[CONTRIBUTION FROM THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY, CALIFORNIA INSTITUTE OF TECHNOLOGY, No. 1226]

Electrophoretic Analysis of Ovalbumin

BY JOHN R. CANN

Introduction

Longsworth, Cannan and MacInnes[1] found that within the *p*H range of 5 to 10 crystalline ovalbumin is resolved electrophoretically into two components, A_1 and A_2, A_2 being the slower moving component. The relative concentration of A_2 in freshly prepared material is reported to vary from 15 to 25%. A third component, A_3, has also been found to be present in small amounts.[2] A_3 is less mobile than A_1 and A_2 at *p*H 6.8.

In connection with the program of protein fractionation by electophoresis-convection being carried out in this Laboratory,[3] it has been necessary to analyze a large number of samples of ovalbumin electrophoretically using the moving boundary technique of Tiselius[4] as modified by Longsworth.[5]

The moving boundary theory recently extended by Dole[6] and Svensson[7] indicates that appreciable error may be made in the usual electrophoretic analyses of proteins due to the superposition of foreign protein and buffer gradients on the concentration gradient of a given electrophoretic component. In the case of protein mixtures in alkaline solution these superimposed gradients would cause the relative concentration of the fastest moving component, *e. g.*, albumin in serum, to appear greater than its real value. Since electrophoretic distributions appear to approach actual distributions at high ionic strengths and low protein concentrations, Longsworth[8] recommends that the true distribution of components be obtained either by extrapolating the apparent electrophoretic distributions to infinite ionic strength at constant protein concentration or zero protein concentration at constant ionic strength. Other workers[9] have suggested extrapolating to zero value of the ratio of protein concentration to ionic strength.

Since the electrophoretic analysis of ovalbumin is complicated by the change of relative mobilities of the components with ionic strength, it appeared desirable to report the findings of this Laboratory with respect to the variations in apparent distribution of the components of ovalbumin as a function of the conditions of electrophoresis.

Experimental

Method.—The electrophoresis apparatus described by Longsworth[5] for use in the analysis of proteins by the Tiselius moving boundary method was used in this investigation. The time and field strength employed are noted under the appropriate pattern in Fig. 1. Photographic records of the refractive index gradients in the boundaries were made by the schlieren scanning method.

The apparent concentrations of the components of ovalbumin were determined by finding the ratio, in each case, of the component area to the total area, exclusive of the ϵ-boundary. The areas were measured on projected tracings of the descending patterns with a planimeter, resolution into components being carried out by the method of Pedersen.[10]

Material.—Crystalline ovalbumin was prepared by the method of Sorensen and Høyrup.[11] Analysis of a 1% solution of a representative preparation in phosphate buffer, *p*H 6.81 and ionic strength 0.1, yielded 76.0% A_1 (-5.93×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1}), 18.2% A_2 (-5.05×10^{-5}) and 5.8% A_3 (-4.4×10^{-5}).

Experimental Results and Discussion

Electrophoretic experiments were carried out in phosphate buffers, *p*H 6.8 and ionic strength 0.05 and 0.1, and in phosphate–chloride buffer, *p*H 6.7 and ionic strength 0.3. In these runs the protein concentration was 0.74%. The resultant electrophoretic patterns are shown in Fig. 1. It will be noted that a marked decrease in resolution into components parallels an increase in ionic strength. Thus, at ionic strength 0.05 excellent resolution into three components was obtained, while at ionic strength 0.3 the pattern consisted of a single asymmetric peak (-4.3×10^{-5}). The apparent distribution of components at ionic strength 0.05 was 78.6% A_1 (-7.08×10^{-5}), 15.2% A_2 (-5.93×10^{-5}) and 6.2% A_3 (-5.2×10^{-5}), and at ionic strength 0.1, 77.9% A_1 (-6.24×10^{-5}), 17.6% A_2 (-5.37×10^{-5}) and 4.5% A_3 (-4.8×10^{-5}). A similar behavior was observed in the case of electrophoresis in acetate buffers. Patterns obtained in phosphate buffer, *p*H 6.8 and ionic strength 0.3, revealed the presence of three components,

(1) L. G. Longsworth, R. K. Cannan and D. A. MacInnes, THIS JOURNAL, **62**, 2580 (1940).

