cell is four molecules long in the c direction, this means that an increase of 8 carbon atoms in the chain direction has caused an increase in d_{001} of 11.02 Å. In order for a change of this magnitude to occur, a change of tilt of the order of 7.5° would have to occur between the two compounds. That this difference in tilt actually exists is not probable, since Buerger[2] has shown that the symmetry properties of the two compounds are the same.

Using the cell constants determined in this Laboratory the following increases in d_{001} between the pairs of substances are noted:

Compounds	Δd_{001}, Å.
NaMy–NaP	10.26
NaP–NaSt	10.19
NaSt–NaAr	10.32

Using a least squares approximation on values for d_{001} *vs.* number of carbon atoms in the chain, the most probable value for change in d_{001} is found to be 10.25 Å.

The work of MacArthur[8] on the extension of the C⟨C⟩C link in normal long chain aliphatic compounds indicates that an addition of 8 carbon atoms to a compound of this type should give an increase in chain length of 10.16 Å. MacArthur also lists the data of a number of other workers in this field, and shows that the average of their results would lead to a value of 10.19 Å. (It is not apparent whether the figures published by MacArthur are in kX or Å. units. If the published figures are in kX units, the two results in Å. would then be 10.18 and 10.21, respectively.)

The results of this Laboratory also show a difference of 0.09 Å. between the a_0 value for NaMy and the corresponding a_0 values for NaP and NaSt. There is no apparent reason for the existence of a real difference in the values, and it is assumed that this difference arises from experimental error since the NaMy crystals obtained were poorly formed.

The agreement in d_{001} increase between pairs of compounds in this series and the agreement with the figures for other normal long chain aliphatic compounds indicates that the long axes of the chains in these compounds are very nearly normal to (001) and that there is little or no difference in tilt in the several crystals. This conclusion is also corroborated by unpublished work in this Laboratory on the hydrazides of n-aliphatic acids. In the hydrazides it is found that an increase of 8 carbon atoms in the chain direction leads to an increase in d_{001} of 10.19 Å.

(8) MacArthur, *Proc. Leeds Phil. Lit. Soc., Sci. Sect.*, **4**, pt. 3, 170 (1944).

Summary

1. The unit cell constants of the hemihydrates of NaMy and NaAr have been determined, and the values for NaP and NaSt redetermined.

2. The increase in d_{001} with increase in chain length has been found to be 1.281 Å. per carbon atom, indicating that the chains are very nearly normal to (001).

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The Electrophoretic Properties of Plakalbumin

BY GERTRUDE E. PERLMANN

Introduction

In a recent note, Linderstrøm-Lang and Ottesen[1] showed that ovalbumin is transformed, by the action of an enzyme from *Bacillus subtilis*, into a protein that crystallizes as rectangular plates from ammonium sulfate. They named this protein plakalbumin.[2] Since the transformation of ovalbumin to plakalbumin, A→P, occurs without serious degradation of the protein,[1] it seemed of interest to compare the electrophoretic behavior of the two materials. Consequently, the preliminary experiments[3] that indicated that plakalbumin differed electrophoretically from its parent substance, ovalbumin, have been extended and the new results are reported below.

Experimental

Plakalbumin was obtained by incubating a 6% solution of thrice recrystallized ovalbumin[4] in 0.02 ionic strength phosphate buffer at *p*H 6.8 with an enzyme preparation from *B. subtilis*, kindly supplied by Linderstrøm-Lang. After incubation for six hours at 30°, the solution was acidified with hydrochloric acid to *p*H 5.4 and the protein crystallized as rectangular plates by the addition of saturated ammonium sulfate solution.[1]

The electrophoretic experiments were carried out at 0.5° in the apparatus described by Longsworth.[5,6] Unless noted otherwise below, a protein concentration of 1% was used in the experiments and prior to electrophoresis the protein solutions were dialyzed for two to three days against liberal portions of an appropriate buffer. The mobilities were computed from the descending patterns and refer to 0°.

(1) Linderstrøm-Lang and Ottesen, *Nature*, **159**, 807 (1947).

