aggregation and therefore the complex electrophoretic pattern must be due to some other phenomena. Apparently the absolute amount of the slow component present in the electrophoretic cell remained approximately constant over the range of concentration examined. Table II also contains some data taken from Polis, *et al.*[5] At 1% protein concentration they found a mobility of 1.8×10^{-5} for β_1 at *p*H 4.8 compared to 1.7×10^{-5} at *p*H 4.85 in the present study. For the *fast* moving component they indicate a mobility of 3.0×10^{-5}; the fast component of β_2 in the present study has a mobility of approximately 3.0×10^{-5} at about 0.5% concentration.

Isoelectric Points.—In Table III are listed the electrophoretic mobilities of the two proteins in acetate buffer at *p*H's near their isoelectric points. From these data the isoelectric points were calculated for both the ascending and descending sides; the averages of these values are reported in the table as the isoelectric points of the β_1- and β_2-lactoglobulins.

TABLE III

ELECTROPHORETIC MOBILITIES OF β_1- AND β_2-LACTOGLOBULIN NEAR THE ISOELECTRIC POINT IN ACETATE BUFFER $I = 0.1$

Concentration of β_1, 0.5%; of β_2, 1.1%

		$\mu \times 10^5$		
	*p*H	Desc.	Asc.	
β_1	5.20	+0.1[a]	+0.4[a]	
	5.32	− .6	− .8	*p*I = 5.23
β_2	5.13	− .4	.0	
	5.23	− .9	− .5	*p*I = 5.09

[a] Differences primarily due to boundary asymmetry.

The difference in isoelectric point may not be impressive, but it is real. It is therefore of interest to compare these isoelectric points with the values of Polis, *et al.*,[5] who found *p*I = 5.3 for β_1-lactoglobulin and *p*I = 5.1 for pooled β-lactoglobulin.

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[CONTRIBUTION FROM THE KERCKHOFF LABORATORY OF BIOLOGY AND THE GATES AND CRELLIN LABORATORIES OF CHEMISTRY OF THE CALIFORNIA INSTITUTE OF TECHNOLOGY]

Electrophoresis and Ultracentrifuge Studies of Milk Proteins. II. α-Lactalbumin[1,2]

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α-Lactalbumin shows various degrees of heterogeneity in boundary electrophoresis at *p*H's outside its isoelectric range. The degree of heterogeneity depends also on the protein concentration and the buffer ions. In the ultracentrifuge α-lactalbumin is homogeneous at room temperature and at 7°. The isoelectric point has been found to be *p*I = 5.1, which lies outside the *p*H range of minimum solubility.

Introduction

The name lactalbumin has been applied to various protein preparations derived from milk even before criteria were adopted for the practical definition of the terms albumin and globulin in protein chemistry. A crystalline material which he called lactalbumin was observed and reported first by Wichmann.[3] He removed the casein and crude globulins by precipitation with magnesium sulfate and acid and obtained crystals from the remaining albumin fractions by addition of ammonium sulfate and acid. Sjögren and Svedberg[4] obtained crystals in a similar way. In both cases, the term lactalbumin was applied to the crystals, but it is now clear that the crystals were not pure and that they consisted primarily of the protein now called β-lactoglobulin.

Pedersen[5] in his extensive ultracentrifuge studies found several components in bovine whey; he called the slowest moving component α, the next β, etc. His β is found in the term β-lactoglobulin, which it represents. The α-component was isolated by Kekwick in 1935 (unpublished results quoted in reference 5). Its sedimentation constant was determined[6] and reported to be $S_{20} = 2 \times 10^{-13}$. The term α-lactalbumin was first used by Svedberg.[6] Sørensen and Sørensen[7] gave a procedure for isolating a "crystalline insoluble substance" from bovine whey, and showed that it was not β-lactoglobulin. This procedure was the basis of the method used by Gordon and Semmett,[8] who suggested the name α-lactalbumin for such preparations. Further variations of this method have been described by Gordon, *et al.*,[9,10] and by Zweig and Block.[11]

The present authors first became interested in α-lactalbumin as an attractive protein for X-ray diffraction investigations; it has a low molecular weight (about 16,000) and readily forms large crystals which can be obtained in abundance. At first, the work reported in the present paper was undertaken as an investigation of a possible heterogeneity of the protein, an indication of which was

(1) Contribution No. 2155 from the Gates and Crellin Laboratories of Chemistry.

