area of a hexagon whose sides are of length 88 Å. A set of such hexagons packed tightly together would have their centers 152 Å. apart, the value obtained for the lattice spacing of TMV by Bernal and Fankuchen.[11] Thus, if we assume that the particles of dried TMV gel are packed in the cross-sectional form of hexagons, we find that a particle weight of 49 × 10^6 g./mole is consistent with the data for lattice spacing, density and particle length.

BERKELEY 4, CALIF. RECEIVED AUGUST 30, 1950

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

The Effect of Serum Albumin on the Polarographic Diffusion Current of Cadmium[1]

BY CHARLES TANFORD

A study has been made of the depression of the polarographic diffusion current of cadmium by bovine serum albumin. It is shown that the effect is due to complex formation between cadmium and the protein, and that adsorption of protein on the mercury drop and other non-specific factors do not play a significant role. The possibility that this type of diffusion current depression might be used to evaluate the thermodynamic constants of protein–metal interaction is discussed.

It is a well-known fact that very small quantities of protein added to a solution of a reducible substance will (provided the sign of the protein charge is correct) eliminate the polarographic maximum which may be exhibited by that substance. If somewhat greater quantities of protein are added however, it is usually found that a reduction in the diffusion current also takes place.[2,3] Kolthoff and Lingane have ascribed this phenomenon to complex formation between the protein and the reducible substance. Alternative explanations, however, have been offered, *e.g.*, that the phenomenon is due to reduction of the effective surface of the drop by protein adsorption.[4,5] It is also possible that viscosity changes may play a role in the phenomenon.[2] The objects of this paper are (a) to bring forward evidence to show that, at least in the example chosen for study, complex formation is indeed the predominant, and probably the only factor in the decrease of the diffusion current, and (b) to investigate the possibility of using this effect to evaluate the thermodynamic constants for complex formation between proteins and reducible molecules or ions.

Experimental

The polarograph used was a modified Sargent Polarograph, Model XX. An H-type cell, designed to hold 2 to 3 ml. of solution, and containing a satd. calomel electrode, was used. Diffusion currents were measured by the extrapolation method, in a constant temperature bath at 25.1°, using a capillary with an m value of approx. 2.65 mg./sec. The drop time at the point of measurement was 3.8 sec. Oxygen was removed from the solutions by a special technique described elsewhere.[6] (The ordinary method of oxygen removal is not possible here, because solutions containing proteins foam if gases are bubbled through them.) The supporting electrolyte for all experiments, except that illustrated by Fig. 1, consisted of 0.15 M sodium chloride.

All ordinary reagents were commercial C.P. reagents. Armour crystalline bovine plasma albumin was used. It was found to contain 5% moisture, which was corrected for in all weighings. Stock solutions of the protein were adjusted to desired pH values by the careful addition of dilute sodium hydroxide.

Theoretical

If there is combination taking place between reducible substance and protein, we can envisage two limiting types. The first is one in which there is a very strong interaction, *i.e.*, in which there is a large negative free energy of combination. This would result in a large displacement of the half-wave potential of the reducible substance, and there would therefore be no difficulty in recognizing and interpreting the effect.[7] On the other hand, the interaction between protein and reducible ion may be relatively weak. To see what would occur then it is convenient to discuss a hypothetical case with appropriate values of the thermodynamic constants. Let us assume, for example, that we have a protein with 40 positions available for combination with a reducible molecule, and that the free energy of combination, per mole of reducible substance, is −4000 calories (average value).[8] If the reduction involves two electrons, the difference in half-wave potential between combined and uncombined reducible substance would then be only 4.186 × 4000/2 × 96,500 or 0.186 volt. If a given solution were to contain both combined and uncombined reducible substance, the reduction waves of the two would therefore overlap, and would, in fact, appear as one, somewhat flattened wave. However, since reducible molecules bound to a protein molecule will have a much smaller diffusion coefficient than free reducible molecules, as well as, possibly, a slow reduction rate due, for example, to the necessity of correct orientation of the protein molecule at the drop, the diffusion current will be markedly decreased over what it would be in the absence of protein.

It remains to show that a weak interaction of this sort will cause an appreciable fraction of a reducible substance in a solution containing protein to be bound to the protein so as to cause the sizable diffusion current reduction which is actually observed. To do this we write the equilibrium constant for the combination of a reducible substance with a protein[9] neglecting for the moment the term for electrostatic interaction

$$\bar{\nu}/(n - \bar{\nu})c = K \qquad (1)$$

where c is the concentration of free reducible substance, n is the total number of sites on the protein available for combination and $\bar{\nu}$ is the average number of such sites covered. In our example n is equal to 40, and K has the value exp.

