Brockman, P. W., and Heppel, L. A. (1968), *Biochemistry 7*, 2554.
Cashel, M., and Freeze, E. (1964), *Biochem. Biophys. Res. Commun. 16*, 541.
Chesbro, W. R., and Lampen, J. O. (1968), *J. Bacteriol. 96*, 428.
Clark, J. M., Ed. (1964), *in* Experimental Biochemistry, San Francisco, Calif., W. H. Freeman, p 32.
DeMoss, R. D., and Moser, K. (1969), *J. Bacteriol. 98*, 167.
Dorn, G. L. (1968), *J. Biol. Chem. 243*, 3500.
Dvorak, H. F. (1968), *J. Biol. Chem. 243*, 2640.
Edelhoch, H. (1967), *Biochemistry 6*, 1948.
Ehrenberg, A. (1957), *Acta Chem. Scand. 11*, 1257.
Fiske, C. H., and Subbarow, Y. (1925), *J. Biol. Chem. 66*, 375.
Friedberg, I., and Avigad, G. (1967), *Eur. J. Biochem. 1*, 193.
Garen A., and Levinthal, C. (1960), *Biochim. Biophys. Acta 38*, 470.
Graber, P., and Williams, Jr., C. A. (1953), *Biochim. Biophys. Acta 10*, 193.
Ghosh, N. K., and Fishman, W. H. (1968), *Arch. Biochem. Biophys. 126*, 700.
Harkness, D. R. (1968), *Arch. Biochem. Biophys. 126*, 513.
Heppel, L. A., Harkness, D R., and Hilmoe, R. J. (1962), *J. Biol. Chem. 237*, 841.
Hirs, C. H. W. (1967), *Methods Enzymol. 11*, 59.
Hulett-Cowling, F. M. (1969), Ph.D. Dissertation, University of Illinois, Urbana, Ill.
Kadner, R. J., Nyc, J. F., and Brown, D. M. (1968), *J. Biol. Chem. 243*, 3076.
LKB 6800 (1963), A Immunoelectrophoresis Operating Manual, pp 8–16.
Lowry, O. H., Rosebrough, N. J., Farr, D. L., and Randall, R. J. (1951), *J. Biol. Chem. 193*, 265.
Malamy, M., and Horecker, B. L. (1961), *Biochem. Biophys. Res. Commun. 5*, 104.
Neu, H. C., and Heppel, L. A. (1965), *J. Biol. Chem. 240*, 3685.
Neumann, H., Boross, L., and Katchalski, E. (1967), *J. Biol. Chem. 242*, 3142.
Reynolds, J. A., and Schlesinger, M. J. (1967), *Biochemistry 6*, 3552.
Reynolds, J. A., and Schlesinger, M. J. (1968), *Biochemistry 7*, 2080.
Schachman, H. K. (1967), *Methods Enzymol. 4*, 69.
Schlesinger, M. J. (1965), *J. Biol. Chem. 240*, 4293.
Schlesinger, M. J. (1968), *J. Bacteriol. 96*, 727.
Schlesinger, M. J., and Barrett, K. (1965), *J. Biol. Chem. 240*, 4284.
Shah, D. B., and Blobel, H. (1967), *J. Bacteriol. 94*, 780.
Simpson, R. T., and Vallee, B. L. (1968), *Biochemistry 7*, 4343.
Simpson, R. T. Vallee B. L., and Tait, G. H. 1968), *Biochemistry 7*, 4336.
Takeda, K., and Tsugita, A. (1967), *J. Biochem. (Tokyo) 61*, 231.
Torriani, A. (1968), *J. Bacteriol. 96*, 1200.
Welker, N. E., and Campbell, L.L. (1963), *J. Bacteriol. 86*, 681.
Wilcox, P. E. (1967), *Methods Enzymol. 11*, 63.
Wilson, I. B., and Dayan, J. (1965), *Biochemistry 4*, 645.
Wolfenden, R., and Spence, G. (1967), *Biochim. Biophys. Acta 146*, 296.