(2) Eeg-Larsen, Linderstrøm-Lang and Ottesen, *Arch. Biochem.*, **19** 340 (1948).

(3) Perlmann, *Nature*, **161**, 720 (1948).

(4) Sørensen and Høyrup, *Compt. rend. Lab. Carlsberg*, **12**, 12 (1917).

(5) Longsworth, *Chem. Reviews*, **30**, 323 (1942).

(6) Longsworth, *Ind. Eng. Chem., Anal. Ed.*, **18**, 219 (1946).

Results

In Fig. 1 are superimposed the tracings of the patterns obtained after electrophoresis of ovalbumin and plakalbumin in a 0.1 ionic strength phosphate buffer at *p*H 6.8 at a potential gradient of 6 volts per cm. for 9900 seconds. Under these conditions superposition of the patterns permits not only a direct comparison of the relative concentrations of the components but also of their approximate mobilities. It is thus clear from Fig. 1 that although the patterns are similar in appearance the plakalbumin has a lower mobility, u, than its parent substance ovalbumin. The actual values are -5.9×10^{-5} and -5.5×10^{-5} cm.^{2} sec.$^{-1}$ volt^{-1} for the main components, A_1 and P_1, of ovalbumin and plakalbumin, respectively, and -4.8×10^{-5} and -4.3×10^{-5} for the components A_2 and P_2. Plakalbumin from a sample of ovalbumin incubated for twenty-four hours gave the same results. Furthermore, as is the case with ovalbumin[7] at *p*H values below 4.0, plakalbumin also migrates over a distance of at least 7.0 cm. in the channel of the electrophoresis cell without resolution of the boundary into more than one peak.

In the preliminary work on plakalbumin it was observed that the relative concentration of the components, P_1 and P_2, but not their mobilities, changed on repeated crystallization of the freshly prepared protein. However, if the time between successive crystallizations is kept at a minimum and if the plakalbumin is stored as a paste under saturated ammonium sulfate, no significant alteration occurs. On the other hand, prolonged dialysis, or storage of plakalbumin in solution, causes marked changes. Thus, in a solution stored at 3° for ten days, the amount of the slowly moving component, P_2, had increased from 16 to 44% at the expense of the faster one, P_1. This result may be considered in connection with the observation of the Danish workers that in plakalbumin solutions at *p*H values above 5, even after repeated recrystallizations and dialysis, a slow liberation of non-protein nitrogen occurs.[2]

Mobilities of Ovalbumin and Plakalbumin at Different *p*H Values.—Much of this work has been directed toward the determination of the mobilities of the two proteins at various *p*H values. The results of these measurements are presented in Table I, in which the nature and composition of the buffers is listed in the first column while the *p*H of the protein solution in equilibrium therewith is given in column 2. The mobilities listed in this table refer in each case to the fast moving component of the proteins, except for those at *p*H values below 4.0 where the proteins migrate as a single peak. In these cases the mobilities are mean mobilities. A comparison of the figures for plakalbumin, column 3, with those for ovalbumin, column 4, shows that the

(7) Longsworth, Cannan and MacInnes, THIS JOURNAL, **62**, 2580 (1940).

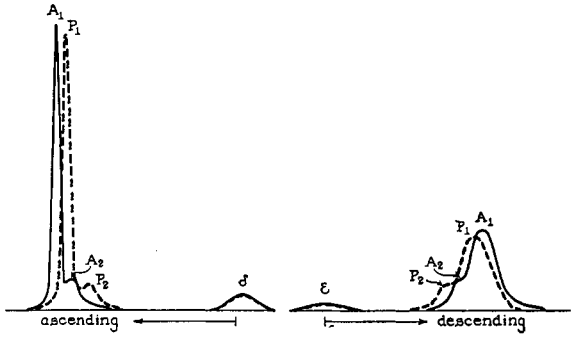

Fig. 1.—Superimposed tracings of electrophoretic patterns of a 1% solution of ovalbumin, —, and plakalbumin, - - -, after electrophoresis in sodium phosphate buffer at *p*H 6.8 and 0.1 ionic strength for 9900 seconds at 6 volts per cm.

mobilities of the two proteins at any given *p*H below about 7.0 differ by 0.5 to 0.6 $\times 10^{-5}$, column 5. At *p*H values above *p*H 7.8 the two proteins migrate with the same velocity.