(2) C. F. C. MacPherson, D. H. Moore and L. G. Longsworth, *J. Biol. Chem.*, **156**, 381 (1944); R. A. Alberty, E. A. Anderson and J. W. Williams, *J. Phys. and Colloid Chem.*, **52**, 217 (1948).

(3) L. E. Nielsen and J. G. Kirkwood, THIS JOURNAL, **68**, 181 (1946).

(4) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

(5) L. G. Longsworth, *Chem. Rev.*, **30**, 423 (1942); *Ind. and Eng. Chem., Anal. Ed.*, **18**, 219 (1946).

(6) V. P. Dole, THIS JOURNAL, **67**, 1119 (1945).

(7) H. Svensson, *Arkiv. Kemi, Mineral. Geol.*, **17A**, No. 14, 1 (1943); **21B**, No. 5, 1 (1945); **22A**, No. 10, 1 (1946).

(8) L. G. Longsworth, *J. Phys. and Colloid Chem.*, **51**, 171 (1947).

(9) G. E. Perlmann and D. Kaufman, THIS JOURNAL, **67**, 638 (1945); S. H. Armstrong, Jr., M. J. E. Budka, and K. C. Morrison, *ibid.*, **69**, 416 (1947).

(10) T. Svedberg and K. O. Pedersen, "The Ultracentrifuge," Oxford Univ. Press, London, 1940, p. 296.

(11) S. P. L. Sorensen and M. Høyrup, *Compt. rend. trav. lab. Carlsburg*, **12**, 12 (1916).