(2) Supported by the Carnation Company and by a grant from the National Science Foundation.

(3) A. Wichmann, *Z. physiol. Chem.*, **27**, 575 (1899).

(4) B. Sjögren and T. Svedberg, THIS JOURNAL, **52**, 3650 (1930).

(5) K. O. Pedersen, *Biochem. J.*, **30**, 948 (1936).

(6) T. Svedberg, *Nature*, **139**, 1051 (1937).

(7) M. Sørensen and S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg, Sér. chim.*, **23**, 55 (1939).

(8) W. G. Gordon and W. F. Semmett, THIS JOURNAL, **75**, 328 (1953).

(9) W. G. Gordon, W. F. Semmett and J. Ziegler, *ibid.*, **76**, 287 (1954).

(10) W. G. Gordon and J. Ziegler, "Biochemical Preparations," Vol. 4, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 16.

(11) G. Zweig and R. J. Block, *Arch. Biochem. Biophys.*, **51**, 200 (1954).

reported by Gordon and Semmett.[8] It subsequently came to include a further characterization of α-lactalbumin by means of electrophoresis and ultracentrifuge techniques under various conditions of *p*H, temperature and protein concentration.

Experimental

Isolation and Purification of α-Lactalbumin.—Pooled milk and milk from a single cow[12] (containing the β_2-type lactoglobulin only) served as starting material.

The method of Gordon, *et al.*,[9,10] was used. In the first crystallization step, however, the addition of salt was discontinued when about 25–30% of the expected yield had crystallized. In this way it was hoped to minimize possible contamination with β-lactoglobulin, which can co-crystallize with α-lactalbumin.[7] Recrystallization and reprecipitation were performed three times and the final crystals were stored under the mother liquor at 4° until used.

Preparation of Solutions.—The crystalline protein was dissolved in the buffer solution and then dialyzed at 4° against a large volume of buffer for 20 hours or more. Protein concentrations were measured with a differential refractometer and *p*H's were measured at room temperature with a glass electrode.

Electrophoresis Experiments.—A Perkin-Elmer model 38 unit with microcell was used. The temperature was about 0° (ice-water). The experiments were carried on until the fast moving boundary had almost crossed the cell. In one extended experiment (16 hours) the Spinco model M unit was used since its compensating device permits experiments of such long duration.

Ultracentrifuge Experiment.—A Spinco Analytical Ultracentrifuge model E with synthetic boundary cell was used. The nominal speed was 59,870 r.p.m. Boundary positions were evaluated with a microcomparator.