TABLE I

MOBILITIES OF PLAKALBUMIN AND OVALBUMIN IN BUFFER SOLUTIONS OF IONIC STRENGTH 0.1

Ac = acetate; Cac = cacodylate; V = diethyl barbiturate

1	2	3	4	5
		$u \times 10^5$		
Buffer	*p*H, 25°	Plakalbumin	Ovalbumin	Δu
0.1 *N* HCl—0.5 *N* glycine	3.10	6.54	5.95	0.59
.1 *N* NaAc—0.5 *N* HAc	3.91	3.25	2.71	.54
.1 *N* NaAc—0.14 *N* HAc	4.50	1.06		
.1 *N* NaAc—0.1 *N* HAc	4.64	0.29	−0.25	.54
.1 *N* NaAc—0.06 *N* HAc	4.89	−0.74		
.1 *N* NaAc—0.01 *N* HAc	5.65	−2.91	−3.50	.59
.02 *N* NaCac—0.004 *N* HCac —0.08 *N* NaCl	6.79	−4.50	−5.16	.66
.02 *N* NaV—0.02 *N* HV —0.08 *N* NaCl	7.82	−5.62	−5.65	
.1 *N* NaV—0.02 *N* HV	8.60	−5.84	−5.78	
.1 *N* NaV—0.005 *N* HV	9.10	−5.93	−5.92	

The Behavior of Mixtures of Plakalbumin and Ovalbumin.—Although the mobility differences below *p*H 7.0 are sufficient to lead one to expect resolution of the ovalbumin and plakalbumin on mixing, attempts in a 0.1 ionic strength phosphate buffer at *p*H 6.8 and in an 0.1 ionic strength hydrochloric acid–glycine buffer at *p*H 3.0 failed. The resulting patterns for the mixture were identical in appearance with those of either protein except that the boundary displacement corresponded to mobility values intermediate between those of the components of the mixture. Resolution is obtained, however, at a *p*H of 4.64, *i.e.*, between their isoelectric *p*H values. This is shown in Fig. 2 where the full line represents the patterns obtained after electrophoresis of a 1.2% solution of a mixture of 33% ovalbumin and 67% plakalbumin at a potential gradient of 6 volts per cm. for 28,800 seconds. The dashed and dotted curves superimposed upon this pattern are those of ovalbumin and plakalbumin, re-

spectively, obtained in separate experiments under identical conditions.

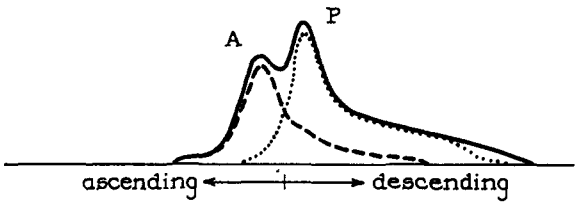

Fig. 2.—Superimposed tracings of electrophoretic patterns of a 1.2% mixture of 33% ovalbumin and 67% plakalbumin —, of a 0.4% solution of ovalbumin - - -, and of a 0.8% solution of plakalbumin . . . . , in 0.1 N sodium acetate buffer at pH 4.64 at 6 volts per cm. for 28800 seconds.

Dependence of the Isoelectric pH of Plakalbumin upon Ionic Strength in Acetate Buffers.—As is shown by the data of Table II the isoelectric pH of plakalbumin, designated here as pI, shifts to higher values at low ionic strength in acetate buffers. The pI values at each ionic strength, column 4, were obtained by linear interpolation of the pH mobility values listed in the two preceding columns of this table. Owing to the complexity of the electrophoretic patterns of plakalbumin at pH values just below the isoelectric pH, the first moment of the entire gradient curve of the descending pattern is used for the computation of the mean mobility, reported in Table II.

