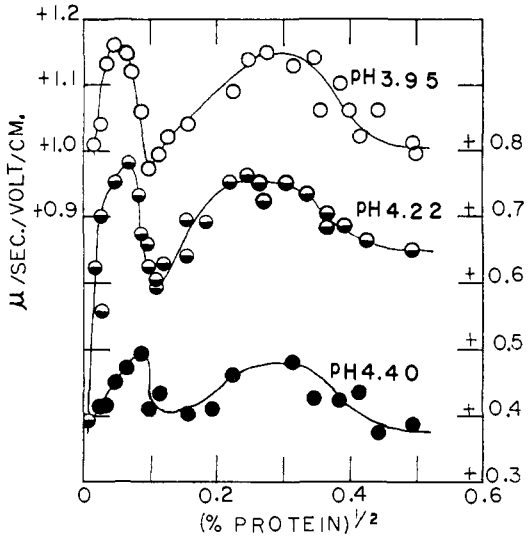

Fig. 1.—Mobilities of Pyrex glass particles as functions of the sq. root of per cent. B.S.A. concentration. Left scale, pH 3.95; right scale, pH 4.22 and 4.40. Sodium acetate buffers ionic strength 0.05 at 25°.

at 25°. Included in Fig. 3 are the mobilities of Nujol particles in B.S.A. solutions of increasing concentration of protein as well as the mobilities of B.S.A. in solution as measured by the moving boundary method under the same conditions of buffer strength, pH (4.22) and temperature as employed for mobility measurements by the micro method.

(4) H. B. Bull, *Biochim. Biophys. Acta*, **19**, 464 (1956).

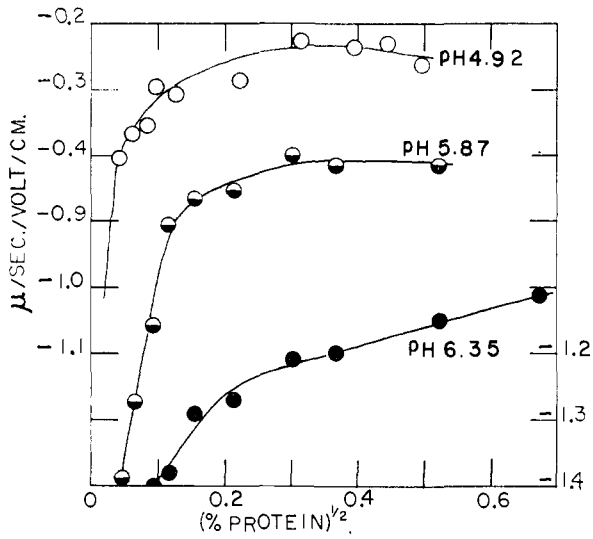

Fig. 2.—Mobilities of Pyrex glass particles as functions of the sq. root of per cent. B.S.A. concentration. Left scale, pH 4.29 and 5.87; right scale, pH 6.35. Sodium acetate buffers ionic strength 0.05 at 25°.

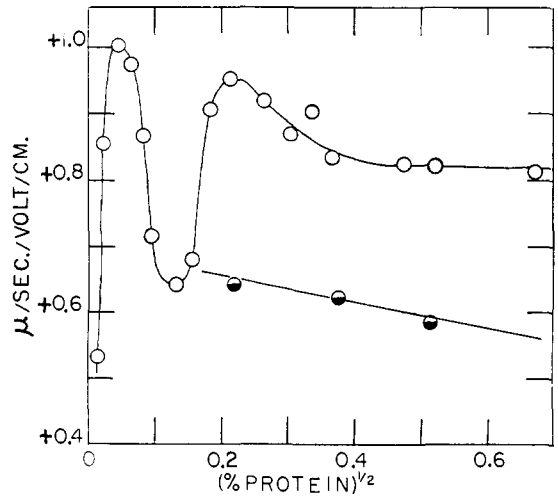

Fig. 3.—Mobilities as functions of sq. root of per cent. B.S.A. concentration. Sodium acetate buffer ionic strength 0.05, pH 4.22 at 25°. O, Nujol particles; ◒, dissolved protein with moving boundary.