Molecular Weight and Subunits of the Alkaline Phosphatase of *Bacillus licheniformis**

F. Marion Hulett-Cowling† and L. Leon Campbell‡

ABSTRACT: The molecular weight of the alkaline phosphatase (orthophosphoric monoester phosphohydrolase, EC 3.1.3.1) of *Bacillus licheniformis* as determined by high-speed sedimentation equilibrium was not dependent on speeds of rotation, concentration of protein, or ionic strength of the solvent within the range of those parameters examined. The calculated molecular weight was 121,000 ± 3000. A 55,000 species resulted from dialysis against 5 M guanidine hydrochloride, 8 M urea, or 0.01 M HCl. These preparations were homogeneous by the criteria of weight and in the case of the urea-treated enzyme by electrophoresis.

Evidence for a species one-fourth the molecular weight of the whole enzyme came from peptide analysis and pH experiments. The 55,000 and 26,000 molecular weight species are antigenically deficient as compared to the whole enzyme.

The subunit structure of alkaline phosphatase from *Neurospora crassa* (Kadner *et al.*, 1968), *Escherichia coli* (Garen and Levinthal, 1960; Torriani, 1968; Schlesinger, 1965, 1967; Schlesinger and Barrett, 1965) and human placental tissue (Gottlieb and Sussman, 1968) has been studied. In each case the high-speed sedimentation equilibrium method of molecular weight analysis revealed a subunit one-half the weight of the whole enzyme. Fingerprinting

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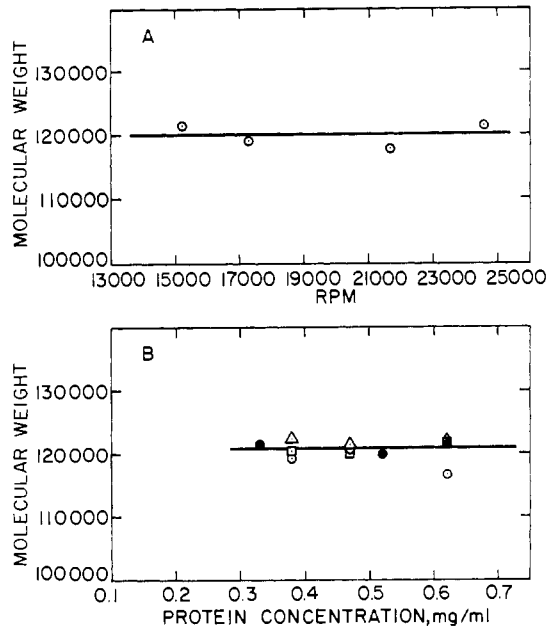

FIGURE 1: Effect of ionic strength of buffer, concentration of protein, and speed of centrifugation on the molecular weight of alkaline phosphatase. (A) Speed dependency of molecular weight. The conditions were as follows: protein concentration, 0.54 mg/ml; buffer, BG; temperature, 20°. (B) Concentration and ionic strength affect. Molecular weights are recorded at specified concentrations. Four molarities of NaCl in BG buffer are designated as follows: ●, no NaCl; △, 0.1 M NaCl; □, 0.5 M NaCl; ○, 1.0 M NaCl.

TABLE 1: Molecular Weight Determinations of the Alkaline Phosphatase of *B. licheniformis* at Several Salt and Protein Concentrations.

NaCl Concn in BG Buffer (M)	Protein Concn (mg/ml)	Mol Wt[a]	Mol Wt Av
None	0.33	121,618 ± 3,000	
	0.52	120,000 ± 1,800	121,270
	0.62	122,193 ± 1,200	
0.1	0.38	122,928 ± 200	
	0.47	121,104 ± 450	122,087
	0.62	121,911 ± 1,700	
0.5	0.38	120,766 ± 2,000	
	0.47	120,031 ± 1,750	121,929
	0.62	122,786 ± 1,100	
1.0	0.38	119,660 ± 1,500	
	0.47	120,485 ± 4,000	119,104
	0.62	117,167 ± 1,500	
		Composite av	121,100

[a] Average of three readings.

of tryptic digests of alkaline phosphatase from *Escherichia coli* indicated that the enzyme was composed of two subunits (Rothman and Byrne, 1963). The subunit of *E. coli* does not give a strong precipitant reaction with antiserum against the whole enzyme; however, it does coprecipitate when the whole enzyme antibody precipitate is formed (Reynolds and Schlesinger, 1967).