(1) Presented at the 118th National Meeting of the American Chemical Society, Chicago, Ill., Sept. 3–8, 1950.

(2) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941.

(3) J. K. Taylor and R. E. Smith, *Anal. Chem.*, **22**, 495 (1950).

(4) B. Keilin, THIS JOURNAL, **70**, 1984 (1948).

(5) K. Wiesner, *Coll. Czech. Chem. Comm.*, **12**, 594 (1947), has shown that the reduction of the diffusion current of certain organic molecules by eosin is due to this effect. The differences between eosin and bovine serum albumin in this respect will be pointed out below.

(6) C. Tanford and J. Epstein, *Anal. Chem.*, in press.

(7) Strong combination of this sort occurs in some specific cases, and some examples of it will be studied in this laboratory in the near future.

(8) It should be mentioned that the polarographic wave obtained will not at any time obey the fundamental equation of Heyrovsky and Ilkovic, $E_{d.e.} = E_{1/2} - 0.0591/n \log i/(i_d - i)$, even if all the reducible molecules are bound to the protein, since the free energy of combination will be different for each successive molecule or ion bound to the protein, *i.e.*, the half-wave potential will differ slightly for each successive molecule or ion reduced. For metals the result is always a curve less steep than that predicted by the Heyrovsky-Ilkovic equation.

(9) G. Scatchard, *Ann. N. Y. Acad. Sci.*, **51**, 660 (1949).

$(4000/RT)$, *i.e.*, 850 at 25°. Using Eq. 1 and the fact that the total concentration of reducible substance must be c plus $\bar{\nu}$ times the concentration of protein we can compute the data summarized in Table I, and we see that the weak interaction assumed will indeed lead to combination of a large fraction of reducible substance with protein, under condition which may be regarded as typical, *i.e.*, about 10^{-4} molar protein and 10^{-2} to 10^{-4} molar reducible substance.

TABLE I

FRACTION OF REDUCIBLE ION BOUND

Total concn.	C_{free}	$\bar{\nu}$	C_{bound}	C_{bound}/C_{total}
0.012	0.0100	22	0.002	0.18
.0022	.0010	11.5	.0012	.54
.0003	.0001	2	.0002	.67

It now becomes possible to make the following predictions about the behavior of this diffusion current decrease, if it is due to combination of the reducible substance with protein:

(1) For a given total concentration of reducible substance the ratio of the diffusion current in the presence of protein to the diffusion current in the absence of protein, which we shall designate by $i_d/(i_d)_0$, should decrease with increasing protein concentration. This would be true, of course, no matter what the cause of the effect.

(2) The ratio $i_d/(i_d)_0$ will, at sufficiently high protein concentrations, reach a limiting value below which it will never fall. This value will be reached when all of the reducible substance is combined with protein.

(3) At a given protein concentration, the ratio $i_d/(i_d)_0$ should decrease with decreasing concentration of reducible substance, since, the greater the ratio of protein to reducible substance, the more of the reducible substance in proportion will be bound by the protein. This can be seen at once from the computations in Table I.

(4) Under otherwise identical conditions, for a negatively charged reducible ion, $i_d/(i_d)_0$ should increase with increasing pH, because of the greater electrostatic repulsion due to the increasing negative charge of the protein. For positively charged ions, similarly, there is greater attraction between protein and ion at higher pH values, and $i_d/(i_d)_0$ should therefore *decrease* with increasing pH.

(5) Finally, the effect of a given protein might well be different for different reducible substances, since different reducible molecules or ions might have different degrees of affinity for the protein.