It is clear from an inspection of Fig. 1 that acid to the isoelectric point, the electrophoretic mobilities of adsorbed B.S.A. exhibit two maxima as functions of protein concentration. Actually, a study of the mobilities at pH 2.1 of B.S.A. adsorbed on Pyrex glass in mixtures of hydrochloric acid and sodium chloride reveals that at this acid pH the maxima are completely missing and the mobilities are a monotonous function of protein concentration reaching saturation at very low protein concentrations (these data are not shown). Figure 2 shows that the mobility–concentration maxima are also missing on the alkaline side of the isoelectric point of B.S.A. However Nujol particles at pH 6.40 do show some small irregularities suggesting possible maxima (these data are not shown). The mobilities of E.A. adsorbed on Pyrex glass particles

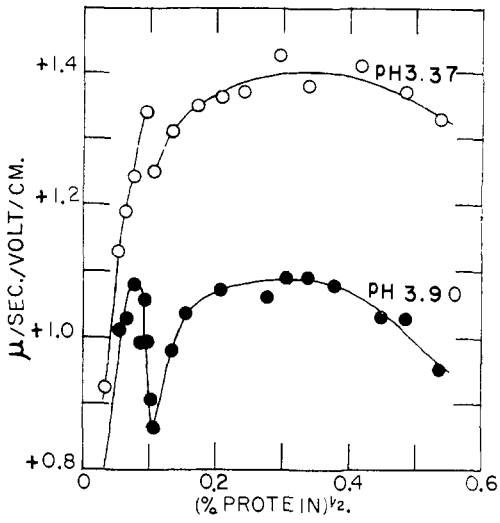

Fig. 4.—Mobilities of Pyrex glass particles as functions of the sq. root of per cent. E.A. concentration. Sodium acetate buffers ionic strength 0.05 at 25°.

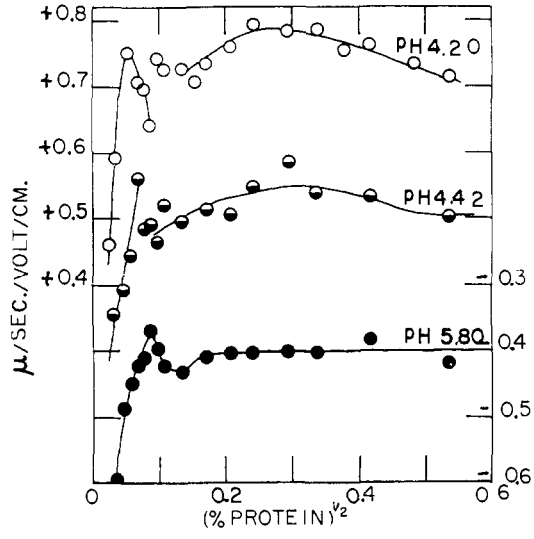

Fig. 5.—Mobilities of Pyrex glass particles as functions of the sq. root of per cent. E.A. concentration. Left scale, *p*H 4.20 and 4.42; right scale *p*H 5.80. Sodium acetate buffers ionic strength 0.05 at 25°.

(Figs. 4 and 5) show two such maxima as functions of the protein concentration on both the acid and alkaline side of the isoelectric point of this protein.

Since we have found from our previous work[4] the amount of B.S.A. adsorbed on Pyrex glass as a function of the protein concentration, it is possible to compare the mobility–concentration with the adsorption–concentration relationship. Figure 6 shows the mobilities of Pyrex glass particles as functions of the amounts of B.S.A. adsorbed in mg. per sq. meter of glass surface at two *p*H values. As noted previously,[4] there is evidence for phase transitions in the adsorbed films of B.S.A. on glass acid to the isoelectric point but none on the alkaline side.[5]

A distinct phase transition is exhibited by an adsorbed layer of E.A. on Pyrex glass at *p*H 4.66,[5]

(5) H. B. Bull, *Arch. Biochem. Biophys.*, **68**, 102 (1957).