The accompanying paper describes the solubilization, purification, amino acid composition, and some general properties of the alkaline phosphatase of a facultatively thermophilic strain of *Bacillus licheniformis* (Hulett-Cowling and Campbell, 1971). This paper reports studies on the molecular weight and subunits of the alkaline phosphatase of this organism. Data are also presented on trypsin digestion and subsequent fingerprinting of the enzyme, and the immunological response between the subunits of the enzyme and antiserum against the native enzyme.

Methods and Materials

Production, Purification, and Assay of the Enzyme. The procedures for the production, solubilization, purification and determination of enzyme activity of the alkaline phosphatase of *Bacillus licheniformis* MC14 were those described by Hulett-Cowling and Campbell (1971).

Sedimentation Equilibrium Molecular Weight Determinations for the Whole Enzyme. High-speed sedimentation equilibrium studies were done using an An-D rotor, in a Beckman-Spinco Model E ultracentrifuge equipped with interference optics. A cell containing a three double-channel equilibrium charcoal-filled Epox centerpiece and sapphire windows was used. Rotor speeds of 35,600 and 37,020 rpm were used for the studies on subunits and 24,630 rpm for the whole enzyme. Pictures were taken after 20 hr and the plates were read on a Gaertner microcomparator. A 360 IBM computer and a FORTRAN IV program using least-squares analysis was employed to calculate the weight-average molecular weight. Ordinate displacements greater than 100 μ were used in the calculations of molecular weights (Yphantis, 1964).

The molecular weight of the enzyme was determined by obtaining the weight-average molecular weight at three protein concentrations in BG buffer (0.01 M Bicine–0.05 M glycine, pH 7.3) for each of four NaCl concentrations (0, 0.1, 0.5, and 1.0 M). The protein samples were dialyzed against 2 l. of the appropriate solvent for *ca.* 18 hr. The density of the BG buffer was determined pycnometrically. The density values of the BG buffers containing NaCl were calculated using density values of NaCl solutions from Svedberg and Pederson (1940). One concentration of protein in BG buffer was also examined at four rotor speeds for homogeneity and nonideality.

Molecular Weight Determinations of the Subunits. PERFORMIC ACID OXIDIZED ENZYME. Enzyme samples were subjected to performic acid oxidation exactly as described by Hirs (1967). The oxidized protein was dialyzed against 60% formic acid for 48 hr at 4°. The sample was centrifuged at 4° for 20 hr at 37,020 rpm in a double-sector interference cell equipped with a Kel-F centerpiece.

FORMIC ACID TREATED ENZYME. Enzyme samples (0.49 mg/ml) were dialyzed against 60% formic acid for 16 hr at 4° and immediately analyzed by sedimentation equilibrium at 4°.

Guanidine Hydrochloride Treated Enzyme. Three guanidine hydrochloride solutions containing enzyme (0.53 mg/ml) were made: 5 M guanidine, 5 M guanidine hydrochloride containing 0.1 M mercaptoethanol, and 5 M guanidine hydrochloride containing 0.03 M dithiothreitol. Each of the solutions were incubated for 1 hr at 37°. After incubation the samples were dialyzed for 24 hr against 50 ml of their respective guanidine solutions at 4°. High-speed sedimentation equilibrium studies were conducted on each sample.

TABLE II: Subunit Molecular Weight Data on the Alkaline Phosphatase of *B. licheniformis*.[a]

Treatment	ρ	Mol Wt
Performic acid oxidized	1.156[d]	57,200 ± 300
Formic acid	1.156	48,600 ± 500
5 M Guanidine hydrochloride	1.122[b]	57,600 ± 2,500
5 M Guanidine hydrochloride + 0.1 M mercaptoethanol	1.122	56,500 ± 500
5 M Guanidine hydrochloride + 0.03 M dithiothreitol	1.122	55,000 ± 250
Dithiothreitol (0.03 M) — guanidine hydrochloride	1.001[c]	124,700 ± 2,000
8 M Urea	1.122	50,200 ± 600
8 M Urea + 0.03 M mercaptoethanol	1.122	52,700 ± 1,200
8 M Urea + 0.03 M dithiothreitol	1.122	53,900 ± 2,000
0.01 M HCl (pH 2.3)	1.005[e]	52,400 ± 600

[a] All treatments resulting in the formation of subunits completely inactivated alkaline phosphatase activity. Centrifugations were at 37,020 rpm for 20 hr at 20°. Readings and calculations were made in triplicate and the average molecular weight is given. All ln J *vs.* R^2 plots were linear indicating homogeneity of the preparations. [b] Value obtained from Kawahara and Tanford (1966). [c] Value determined for the BG buffer. [d] Value from International Critical Tables. [e] Value from Svedberg and Pederson (1940).