Results and Discussion

Effect of Protein and Cadmium Concentration and of pH.—The results obtained from the effect of bovine serum albumin on the diffusion current of cadmium solution show that the reduction of the diffusion current in this particular case is indeed due to protein–metal interaction. Figure 1 shows the polarograms of 1.2×10^{-3} molar cadmium ion without added protein, and in the presence of 1.5×10^{-4} molar serum albumin (pH about 6). It shows the typical diffusion current reduction and the slight flattening of the reduction wave. Fig. 2 is a plot of $i_d/(i_d)_0$ *versus* protein concentration for 4.9×10^{-4} molar cadmium, at a pH of 8.7. The low cadmium concentration and high pH were chosen so that conditions would be reached under which all the cadmium is protein-bound. A limiting value of $i_d/(i_d)_0$ is obtained, as predicted by (2) in the theoretical discussion. The limiting value is 0.186. At lower pH values and higher cadmium concentrations $i_d/(i_d)_0$ falls off less rapidly, as illustrated by the upper curve in Fig. 2. Figure 3 shows the effect of total cadmium concentration upon the ratio $i_d/(i_d)_0$ at a constant protein concentration of 1.03×10^{-4} molar (0.70 g./100 ml. solution). The results are entirely in accord with prediction (3) above. Furthermore, the effect of pH is shown to be precisely as expected from prediction (4).

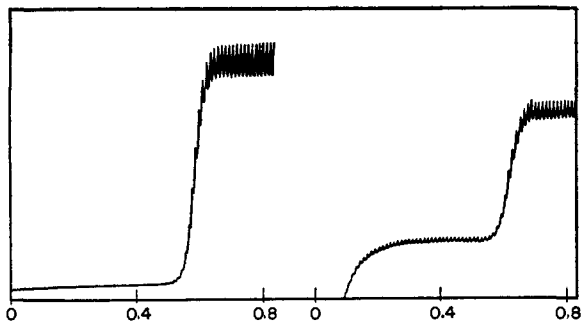

Fig. 1.—Polarograms of 1.2×10^{-3} M Cd^{++} without added protein, and in the presence of 1.5×10^{-4} M serum albumin (pH about 6). (In the solution containing protein, sulfite was added to remove oxygen. This is responsible for the observed shift of the anodic mercury wave to a more negative potential.)

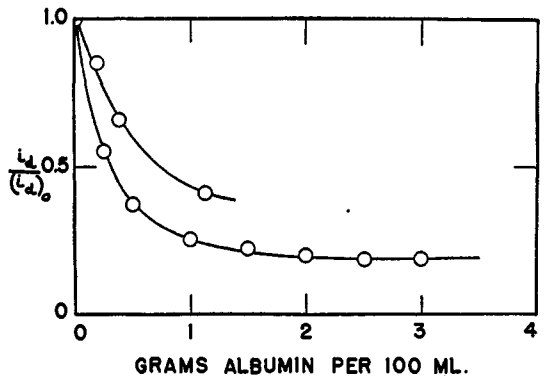

Fig. 2.—Effect of protein concentration on diffusion current; lower curve, 4.9×10^{-4} M Cd^{++}, pH 8.7; upper curve, 1.0×10^{-3} M Cd^{++}, pH 7.7.

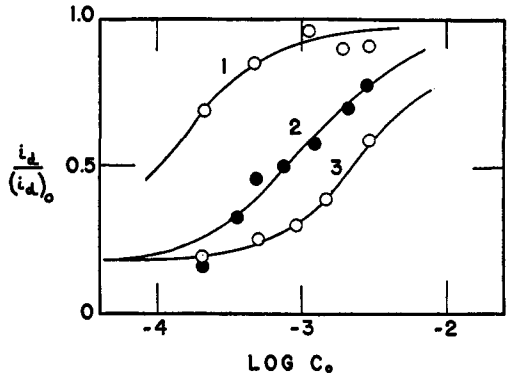

Fig. 3.—Effect of total cadmium concentration on diffusion current depression at constant protein concentration of 1.03×10^{-4} M. Curve 1: pH 4.3; curve 2: pH 7.1; curve 3: pH 8.9.

It is instructive to compare these results with what would have been obtained if protein adsorption were responsible for the diffusion current decrease. Firstly, $i_d/(i_d)_0$ should, at a given protein concentration, be independent of the concentration of reducible substance. This was actually found to be the case in the reduction of the diffusion current of organic substances by eosin.[5] Secondly,

the *p*H effect would be in the opposite direction from that observed. Since the cadmium diffusion current is measured on the negative side of the electrocapillary zero, the extent of protein adsorption should decrease with increasing *p*H because of repulsion between the negative drop and the increasingly negative protein molecule. This would result in an *increase* in $i_d/(i_d)_0$, instead of the decrease actually found. It might be mentioned here that the effect of *p*H upon the depression of the lead diffusion current by gelatin[3] is similar to that observed in this work, indicating that metal–protein complex formation plays the major role in that case also.