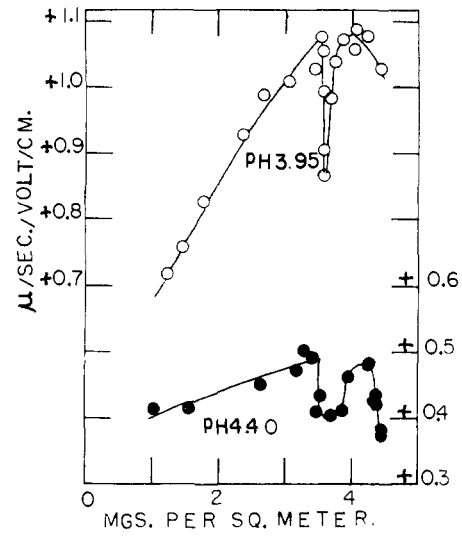

Fig. 6.—Mobilities of Pyrex glass particles as functions of the amount of adsorbed B.S.A. in mg. per sq. meter of glass. Left scale, *p*H 3.95; right scale, *p*H 4.40. Sodium acetate buffers ionic strength 0.05 at 25°.

but we are without information on the behavior of this protein at other *p*H values. It is to be noted, however, that the phase transition in the adsorbed monolayer of E.A. at a *p*H of 4.66 occurs[5] in the protein concentration range of 0.06–0.1% which corresponds to the concentration range of the second maximum in the mobility–concentration curve (Figs. 4 and 5). Naturally, we have devoted considerable thought and effort to understand the complex course of the mobility–concentration curves of the adsorbed protein. As a working hypothesis, we suggest the following explanation.

At very low protein concentrations, less than about 0.002%, the adsorbed protein exists at the interface as a spread monolayer and resembles closely the type of spread monolayer formed by spreading a protein at the air–water surface, which has been extensively studied in terms of force–area measurements. As the protein concentration in solution is increased, the adsorbed and greatly expanded protein molecules are progressively compressed until at a concentration of about 0.02 to 0.03% the compressed molecules are forced into dimensions corresponding to those of the native protein molecules. This change in dimensions of the adsorbed protein molecules results in a notable drop in the electrophoretic mobility and which, according to our view, in turn results from less of the particle surface being covered by the more compact protein molecules. Thus, we interpret the change of the adsorbed film in this concentration range as essentially a reversal of surface denaturation with increasing protein adsorption. The increase in electrophoretic mobility following the abrupt decrease of the mobility as the protein concentration is increased is attributed to a completion of a condensed film of native protein molecules in response to increasing protein concentration.

The decrease of the mobility of the particles beyond the second maximum is difficult to interpret

with confidence. We have considered the possibility that the dissolved protein might contribute sufficiently to the ionic strength to produce the observed decrease of the mobility but it is clearly evident from Fig. 3 that at higher protein concentrations the mobilities of the particles become independent of protein concentration and obviously this would not be true if the dissolved protein were adding significantly to the ionic strength. We think it likely that the decrease of the mobility beyond the second maximum is due to a rearrangement of the protein molecules within the adsorbed monolayer itself and this rearrangement can be regarded as a surface crystallization of the native adsorbed molecules in such a way that some of the ionogenic groups of the adsorbed protein which in the adsorbed but amorphous state were exposed to the aqueous phase are now buried in the two dimensional surface crystal.

We have exposed Pyrex glass to 0.25% B.S.A., at *p*H 4.22, determined the mobility of the powdered glass and then progressively diluted the suspension of glass–protein solution with acetate buffer at *p*H 4.22, measuring the mobility of the glass particles after each dilution. As can be seen from Fig. 7 the mobilities of the particles as the protein

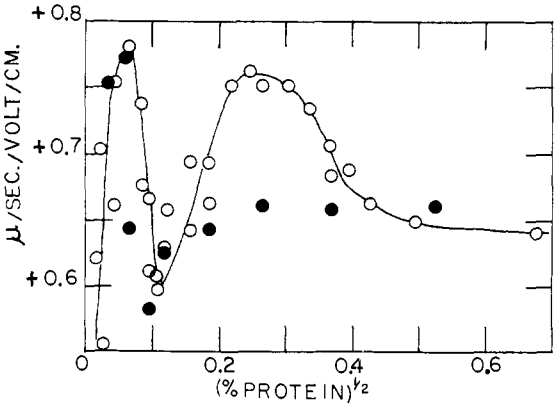

Fig. 7.—Mobilities of Pyrex glass particles as functions of the sq. root of B.S.A. concentration. Sodium acetate buffers ionic strength 0.05 *p*H 4.22 at 25°. O, increasing protein concentration; ●, decreasing protein concentration.

concentration is decreased do not exhibit a maximum in the mobility at a B.S.A. concentration of about 0.1% but the mobility maximum at somewhat less than 0.01% is observed as the protein concentration is decreased; we consider that the adsorbed crystalline protein does not revert to an amorphous state before it begins to be desorbed. On the other hand, the native adsorbed protein molecules do expand on the surface as part of the native molecules are desorbed with decreasing concentration and hence the mobility maximum at the lower protein concentration is observed.