UREA-TREATED ENZYME. Protein solutions (0.36 mg/ml) were dialyzed for 16 hr against 8 M urea, 8 M urea plus 0.1 M mercaptoethanol, and 8 M urea plus dithiothreitol (0.03 M) at 4°. Sedimentation equilibrium studies were carried out in the usual manner.

pH Experiments. The enzyme (0.48 mg/ml) was dialyzed for 24 hr at 4° against 0.01 M HCl containing 0.1 M NaCl (final pH 2.3) and analyzed by sedimentation equilibrium.

A second enzyme sample subjected to the same treatment as above was then dialyzed against BG buffer. It was assayed for per cent activity recovery and the molecular weight was determined by sedimentation equilibrium.

Trypsin Digest of the Enzyme. Alkaline phosphatase (2.5 mg) per fingerprint was dialyzed against 5 M guanidine hydrochloride (Mann Ultra Pure) for 17 hr and then dialyzed exhaustively against water. The sample was heated at 100° for 5 min, cooled, and 1 M NH_4HCO_3 was added to a final concentration of 0.01 M. 1% trypsin (Worthington Biochemical Corp.) to total weight of protein was added three times at 2-hr intervals. The trypsin digest was lyophilized, suspended in water, and relyophilized.

Paper fingerprinting was done according to Kimmel *et al.* (1962). The digested protein (*ca.* 2 mg) was suspended in 30 μl of pH 6.6 buffer (Ingram, 1963) and applied to the paper (previously wet with the same buffer). Electrophoresis was run for 90 min at 40 mA (1500 V). The dried papers were chromatographed (descending, 16 hr) in a 1-butanol–glacial acetic acid–pyridine–distilled water (600:120:400:480, v/v) buffer. The dried chromatograms were stained with ninhydrin or with special stains for methionine, arginine, and histidine plus tyrosine.

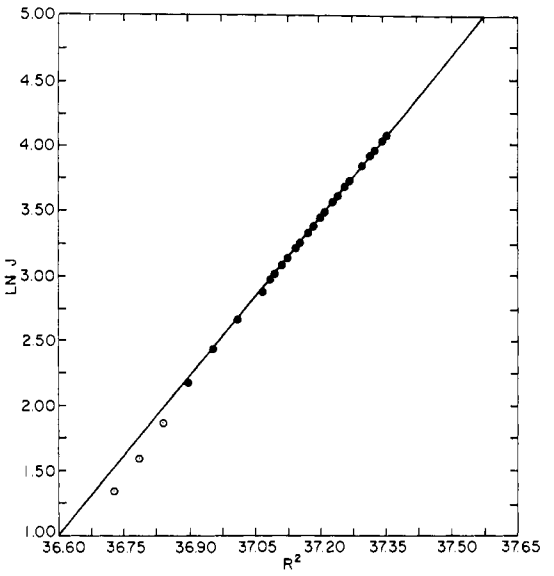

FIGURE 2: Representative ln J *vs.* R^2 plot of native alkaline phosphatase of *B. licheniformis*. The sedimentation equilibrium fringe displacement plot of ln J *vs.* R^2 gave a slope which yielded a calculated molecular weight of 120,800 for the whole enzyme in BG buffer containing 0.5 M NaCl and at a protein concentration of 0.38 mg/ml. The open circles represent points with a fringe displacement of less than 100 μ. Those points were plotted by the computer but were not used in the calculation of the molecular weight (Yphantis, 1964). This convention was followed for all ln J *vs.* R^2 plots composed of open and closed circles.

Immunology. Antiserum against the pure alkaline phosphatase was prepared as described by Hulett-Cowling and Campbell (1971) for the crude soluble enzyme, except that rabbits were injected subcutaneously (twice weekly) with 1 mg of enzyme in a 1-ml emulsion of Freund's complete adjuvant (1:1, v/v). After eight injections the rabbits were bled through the ear vein. The procedures employed for Ouchterlony double diffusion and immunoelectrophoresis were described by Hulett-Cowling and Campbell (1971).