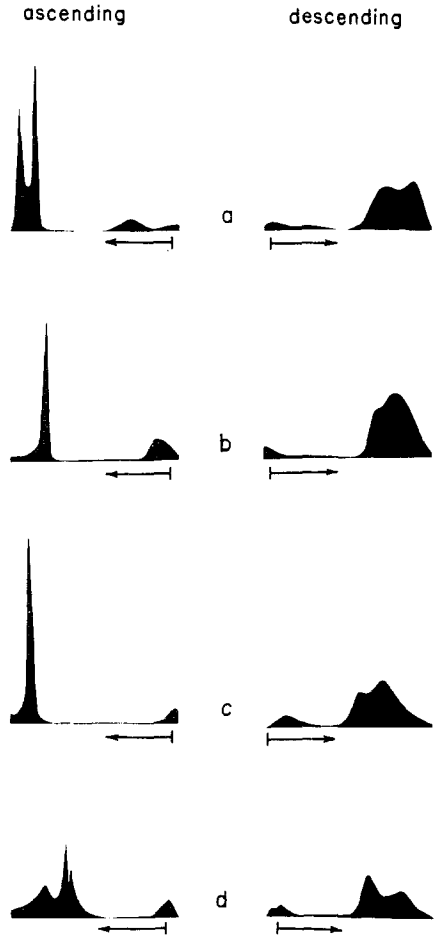

Fig. 1.—Electrophoretic pattern of α-lactalbumin solutions listed in Table I: (a) lactate, C = 1.25%; (b) phosphate, C = 1.4%; (c) "tris," C = 1.25%; (d) veronal, C = 1.0%.

(12) All milk was supplied through the courtesy of the Carnation Milk Farms, Carnation, Washington.

Results and Discussion

Electrophoretic Experiments.—Figure 1, showing patterns of α-lactalbumin obtained in lactate, phosphate, "tris" and barbitol buffers, demonstrates heterogeneity of the protein in all cases. When lactate buffer was used the heterogeneity was apparent at an earlier stage in the experiment than when the other buffers were used. This may explain the fact that Gordon and Semmett[8] detected heterogeneity in lactate buffer only.

Figure 2 shows that the heterogeneity of α-lactalbumin in lactate buffer varies with protein concentration. This also was true to a lesser extent in "tris" buffer.

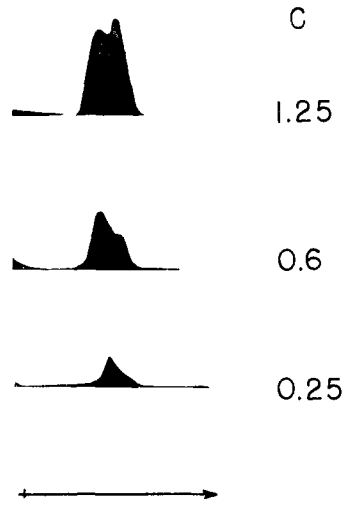

Fig. 2.—Descending boundaries of α-lactalbumin in lactate buffer, I = 0.1, *p*H 3.3. Photographs are taken after the experiments had proceeded the same length of time. The numbers indicate the protein concentration in percentage.

The mobilities of the fast and slow moving components in the buffers mentioned showed some dependence on the protein concentration. Average values for the descending side are given in Table I, mainly to illustrate the changes observed with different *p*H and buffer ions. The general lability of the protein makes it difficult, however, to reproduce the values with great accuracy.

TABLE I

ELECTROPHORETIC MOBILITIES OF α-LACTALBUMIN[a] IN SOME BUFFER SOLUTIONS, I = 0.1

Buffer soln.	*p*H	Slow component $\mu \times 10^5$	Fast component $\mu \times 10^5$
Lactate	3.3	+6.5	+8.0
Phosphate	7.5	−5.3	−5.9
"Tris"	7.5	−3.1	−3.9
Barbitol	8.5	−4.8	−6.6

[a] Measured on descending side.

The nature of the heterogeneity in lactate buffer was investigated in an extended electrophoresis

experiment lasting 16 hours. Figure 3 shows that the resolution of the two main components was not complete although the peaks were spaced about 30 mm. apart and the fast component had travelled close to 200 mm. It seems therefore that the two components remain interdependent throughout the experiment. In addition material corresponding to the fast peak was removed with a syringe and equilibrated against the lactate buffer. This material gave in the Perkin-Elmer unit the results shown in Fig. 3a. Again the interdependence is strongly suggested.

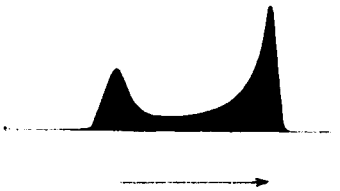

Fig. 3.—(Top) ascending boundary of 1.55% α-lactalbumin in lactate buffer, I = 0.1, pH 4.83. Picture taken after 16 hours using 10 v. cm.$^{-1}$. Initial boundary lost on compensation. Descending boundary was like an extended version of Fig. 1a descending. Fig. 3a (Bottom) Electrophoretic pattern of "fast component" of Fig. 3 (C = 0.25%) in lactate buffer.