A word might be said at this stage about the extrapolation of the data of Fig. 3. While these data are not precise enough to permit an accurate extrapolation to very dilute cadmium solutions (in which again all the cadmium would be expected to be protein-bound) an extrapolation to a value of 0.186, *i.e.*, the same value as was obtained in Fig. 2, is not at all unreasonable. This fact is of importance in the discussion below.

Results with Thallous Chloride.—To see if different reducible substances lead to different degrees of diffuion current depression, polarograms were obtained for 0.001 molar thallous chloride both with and without added serum albumin (0.9 g./100 ml. solution, *p*H 5.5). In a similar experiment with cadmium chloride the diffusion current was reduced in the presence of protein to 89% of its original value, but in the case of thallous chloride, *no change in diffusion current was observed at all.* This is easy to understand, since thallous ion displays little tendency toward complex formation,[10] but would be impossible to explain on the basis of adsorption. In fact, Wiesner[5] uses as one of his most important pieces of evidence in favor of the theory that adsorption causes diffusion current depression by eosin the fact that the per cent. depression is identical for all of the organic substances he studied.

Number of Cadmium Ions Bound per Protein Molecule.—While the results just presented indicate that formation of cadmium–protein complexes is largely responsible for the diffusion current lowering, it remains possible that, in addition, the protein exerts a non-specific effect, *e.g.*, a viscosity effect, or an adsorption effect. While the results obtained with thallium would indicate that this is not so, it is desirable to eliminate this possibility for the cadmium data also. To do so, the fundamental equation for protein-ion interaction, which, in its complete form, including electrostatic interactions, is[9]

$$\bar{\nu}/(n - \bar{\nu})c = Ke^{-2w\bar{\nu}} \qquad (2)$$

Since, for any given protein and an ion of given valence, w, the electrostatic free energy term, is a constant, Eq. (2) shows that the average number of cadmium ions bound per albumin molecule, $\bar{\nu}$, depends only on the concentration of free cadmium in solution, and must be independent of the protein concentration. The most searching test, therefore, is to determine $\bar{\nu}$ as a function of free cadmium concentration at different protein concentrations. If no non-specific factors enter in, the values so obtained should be the same at all protein concentrations.[11] To determine $\bar{\nu}$ and c from the observed diffusion current we make use of the fact that the Ilkovic constant for the free metal ion, *i.e.*, the constant in the equation $i_d = Ac$, is known from determination of the diffusion current in the absence of protein. (A correction for change in drop time must be made where necessary.) This, however, is not the only contribution to the diffusion current, since bound metal is also reduced, though at a lower rate. As a first approximation we can assume that this lowering of the rate of reduction is a constant (see discussion below), *i.e.*, that the total diffusion current is given by

$$i_d = A\,(c + kc_b) \qquad (3)$$

where c is the concentration of free cadmium in the solution, c_d that of protein-bound cadmium, while k is a fractional coefficient. If this coefficient is taken to be a constant, it must clearly be equal to the limiting value of $i_d/(i_d)_0$ observed in Figs. 2 and 3, *i.e.*, its value is known. Since the sum of c and c_b must be the total cadmium concentration originally in the solution, designated by c_0, it becomes possible to solve Eq. (3) for c and c_b. Furthermore, dividing c_b by the protein concentration in millimoles per liter will yield a value of $\bar{\nu}$. The result of such calculation applied to the data of Fig. 3 is a plot of $\bar{\nu}$ against log c, given in Fig. 4. The three curves drawn through the points with open circles are the curves for three *p*H values and a protein concentration of 1.03×10^{-4} M. The fourth curve is calculated from a set of data at varying protein concentration, ranging from 6.8×10^{-5} M to 2.8×10^{-4} M. If the protein were to cause a diffusion current reduction by some non-specific process in addition to complex formation, then the apparent values of $\bar{\nu}$ computed at higher protein concentrations would be higher than expected. Actually, Fig. 4 shows that this is not the case. All the points fall just about where expected, even though the protein concentration varies by a factor of more than 4. The experimental error is quite large, but at least we can be sure that no very appreciable non-specific effect exists.

Diffusion Current or Kinetic Current?—Another point of interest concerns the real cause of the diffusion current reduction. Is it due entirely to a decrease in the diffusion coefficient, or is it due in part or altogether to a slow reduction process at the electrode? If it is due entirely to the former, then the ratio of the limiting diffusion current in the presence of an excess of protein to the diffusion current in the absence of protein should be proportional to the ratio of the square roots of the appropriate diffusion coefficients. This ratio can be calculated by means of the Stokes–Einstein equation.[12] Stricks and Kolthoff[13] in a brief note on the depression by

(10) Ref. 1, p. 266.