We are not inclined to attempt an explanation of the electrophoretic behavior of the adsorbed protein in terms of the formation of a duplex adsorbed protein film. For example, from Fig. 6, it is noted that the ratio of the amount of protein adsorbed at the end of the first film transition (at about 0.01% protein) to that at the end of the second transition (at about 0.25% protein) is about 1.25 both at *p*H 3.95 and at *p*H 4.40. This ratio of the amounts of B.S.A. adsorbed should have been much larger if duplex film formation had occurred.

In Fig. 8 is shown a plot of the electrophoretic mobilities of bare Pyrex glass particles, of Pyrex glass particles in the presence of 0.173% E.A. and

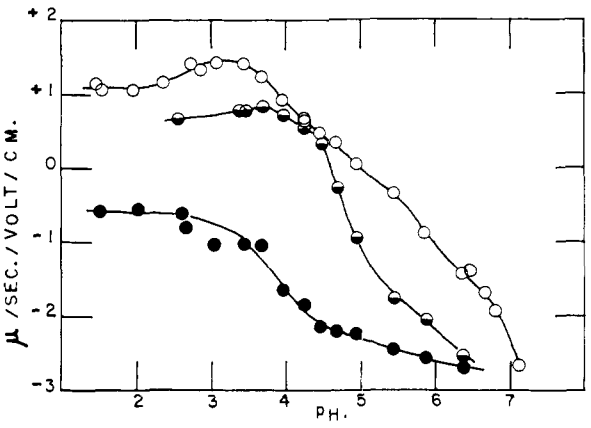

Fig. 8.—Mobilities of Pyrex glass particles as functions of *p*H. Ionic strength 0.05. Above *p*H 3.4 sodium acetate buffers; below *p*H 3.4 hydrochloric acid–sodium chloride solutions. O, 0.173% E.A.; ◒, adsorbed and washed E.A.; ●, bare Pyrex glass particles.

of Pyrex glass particles first exposed to 0.866% E.A. and then washed by repeated decantations with 0.05 ionic strength buffer at *p*H 4.66 until the calculated protein concentration of 4.61×10^{-3} % had been attained. One ml. of this suspension was then transferred to 50-ml. volumes of 0.05 ionic strength buffers at the indicated *p*H values and the mobilities measured. Notable is the maximum in the mobility in the 0.178% E.A. at a *p*H of about 3. We suggest that the adsorbed E.A. molecules have expanded and have become surface denatured permitting sufficient freedom of motion of the peptide chains so that many of the positive groups of the protein have been neutralized by the negative charge on the glass. The washed E.A. surface represents a still greater interaction between the negatively charged glass and the adsorbed protein.

Figure 9 shows a comparison of the electrophoretic mobilities of glass particles and of solid paraffin particles in 0.25% B.S.A. as a function of the *p*H. We see that the mobilities of the paraffin particles are significantly greater than the mobilities of the Pyrex glass particles at a given *p*H except, of course, at the *p*H of intersection of the two mobility curves. We think it is probable that one of the causes for the difference in mobilities of these two types of particles rises from the fact that the paraffin particles expose smooth surfaces to the solution and, accordingly, the streamline of flow and the electrical field are parallel to the particle surface whereas the crushed glass particles are irregular in shape with many cracks and crevices. These surface concavities involve resistance to flow of the liquid past the glass particle surface but the film of protein lining the concavities cannot exert full electrostatic effect and, accordingly, the net mobility of the glass particles is decreased as