Gel Electrophoresis. The procedure used for gel electrophoresis was described by Hulett-Cowling and Campbell (1971).

Results

Molecular Weight of Native Enzyme. A molecular weight of 120,000 ± 3000 was determined using an enzyme concentration of 0.54 mg/ml in BG buffer at 20° and the following rotor speeds: 15,215, 17,248, 21,730, and 24,630 rpm. These data (Figure 1A) suggest that the enzyme is monodisperse and is nonassociating under the conditions employed. The enzyme molecular weight was not concentration dependent over the range studied (Figure 1B). These data also show no nonideality due to charge effects over the NaCl concentrations examined. Table I shows the molecular weights obtained at three protein concentrations in BG buffer of four different ionic strengths. A typical ln J *vs.* R^2 plot used in the calculation of molecular weights of the enzyme is shown in Figure 2.

A $\bar{v}$ of 0.733 ml/g and a density of 1.001 for the BG buffer were used in the calculations (Hulett-Cowling and Campbell, 1971). The average molecular weight from these determinations was 121,000 ± 3000.

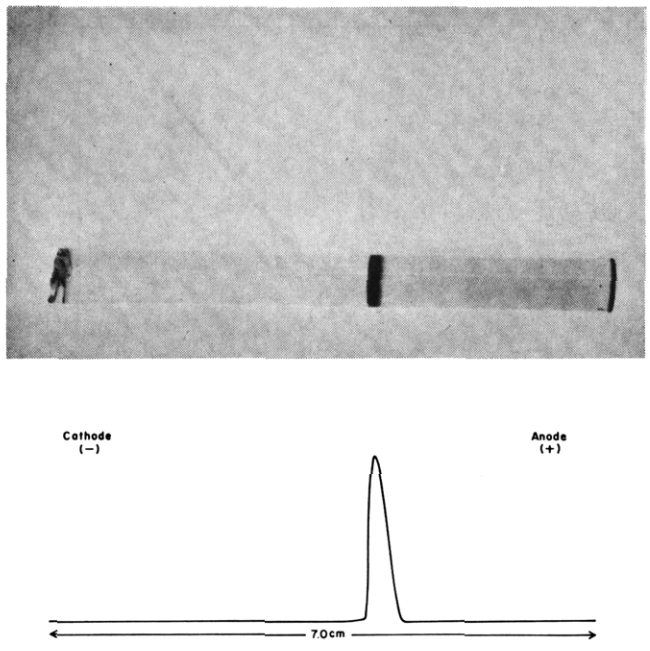

FIGURE 3: Disc gel electrophoresis of the urea-treated alkaline phosphatase of *B. licheniformis* and a densitometer tracing of the analytical gel. Polyacrylamide gel electrophoresis of alkaline phosphatase in 8 M urea shows a single protein band. The protein (50 μg) was run for 8 hr at 3.2 mA/tube. The enzyme moved from the anode to the cathode.

Subunit Molecular Weights. Molecular weights of the enzyme subunits are shown in Table II. It should be noted that all treatments resulting in the formation of subunits inactivated the enzyme.

A molecular weight of 57,200 was obtained for the subunit formed by performic acid oxidation. After oxidation the enzyme was dialyzed against 60% formic acid (48 hr) and ultracentrifugation carried out in 60% formic acid.

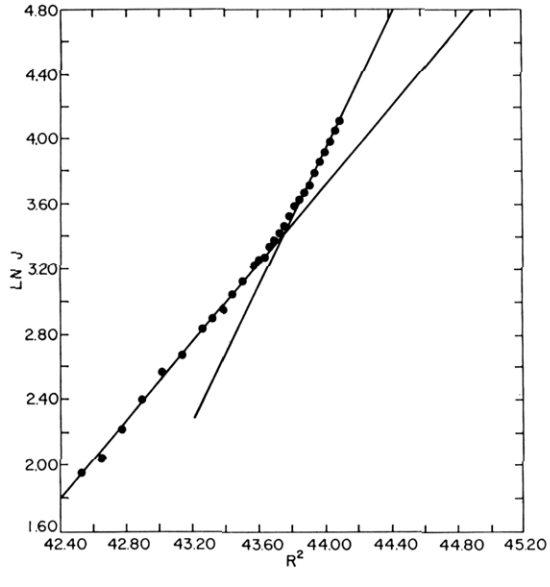

FIGURE 4: Representative plot of alkaline phosphtase of *B. licheniformis* which has been subjected to the following pH changes: pH 7.3 → 2.3 → 4.5 → 7.3. The sedimentation equilibrium fringe displacement plot of ln J *vs.* R^2 of the 0.01 M HCl-treated protein (55,000) after dialysis against BGC buffer containing 0.1 M NaCl showed two slopes.