(11) This test should be made in all studies of the interaction of proteins with small molecules or ions. It has not been made in some studies; for example, in the study of copper-albumin interaction by the dialysis technique by I. M. Klotz and H. G. Curme, THIS JOURNAL, **70**, 939 (1948). One can never be sure then that interaction is the only factor involved in the measurement. In the dialysis technique, for example, protein adsorption on the membrane may also play a role.

(12) Ref. 1, p. 49.

(13) W. Stricks and I. M. Kolthoff, THIS JOURNAL, **71**, 1519 (1949).

serum albumin of the diffusion current of methyl orange, showed that, in that instance, the observed limiting value of $i_d/(i_d)_0$ was roughly the same as that calculated from the diffusion coefficient ratio. In the experiments here described this is not true, however. The calculated ratio is about 0.3; that observed is 0.186. Hence we must assume that a process slower than diffusion, perhaps one of orientation, plays a role in this case.

A further test would be to observe the effect of the height of the mercury column above the drop upon the current observed for a particular solution. This current should vary as the square root of h if it is a true diffusion current, but should be independent of h if it is a "kinetic" current,[14] into which class a current limited by an orientation process would fall. This test has accordingly been performed on one of our solutions containing very little free cadmium, and the results are presented in Table II. It is seen that the dependence of the limiting current upon mercury height is neither that characteristic of a pure diffusion current, nor that characteristic of a pure kinetic current, but, instead, lies somewhere in between. This probably means that both diffusion and slow reduction are important in reducing the limiting current of the protein-bound cadmium ion.

TABLE II

DIFFUSION OR KINETIC CURRENT[a]

Height of Hg column (cor.), cm.	i_d, mm.	i_d (predicted) Diffusion current	Kinetic current
19.0	23.3	..	..
24.3	26.0	26.3	23.3
29.4	27.5	28.9	23.3
34.1	29.0	31.2	23.3
40.2	30.5	33.9	23.3

[a] Solution of $5 \times 10^{-4}M$ Cd^{++} and about $2.5 \times 10^{-4}M$ protein in $0.15M$ KCl; pH 8.8.

The Calculation of Equilibrium Data for Protein–Metal Interaction.—The question now arises whether it is possible to use the observed depression of the diffusion current to measure accurately the extent of protein–cadmium interaction. This reduces to the question of whether it is indeed possible to represent the observed diffusion current by Eq. 3. At least as a first approximation, it would seem that the answer is yes. While the effective diffusion rate for cadmium would be greater, the more cadmium ions are attached, on the average, to a single protein molecule, the effective concentration gradient would be correspondingly reduced. Furthermore, the reduction rate of bound cadmium is likely to depend only little, if at all, on whether a single protein molecule carries one or more cadmium ions. Thus k in Eq. 3 can be regarded as constant, and we can obtain curves of $\bar{\nu}$ as a function of c, as was done in the preparation of Fig. 4 above, and, hence, can compute the thermodynamic constants for the reaction.

It might be mentioned that some support for the constancy of k can be obtained from the data presented above. The lower curve of Fig. 2 levels off when the molar ratio of cadmium to serum albumin is 2:1. If, as is logical to conclude, the cadmium is then all protein-bound, $\bar{\nu}$ must have the value 2. As more protein is added, $\bar{\nu}$ will reach the value unity. No change in $i_d/(i_d)_0$, *i.e.*, no change in k, is observed. Furthermore, the limiting values of $i_d/(i_d)_0$ for two curves in Fig. 3, are within the limits of experimental error, equal to one another and to the value obtained from Fig. 2.