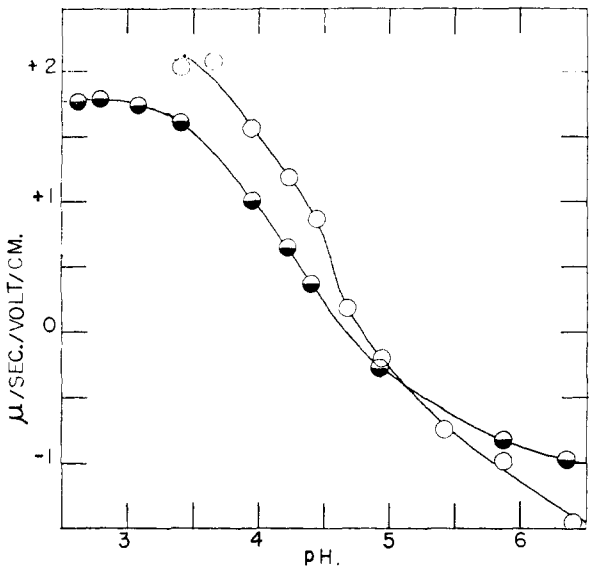

Fig. 9.—Mobilities of adsorbed B.S.A. as functions of *p*H 0.05 ionic strength at 25°; 0.25% protein. Above *p*H 3.5 sodium acetate buffers; below *p*H 3.5 hydrochloric acid–sodium chloride solutions. O, solid paraffin particles; ◒, Pyrex glass particles.

compared with that of the smooth (paraffin) surface.

In view of the dependence of the mobilities of particles upon protein concentration, upon the nature of the underlying surface and the complex relationships revealed in Figs. 1, 2, 3, 4 and 5, it is perhaps unrealistic to attempt a comparison between the electrophoretic mobilities of adsorbed protein and those of protein in solution. However, for what they are worth, such comparisons have been attempted and are shown in Fig. 3 which contrast the mobilities of B.S.A. adsorbed on Nujol particles with the mobilities of dissolved B.S.A. measured by the moving boundary method both at *p*H 4.22 and as functions of the protein concentration. The mobility of the Nujol particles at the highest protein concentration is about 1.45 times greater than is the mobility of the protein in solution.

A more detailed comparison between the mobility of adsorbed and dissolved B.S.A. as a function of *p*H is shown in Fig. 10. We have used the data of Schlessinger[6] which were obtained on an Armour preparation of B.S.A. and which exhibited only one boundary over the *p*H range considered and at 0.05 ionic strength acetate buffer. These data have been adjusted from 0°, the temperature of the measurements, to 25° by multiplying the values of Schlessinger by the viscosity ratio of water at 0° to that of water at 25°. The mobilities thus calculated are shown plotted in Fig. 10; also shown in Fig. 10 are the mobilities of solid paraffin particles in 0.247% B.S.A. as a function of *p*H in 0.05 ionic strength acetate buffer. It will be noted immediately that the mobility of the B.S.A. adsorbed on paraffin particles at *p*H 4.22 as 1.18 μ per sec. per volt per cm. whereas the corresponding mobility of the B.S.A. adsorbed on Nujol particles is 0.82 μ per sec. per volt per cm. (see Fig. 3) and the corresponding mobility of B.S.A. adsorbed on glass at this *p*H and protein concentration is about 0.60 μ per sec. per volt per cm. (see Fig. 9). Evidently, the nature of the underlying surface has a considerable influence on the mobility of the particle upon which protein has been adsorbed. The data of Schlessinger, already corrected to 25°, have been further adjusted to yield protein mobilities with the same charge density per unit area of surface with κr very much larger than unity, *i.e.*, for a flat surface characteristic of that of the adsorbed protein. The relation used to make this conversion is (2)

$$\frac{U_s}{U_p} = \frac{2}{3}\frac{f(\kappa r)\kappa r}{(1 + \kappa r + \kappa r_1)} \qquad (1)$$

where U_s is the mobility of a spherical particle of radius r, κ the Debye–Hückel reciprocal distance, r_1 the average radius of the small ions (assumed to be 2.5×10^{-8} cm.), $f(\kappa r)$ the Henry factor and U_p is the mobility of the very large particle for which $\kappa r >> 1$. The effective electrophoretic radius (r) of the dissolved B.S.A. molecules has been set equal to 48×10^{-8} cm.[7] The values of U_p as calculated from the Schlessinger data are shown in Fig. 10 as a broken line without experimental points.