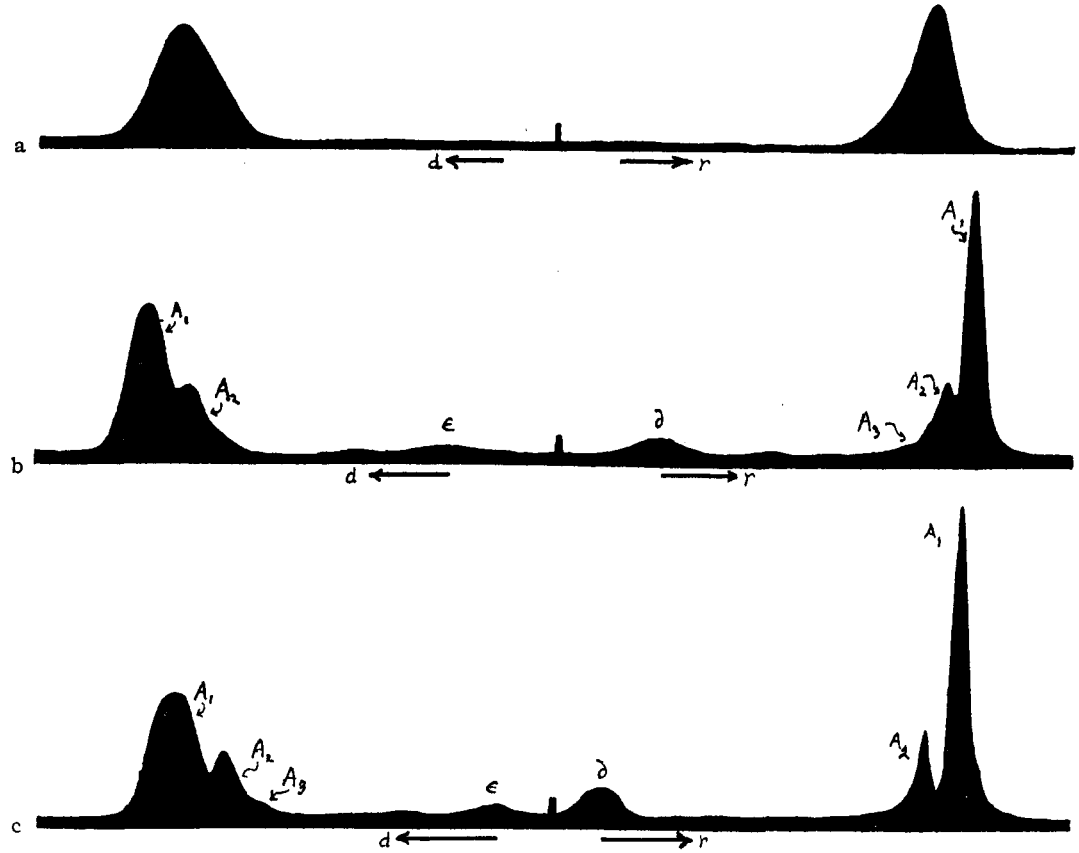

Fig. 1.—Electrophoretic patterns showing the effect of ionic strength on resolution of ovalbumin at a protein concentration of 0.74%; a, phosphate–chloride buffer of ionic strength 0.3, 28 hr. at a field strength of 1.1 volts cm.$^{-1}$; b, phosphate buffer of ionic strength 0.1, 4 hr. at a field strength of 5.0 volts cm.$^{-1}$; c, phosphate buffer of ionic strength 0.05, 2.2 hr. at a field strength of 8.9 volts cm.$^{-1}$.

although considerably less resolution was obtained than at ionic strength 0.1. In accordance with theory the ϵ- and ∂-boundary anomalies were diminished and the enantiography of the patterns were increased as a result of increasing the ionic strength.

Because of the strong dependence of relative mobilities upon ionic strength, the "true"[12] electrophoretic distributions of components were determined by extrapolation of apparent distributions to zero protein concentration at constant ionic strength instead of to infinite ionic strength at constant protein concentration or to zero ratio of concentration to ionic strength. The apparent distributions were obtained by electrophoresis in phosphate buffer, *p*H 6.8 and ionic strength 0.1. A representative series of determinations is presented in Table I. The deviations of the apparent from extrapolated relative concentrations of component A_1 when plotted against the protein concentration fall on straight lines, arising from the origin. Theoretical deviations for ovalbumin samples containing 82.7 and 68.8% of A_1 were calculated by the equations of Dole, and are compared with experimental data in Fig. 2. The agreement between the computed and observed deviations is as good as can be expected considering the nature of the approximations made in the computations.[13] It would appear that Dole's the-

(12) It should be borne in mind that the extrapolated composition has not been shown to be the true value.

(13) The calculations were simplified by including the small contribution of component A_3 to the electrophoretic pattern with that of component A_2. Thus, the protein was considered as being composed of two components of mobilities -6.45×10^{-5} and -5.88×10^{-4}, which correspond to the mobilities of A_1 and A_2, respectively, at zero protein concentration. These mobilities were obtained by extrapolation of the plot of the reciprocal of the mobility *vs.* the square root of the protein concentration, linear in the range of protein concentration 2 to 0.4%, to zero concentration. This method of extrapolation was suggested by Dr. A. Bueche, Baker Chemistry Laboratory, Cornell University.

In the calculations of the $V\sigma$ products for the boundaries (Dole,[6] equation 33) and of the component concentration ratios across them (Dole,[6] equation 31), (whose summations define the deviations of apparent from true electrophoretic distributions), the following relative mobilities were used: sodium + 1, monohydrogen phosphate -1.10, dihydrogen phosphate -0.66, component A_1 -0.322, component A_2 -0.293.

The equivalent concentration of component A_1 was arbitrarily taken from the net charge per g. of ovalbumin (-2.7×10^{-4} faraday equivalent per g. of protein) as given by the titration data of Cannan, Kibrick and Palmer (*Ann. N. Y. Acad. Sci.*, **41**, 243 (1941)). For component A_2 the net charge per gram was assigned the same ratio with that of A_1 as between its mobility and the mobility of A_1.

ory adequately accounts for the errors involved in ordinary electrophoretic analyses.