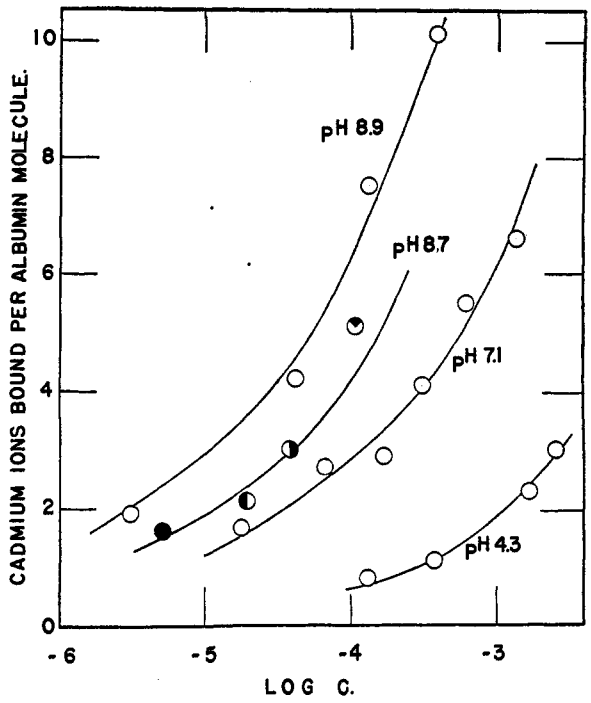

Fig. 4.—Number of cadmium ions bound at various pH values. Serum albumin concentrations: ○ 1.03×10^{-4} M, ◔ 6.8×10^{-5} M, ◑ 1.38×10^{-4} M, ◐ 2.06×10^{-4} M, ● 2.76×10^{-4} M.

It remains only to estimate the accuracy with which equilibrium data can be obtained. With the polarograph used in this work diffusion currents were reproducible at best to within 1%, even if the sensitivity of the instrument was checked at the time of each determination. From Eq. 3 we see that this means that the greatest possible accuracy in c and c_b is 1%, and the greatest possible accuracy in $\bar{\nu}$ (which is c_b divided by the protein concentration) is therefore also 1%. Actually, however, except where c and c_b are about equal, only the larger of the two quantities can be obtained with 1% accuracy. The smaller will be the difference between c_0 and either c or c_b, and, as the difference between two relatively large numbers, one of which is known with only 1% accuracy, will have a probable error which may be much greater than 1%. For this reason the points in Fig. 4 at the extreme ends of the curves are considerably less accurate than those in the middle. It would be a great advantage, therefore, if diffusion currents could be measured with an accuracy, say, of 0.1% rather than 1%, so that plots of $\bar{\nu}$ *versus log c* could be extended over a greater range.

Acknowledgment.—Preliminary experiments leading up to this work were performed by the

(14) R. Brdicka and K. Wiesner, *Coll. Czech. Chem. Comm.*, **12**, 138 (1947).

author early in 1948, at Harvard University. The author wishes to express his appreciation to Prof. J. J. Lingane, both for his generous provision of materials and facilities at that time, and for a number of very helpful discussions.

IOWA CITY, IOWA RECEIVED SEPTEMBER 11, 1950

[CONTRIBUTION FROM THE DEPARTMENTS OF BIOCHEMISTRY AND MEDICINE, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY]

The Deamination of Crystalline Egg Albumin.[1,2] I. Preparation and Properties of Various Soluble and Denatured Derivatives

BY PAUL H. MAURER AND MICHAEL HEIDELBERGER

The removal of 27 to 36% of amino groups from crystalline egg albumin (Ea) under mild conditions leads to a soluble deaminated Ea. At the same time a denatured derivative is formed and in this the extent of deamination is greater than in the soluble fraction. Upon further deamination the soluble deaminated Ea becomes insoluble at its isoelectric point, indicating that removal of this portion of the free $-NH_2$ groups which may participate in holding the native molecule in corpuscular form leads to partial unfolding of the molecule. Complete deamination was not achieved.

The immediate purpose of the present investigation was to determine whether or not crystalline egg albumin (Ea) could be deaminated without being denatured. Since the very meaning of the term "denatured" is under dispute, it was felt that, in spite of the large number of investigations on denatured proteins,[3] a careful study of the deamination of this easily irreversibly denatured protein might lead to additional knowledge of protein structure and the changes involved in denaturation. Because of the drastic conditions employed in previous deamination studies[4,5] it appeared that a deaminated undenatured Ea had never been prepared. For the purposes of the present papers, denaturation is defined as the conversion of the protein used, soluble in the native state at its isoelectric point, into a form or forms insoluble at their isoelectric points, a definition which has been shown to be useful in previous studies from this laboratory.[6-8]

Experimental

1. **Preparation of Ea and Its Derivatives.**—The Ea, prepared and five times recrystallized by the method of Kekwick and Cannan,[9] was dissolved in H_2O and dialyzed against water in the presence of toluene until sulfate-free, and allowed to stand at room temperature until all insoluble material settled, leaving a water-clear supernatant.[8]