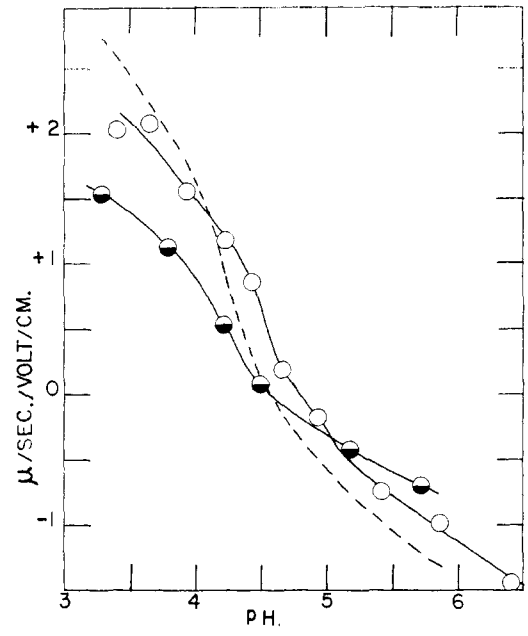

Fig. 10.—Mobilities of B.S.A. as functions of *p*H. Sodium acetate buffers ionic strength 0.05 at 25° O, solid paraffin particles in 0.25% protein; ◒, dissolved protein (Schlessinger); broken line mobilities of dissolved protein adjusted to that of a flat surface.

Examining Fig. 10, it is clear that the isoelectric point of the adsorbed B.S.A. is about 0.2 *p*H unit higher than that of the dissolved protein. If the entire mobility–*p*H curve of the adsorbed protein be shifted to the left by 0.2 *p*H unit, it is seen that values for the calculated mobilities of the dissolved protein corresponding to those for a plane surface (U_p) by the use of eq. 1 do, in fact, coincide in the

(6) B. S. Schlessinger, *J. Phys. Chem.*, **62**, 916 (1958).

(7) K. Aoki and J. F. Foster, THIS JOURNAL, **79**, 3385 (1957).

region alkaline to the isoelectric point but acid to the isoelectric point there is disagreement, the mobilities of the adsorbed protein being too low in the more acid region. It is evident that the mobilities of the protein adsorbed on Nujol would be still lower and those for B.S.A. adsorbed on glass particles would be less than those of Nujol. It is our view that the negative charge on the underlying surface upon which protein has been adsorbed serves to neutralize a substantial part of the positive charge of the adsorbed protein in the region acid to the isoelectric point and in the case of glass, the roughness of the surface is also of importance in reducing the mobilities at any *p*H.

We are of the opinion that it is a mistake to expect quantitative agreement between the electrophoretic mobilities of adsorbed and of dissolved protein even after correction has been made for the difference in particle size. The adsorbed protein molecules have, no doubt, been oriented and or distorted in various ways and, in general, represents a very complex situation. Electrophoresis of adsorbed protein has an inherent interest of its own and can reveal changes in the adsorbed molecules which are difficult to detect by other techniques.

Acknowledgment.—Financial support for this research was provided by the National Science Foundation for which we wish to express our thanks.

IOWA CITY, IOWA

[CONTRIBUTION FROM THE BIOCHEMISTRY DEPARTMENT, STATE UNIVERSITY OF IOWA]

Electrophoresis of Ribonuclease: Isoelectric Points on Various Adsorbents

BY LEWIS B. BARNETT[1] AND HENRY B. BULL

RECEIVED FEBRUARY 18, 1959

The isoelectric point of ribonuclease adsorbed on glass, Dowex 50 cation-exchange resin, Dowex 2 anion-exchange resin, paraffin and Nujol has been determined in various buffer systems at an ionic strength of 0.05 and at 25°. Comparison of these data with the isoelectric points obtained by the moving boundary (Tiselius) method indicates significant shifts after adsorption. These shifts depend upon the buffer system employed and the adsorbing medium.