The possible presence of β-lactoglobulin as an impurity was investigated by electrophoresis of the α-lactalbumin stock material with and without the addition of 10% of β-lactoglobulin. Acetate buffer at pH 4.8 turned out to be an excellent medium for this investigation. α-Lactalbumin shows no heterogeneity at this pH, and its mobility differs sufficiently from that of β-lactoglobulin. The results (Fig. 4) gave no evidence of β-lactoglobulin being present in the α-lactalbumin used, and showed in addition that the proteins move in the same direction at pH 4.8, namely, as cations.

The isoelectric point pI = 4.1-4.8 of α-lactalbumin listed by the Committee of Milk Protein Nomenclature, Classification, and Methodology of the Manufacturing Section of the American Dairy Science Association[13] probably was not based on electrophoretic measurements but inferred from the observed minimum solubility of the protein over this range. Gordon and Semmett[8] state that the "isoelectric protein is only slightly soluble in water."

(13) R. Jenness, *et al.*, *J. Dairy Sci.*, **39**, 536 (1956).

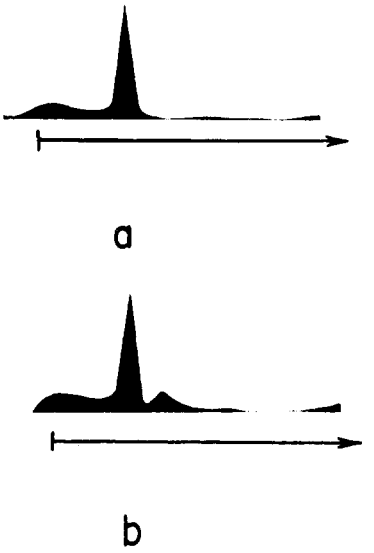

Fig. 4.—Ascending boundary of α-lactalbumin in acetate buffer, I = 0.1, pH 4.83: (a) 0.5% α-lactalbumin, (b) 0.5% α-lactalbumin + 0.05% β-lactoglobulin. Picture taken after 6 hours using 10 v. cm.$^{-1}$.

In the present study the isoelectric point was measured in acetate buffer. At pH 5.25 the mobility was found to be -0.6×10^{-5}, at pH 4.98 it was $+0.5 \times 10^{-5}$. By interpolation of these data the isoelectric point is found to be about 5.1. The solubility of α-lactalbumin at this pH is appreciably higher than it is in the pH range 4.0–4.6.

Ultracentrifuge Experiments.—Preliminary experiments in phosphate buffer pH 7.5, I = 0.1, t = 25° gave homogeneous pattern with S_{20} = 1.79–1.83 S. Since heterogeneity in the electrophoretic experiments was most pronounced in lactate buffer at about 1.5% protein concentration this condition was chosen for a low temperature (7°) experiment in the ultracentrifuge. The pattern was again homogeneous and the boundary was not unusually broad (S_{20} = 2.0).

Lability of α-Lactalbumin.—The electrophoretic experiments gave a determination of the isoelectric point of α-lactalbumin and also indicated its heterogeneous nature in various buffer solutions. The results of the ultracentrifuge experiments exclude the explanation of the electrophoretic heterogeneity as a dimerization or similar molecular interaction. Apparently when α-lactalbumin is dissolved in buffer solutions changes take place as a result of which the final solution contains the protein in more than one molecular form. The observations of Zittle and Della-Monica[14] and of Zittle[15] on the effects of lyophilizing and salts on α-lactalbumin point in the same direction.

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(14) C. A. Zittle and E. S. Della-Monica, *Arch. Biochem. Biophys.*, **58**, 31 (1955).

(15) C. A. Zittle, *ibid.*, **64**, 144 (1956).