The following conditions for deamination were found advantageous: Ea was treated in 0.5 *M* acetate buffer at *p*H 4.0,[10] with *M* $NaNO_2$ for varying periods at 0–3° (Table I). The reaction was stopped by careful neutralization with 2 *N* NaOH to *p*H 7.5. After dialysis until free from nitrite the solution was treated with neutralized thioglycolic acid at a final concentration of 1 *M* at 0–2° for 18 hours[11] to reduce any –S–S– linkages formed by the oxidizing action of HNO_2.[12,13] The solution was dialyzed free from thioglycolate as tested for by $CoSO_4$,[14] and 1-ml. aliquot portions were

TABLE I

PREPARATION AND PROPERTIES OF Ea, DEAMINATED Ea, AND DENATURED Ea

Preparation	Conditions for preparation: Reaction mixture	Temp., °C.	Time, hr.	Rela. proportions of A(1.0) and B	Deamination, %, ninhydrin method
Ea	Ref. (9)				0[a]
3A	*p*H 4.0 OAc^-, NO_2^-	0–3	6	1.0	33
3B	*p*H 4.0 OAc^-, NO_2^-	0–3	6	1.4	27
3B (A)	*p*H 4.0 OAc^-, NO_2^-	0–3	8 more		49
3B (B)	*p*H 4.0 OAc^-, NO_2^-	0–3	8 more	[b]	27
4A	*p*H 4.0 OAc^-, NO_2^-	0–3	8	1.0	42
4B	*p*H 4.0 OAc^-, NO_2^-	0–3	8	1.3	31
5A	*p*H 4.0 OAc^-, NO_2^-	0–3	17.5	1.0	56
5B	*p*H 4.0 OAc^-, NO_2^-	0–3	17.5	0.4	36
6A	*p*H 4.0 OAc^-, NO_2^-	0–3	7.5	1.0	42
6B	*p*H 4.0 OAc^-, NO_2^-	0–3	7.5	0.9	29
8A	*p*H 4.0 OAc^-, NO_2^-	0–3	8		44
8B	*p*H 4.0 OAc^-, NO_2^-	0–3	8		36
10A	*p*H 4.0 OAc^-, NO_2^-	R.T.[c]	18.5		80
5A Dn	*p*H 1.5 HCl	R.T.	72		
6A Dn	*p*H 1.5 HCl	R.T.	72		
8A Dn	*p*H 1.5 HCl	R.T.	144		
6B Dn	*p*H 1.5 HCl	R-T.	96		
8B Dn	*p*H 1.6 HCl	R.T.	144		
DnEa 105	*p*H 1.5 HCl	R.T.	72		
DnEa 105 Deam (SH)	*p*H 3.0 HNO_2	0–2	18		
DnEa 105 Deam (SS)	*p*H 3.0 HNO_2	0–2	18		38
DnEa 106	*p*H 1.7 HCl	R.T.	72		
DnEa 106 Deam (–SH)	*p*H 3.5 HNO_2	0–2	17		
DnEa 106 Deam (SS)	*p*H 3.5 HNO_2	0–2	17		58
8A FNA	*p*H 3.5 HNO_2	0–2	17		67
Ea FNA	*p*H 3.5 HNO_2	0–2	16		56

[a] Ratio of amino N/total in Ea found, 0.045; as also in Ref. (15). [b] Very little isolated. [c] R.T. = room temperature.

(1) Submitted by Paul H. Maurer in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University.

(2) Presented before the 40th Annual Meeting of the American Society of Biological Chemists, Detroit, Michigan, April 18–22, 1949, and before the 41st Annual Meeting of the American Society of Biological Chemists, Atlantic City, New Jersey, April 17–21, 1950.

(3) Reviewed by H. Neurath, J. P. Greenstein, F. W. Putnam and J. O. Erickson, *Chem. Rev.*, **34**, 157 (1944).

(4) H. Schiff, *Ber.*, **29**, 1354 (1896); H. Skraup and K. Kaas, *Ann. Chem.*, **351**, 379 (1907); Z. Treves and G. Salmone, *Biochem. Z.*, **7**, 11 (1907).

(5) B. Jirgensons, *J. prakt. Chem.*, **161**, 181, 293 (1943); **162**, 224, 237 (1944).

(6) M. Heidelberger, B. Davis and H. P. Treffers, THIS JOURNAL, **63**, 498 (1946).

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