TABLE II

DEPENDENCE OF THE ISOELECTRIC pH ON IONIC STRENGTH

1	2	3	4	5	6
			pI		
μ	pH[a]	$u_p \times 10^5$	Plakalbumin	Ovalbumin	ΔpI
0.1	4.50	1.06			
	4.67	0.29	4.72	4.58	0.14
	4.90	−0.74			
.05	4.68	0.82	4.77	4.63	.14
	4.88	−0.75			
.02	4.73	0.7	4.82	4.68	.14
	4.96	−1.54			
.01	4.68	1.95	4.86	4.71	.15
	4.96	−1.06			

[a] pH values refer to 0° by applying a correction of 0.025 to the values measured at 25°.[8]

For comparison, the pI values of ovalbumin obtained by Tiselius and Svensson[8] are listed in column 5 of Table II. Except for a constant difference of 0.14 pH unit, it is apparent that the isoelectric pH of plakalbumin changes with the buffer salt concentration in the same manner as does that of ovalbumin.

The response of plakalbumin to the partial substitution of chloride for acetate in these buffers is also similar to that of ovalbumin; thus if 80% of the acetate ion in a 0.1 N buffer is replaced by chloride ion, the isoelectric pH of plakalbumin is reduced to 4.58, whereas that of ovalbumin to 4.44, a difference of 0.14 pH unit as in the case of the pure acetate buffers, Table II. This displacement of the isoelectric pH on substitution of chloride for acetate toward a more acid reaction is comparable with that observed in the case of other proteins.[9]

(8) Tiselius and Svensson, *Trans. Faraday Soc.*, **36**, 16 (1940).

Discussion

Since there is considerable evidence for a constant proportionality between mobility and the net charge of the protein ion[10,11] it is of interest to compare the change in mobility Δu, accompanying the A→P transformation, with other properties of these two proteins. Of the many explanations for this change that might be advanced the following is a reasonable one at the present time. It is based, in part, on the observation of Eeg-Larsen, Linderstrøm-Lang and Ottesen[2] that the fragments that are lost by the ovalbumin in the A→P process contain a glutamic and possibly an aspartic acid residue in addition to other amino acid residues that would not have ionizing groups when linked, in the α-position, with peptide bonds in the intact protein molecule. In the following discussion the number of ionizing groups that are lost in the A→P process will simply be designated as a. If, therefore, ovalbumin can be idealized as peptide chain, $(HOOC)_mR(NH_2)_n$ with m carboxyl groups that dissociate in the region from pH 2.0 to 6.5 and n amino groups that dissociate above pH 8.5, plakalbumin would be $(HOOC)_{m-a}R'(NH_2)_n$. In strongly acid solution the ovalbumin ion would be $(HOOC)_mR(NH_3^+)_n$ with a net charge, z, of $+n$. Under these conditions plakalbumin should have the same net charge as ovalbumin and if the friction coefficients of the two molecules were the same the mobilities should be identical at a given low pH. A neutral reaction the ovalbumin ion would be $(^-OOC)_mR(NH_3^+)_n$ with a net charge of $n = m$ and since the mobility is negative at this pH one can conclude that $m > n$. Under these conditions the net charge of plakalbumin would be $n - m + a$ and a curve of z against pH, as abscissas, for this protein should be above that of ovalbumin except in acid solutions where the two curves approach the common limiting value of $z = n$. If the a carboxyl groups removed in the A→P reaction are strong ones they will lose their protons at pH values below the isoelectric zone and ovalbumin will thus have an isoelectric pH below that of plakalbumin in which these groups are missing. The experimental observation, Table II, that this is the case thus supports the view that the carboxyl groups that are involved in the A→P transformation are relatively strong ones.

However, the mobility values of plakalbumin

(9) Longsworth and Jacobsen, *J. Phys. and Coll. Chem.*, **53**, 126 (1949)

(10) Abramson, *J. Gen. Physiol.*, **15**, 575 (1931–1932).

(11) Longsworth, *Ann. N. Y. Acad. Sci.*, **41**, 267 (1941).

approach those of ovalbumin at neutrality instead of at acid reactions as the idealized case described above would require. This suggests that the friction coefficients, f, of the two proteins are not identical but that for plakalbumin, f_P is enough lower than that for ovalbumin, f_A, to compensate for the smaller net negative charge at neutrality. At acid reactions where the net charge of both proteins approaches the common value, n, the smaller value of f_P, as compared with f_A, would lead to the higher mobilities for plakalbumin that are observed.