Various investigators have noted the similarity between the mobilities of dissolved and adsorbed proteins. Abramson, Moyer and Gorin[2] cite studies on horse serum albumin[3-5] and horse pseudoglobulin.[4,6] Ribonuclease and bovine fibrinogen have been investigated as adsorbed proteins by Douglas and Shaw[7] and the similarity of isoelectric points noted between adsorbed and dissolved molecules. Bull[8] has concluded that the effective electrophoretic radii of adsorbed protein molecules are very much larger than the radii of the protein molecules in solution. One would therefore expect the mobilities of dissolved and adsorbed proteins to be different, as a general rule.

It is the purpose of the paper to study the isoelectric points of adsorbed ribonuclease as a function of the adsorbing medium and ionic environment and compare the findings with the isoelectric points of dissolved ribonuclease.

Methods

Ribonuclease.—The crystalline ribonuclease was obtained from Armour and Co. (Lot No. 381-059). This protein was rendered isoionic by passage of a solution through an ion-exchange column of the Dintzis type.[9] Concentrations were determined by drying in a vacuum oven at 105°.

Electrophoretic Mobilities.—Electrophoretic measurements of the particles were conducted in a microelectrophoretic cell supplied by Arthur H. Thomas and Co. (See Bull[8] for a more complete description of the apparatus.) The cell was mounted laterally. Komagatas' correction was applied to find the correct position for observing mobilities.[10] Mobilities were taken at room temperature and corrected to 25° by multiplying by the ratio of the viscosity of water at the two temperatures. Conductances were measured at room temperature. The *p*H was determined with a Beckman Model GS *p*H meter. Concentration *vs.* mobility curves were plotted for each set of conditions and the concentration at which maximum adsorption occurred was used to obtain mobilities of adsorbed ribonuclease. (This protein concentration for maximum adsorption is listed with each adsorbing medium.)

Glass.—Powdered Pyrex glass in a standard suspension was used as a stock solution of particles. The diameters of the particles were about 2 μ. Maximum adsorption occurred at 2.55 mg. ribonuclease/g. glass. Solutions were equilibrated overnight.

Dowex Resins.—Dowex 50-X12 cation-exchange resin (Lot No. 3648-16) and Dowex 2-X10 anion-exchange resin (Lot No. 4034-34) of 200-400 mesh were obtained from the Dow Chemical Company. The Dowex 50 and Dowex 2 resins were of low porosity and medium porosity, respectively.

The resins were washed thoroughly and used in the salt form. The dry particles were powdered with a mortar and pestle and fractionated by sedimentation, discarding the larger pieces. The diameters of final particles were about 5 μ. The concentration of the final suspension was determined by drying in a vacuum oven at 105°. After equilibration of the resin and protein over-night, maximum adsorption occurred at 0.50 mg. and 94 mg. ribonuclease/mg. dry resin for Dowex 50 and Dowex 2, respectively. (The latter adsorption was measured at *p*H 7.76, at which *p*H both the protein and anion-exchange resin are positively charged.) Experiments have shown that the conductance of the resin particles is greater than that of 0.05 *M* NaCl.[11] This observation introduces a serious reservation concerning the electrophoresis of resin particles with and without a cover of protein. The difficulty arises in two ways: (1) Since the conductance of the particle is greater than that of the solution of 0.05 *M* NaCl, the current density through the particle will be greater than that through the solution and the

(1) Predoctoral Fellow of the United States Public Health Service.

(2) H. A. Abramson, L. S. Moyer and M. H. Gorin, "Electrophoresis of Proteins," Reinhold Publ. Corp., New York, N. Y., 1942, p. 83.

(3) H. A. Abramson, *J. Gen. Physiol.*, **15**, 575 (1932).

(4) L. S. Moyer, *J. Biol. Chem.*, **122**, 641 (1938).

(5) A. Tiselius, *Nova Acta soc. sc. Upsaliensis*, **7**, No. 4 (1930).

(6) A. Tiselius, *Biochem. J.*, **31**, 313 (1937).

(7) H. W. Douglas and D. J. Shaw, *Trans. Faraday Soc.*, **53**, 512 (1957).

(8) H. B. Bull, THIS JOURNAL, **80**, 1901 (1958).

(9) H. M. Dintzis, Ph.D. Thesis, Harvard University, 1952.

(10) S. Komagata, Researches Electrotech Lab. Tokyo No. 348, 1933.

(11) H. B. Bull, unpublished data.