TABLE I

ELECTROPHORETIC ANALYSES OF OVALBUMIN

Experiment		Protein concentration, % by wt.	Apparent electrophoretic concentration of A_1, %	Deviation of apparent electrophoretic from extrapolated concentration of A_1, %
I	1	1.41[a]	88.4	5.7
	2	0.89[a]	86.8	4.1
	3	0.72[a]	85.5	2.8
Extrapolation		0	82.7	
II	1	2.6[b]	90.3	12.9
	2	17.[b]	86.9	9.5
	3	1.2[b]	83.2	5.8
Extrapolation		0	77.4	
III	1	1.96[a]	85.3	11.8
	2	0.95[a]	79.2	5.7
Extrapolation		0	73.5	
IV	1	1.4[b]	81.1	9.4
	2	1.2[b]	79.4	7.7
	3	0.8[b]	76.8	5.1
Extrapolation		0	71.7	
V	1	1.87[a]	81.9	13.1
	2	1.05[a]	76.1	7.3
Extrapolation		0	68.8	

[a] From nitrogen determination. [b] From area of electrophoretic pattern. The relation between the protein concentration and the area of the electrophoretic pattern was determined empirically over the concentration range of 0.7 to 2%.

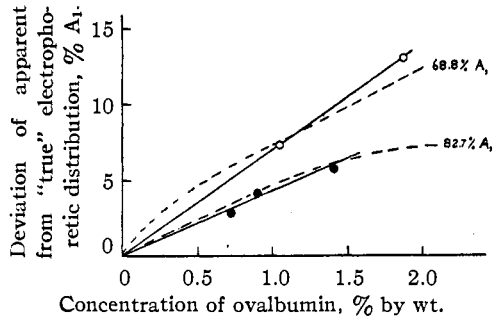

Fig. 2.—Deviations of apparent from "true" electrophoretic distributions: solid line, experimental deviations; broken curve, deviations calculated from the equations of Dole.

Acknowledgment.—The author is indebted to Dr. J. G. Kirkwood for his interest in this work and for his review of the manuscript.

Summary

The electrophoretic analysis of ovalbumin is complicated by the fact that the change of relative mobilities of the components with ionic strength is such that, while resolution into components is obtained at *p*H 6.8 and ionic strength 0.1, only slight if any resolution is found at ionic strength 0.3. "True" electrophoretic distributions of components were obtained by extrapolation of apparent distributions to zero protein concentration at constant ionic strength of 0.1. The moving boundary theory of Dole adequately accounts for the deviations of apparent from "true" distributions.

PASADENA, CALIFORNIA RECEIVED JULY 28, 1948

[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TEXAS]

Spectrophotometric Studies of Complex Formation with Sulfosalicylic Acid. II. With Uranyl Ion

BY ROBERT T. FOLEY AND ROBBIN C. ANDERSON

As part of a series of studies on complex formation with sulfosalicyclic acid, it has been of interest to compare the reactions of uranium—as a member of another transition series of elements—with that of iron. Weil and Rozenblum[1] reported a solid compound of uranium and sulfosalicylate, $[C_6H_3(OH)(SO_3)(COO)]_2U$, but no observations in solution were made. Muller, Fernandes, Mai and Weinland and Hager[2] have reported complex formation between uranyl ion and salicylic acid or various salts.

In this particular investigation reactions of sulfosalicyclic acid in solutions containing uranium as uranyl ion have been studied.

(1) St. Weil and St. Rozenblum, *Bull. tran. inst. pharm. (Poland)*, **1**, 1 (1902).

(2) See R. Weinland and K. Hager, *Z. anorg. allgem. Chem.*, **160**, 193 (1927).

Experimental

Materials.—Uranyl nitrate dihydrate was obtained by vacuum drying the hexahydrate (Eimer and Amend C. P grade) over sulfuric acid. A 0.005 *M* uranyl nitrate solution was prepared by dissolving the proper weight of the dihydrate in water at 25°.

Uranyl acetate dihydrate was also simply weighed and dissolved in water. The salt was Eimer and Amend C. P grade.

The sulfosalicylic acid was a special sample from Eastman Kodak Co. with melting point 112–120° (reported in literature, 108–113°.[3]) Analysis for sulfate gave 0.06%. Solutions of this acid were standarized by titration against a National Bureau of Standards sample of potassium acid phthalate.

Apparatus.—Measurements of optical density were made with a Beckmann Model DU Quartz Spectrophotometer, using a tungsten lamp and Corex cells of 10-mm. light path. The cells were held in a thermostatted cell

(3) Schulze, *Apoth. Ztg.*, **51**, 319 (1936).