If at any given pH, the values of u_A and u_P refer to the same buffer solvent, the friction coefficients may be defined so that $u_A = z_A/f_A$ and $u_P = z_P/f_P$, where the ratio, f_A/f_P, should be independent of the pH, the ionic strength and the nature of the buffer ions. At the isoelectric pH of plakalbumin, $u_P = z_P = 0$ and since the valence of ovalbumin at this pH, *i.e.*, 4.72, is assumed to differ from that of plakalbumin only by the a ionized carboxyl groups that are present in the one protein but missing from the other, $z_A = -a$, and the mobility of ovalbumin, $u_A = -0.6 \times 10^{-5} = a/f_A$ may then be used to estimate the value of a. Here $1/f_A$ may be taken as the slope, 3.0×10^{-6}, of the line that is obtained by plotting[9] as shown in Fig. 3, the mobilities of ovalbumin against the equivalents, h, of acid in alkali bound per mole of protein at an ionic strength 0.1 and at the given pH.[12] Hence $a = 2$, is in agreement with the result by Eeg-Larsen, Linderstrøm-Lang and Ottesen.[2] In view, however, of the arbitrary nature of the assumptions that have been made in the use of the mobility data for the evaluation of a, this agreement should not necessarily be interpreted as confirmation of the chemical analysis. Probably the most that should be said is that the electrophoretic results are not inconsistent with the analytical ones.

In experiments described elsewhere,[3] the author has also observed that plakalbumin differs electrophoretically from the ovalbumin modification reported by MacPherson, Moore and Longsworth.[13] Unpublished results further indicate that their modification is also a product of an enzymatic reaction. It, therefore, appears possible to transform, without serious degradation, a protein like ovalbumin into several other well-defined proteins which differ from each other in at least one property, namely, in their behavior in the electric field.

(12) Cannan, Kibrick and Palmer, *Ann. N. Y. Acad. Sci.*, **41**, 243 (1941).

(13) MacPherson, Moore and Longsworth, *J. Biol. Chem.*, **156**, 381 (1944).

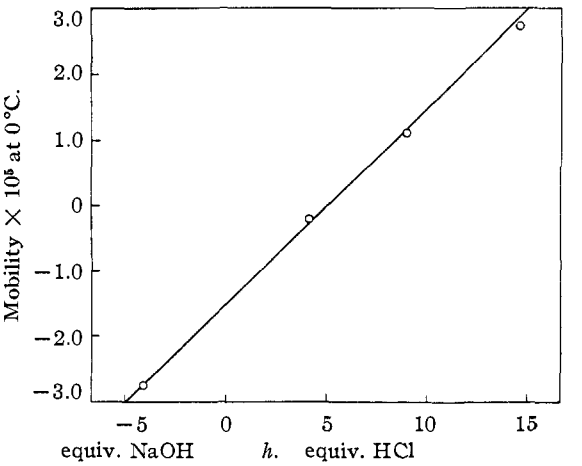

Fig. 3.—Plot for the evaluation of the proportionality factor between mobility and titration increment for ovalbumin.

Acknowledgment.—The author is much indebted to Dr. L. G. Longsworth of these Laboratories for suggestions as to the interpretation of the experimental results and also to him and to Dr. D. A. MacInnes for criticism of the manuscript. My thanks go also to Professor K. Linderstrøm-Lang of the Carlsberg Laboratory, Copenhagen, for his constant stimulating interest in this research.

Summary

The electrophoretic behavior of plakalbumin, a protein derived from ovalbumin by the action of an enzyme from *B. subtilis*, has been compared with that of the parent substance. At pH values below about 7.0, the mobilities differ by 0.5 to 0.6×10^{-5} whereas at more alkaline reactions plakalbumin and ovalbumin are indistinguishable electrophoretically. The isoelectric pH of plakalbumin at all ionic strengths investigated is 0.14 pH unit above that of ovalbumin.

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