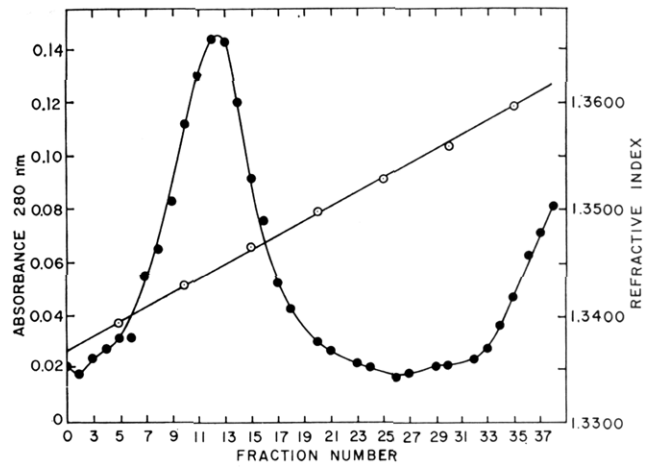

FIGURE 5: Isolation of low molecular weight subunit of alkaline phosphatase of *B. licheniformis* on a preparative sucrose gradient. The closed circles represent the absorbance at 280 nm; the open circles represent the refractive index. Tube 37 is the bottom of the gradient tube. Enzyme (8.3 mg) was dissolved in 1 ml of 0.01 M HCl containing 0.1 M NaCl and dialyzed against 1 l. of the same solution for 16 hr with one change of solution. The final pH of the dialyzed material was 2.3. The volume of the dialyzed solution was brought to 8 ml and the protein sample was dialyzed for 6 hr against BGC buffer at pH 4.5 followed by dialysis against BGC buffer at pH 7.3 for 12 hr. A 5-ml portion (5 mg of protein) of this material was placed on a 60-ml, 5–20% sucrose bucket in the SW-25.2 rotor and centrifuged at 106,000g for 50 hr in a Spinco Model L-2 ultracentrifuge. Fractions (1.5 ml) were collected using an Isco gradient collector and pump with a flow rate of 3 ml/min. The OD_{280nm} of each fraction was read on a Gilford 240 spectrophotometer. The refractive index was also read using a Bausch and Lomb refractometer.

To determine if subunit formation was dependent on the oxidation or the acid pH of the formic acid an enzyme sample was dialyzed against 60% formic acid for 16 hr and the molecular weight was determined. A value of 48,600 was calculated from the data obtained. This indicates that performic acid oxidation was not necessary for subunit formation.

The reducing agents, dithiothreitol and β-mercaptoethanol, were not necessary to form the 55,000 molecular weight subunit and did not reduce this subunit to a smaller subunit when present. The enzyme containing dithiothreitol without guanidine was not reduced to the 55,000 subunit. These data are consistent with the fact that the enzyme does not contain cystine–half-cystine (Hulett-Cowling and Campbell, 1971) and the subunits are, therefore, not covalently linked by disulfide bonds.

The urea experiments also show that reducing agents are not necessary for the formation of subunits in urea. Evidence that the enzyme is one component in urea was shown by gel electrophoresis (Figure 3). The enzyme was placed in 8 M urea 24 hr before gel electrophoresis.

The sedimentation equilibrium plot of the low pH experiment showed a homogeneous protein preparation with a molecular weight of 52,400 (Table II). The enzyme sample retained no activity under these conditions. This sample was then dialyzed at 4° for 20 hr against BG buffer containing 0.1 mM $CoCl_2$ (BGC buffer), pH 7.3. The sedimentation equilibrium fringe displacement plot of this material was polydisperse as shown in Figure 4. The approximate molecular weights determined from this plot were 32,600 for the lower slope and 58,000 for the steeper slope. The appearance of the 32,600 molecular weight species was the first indication

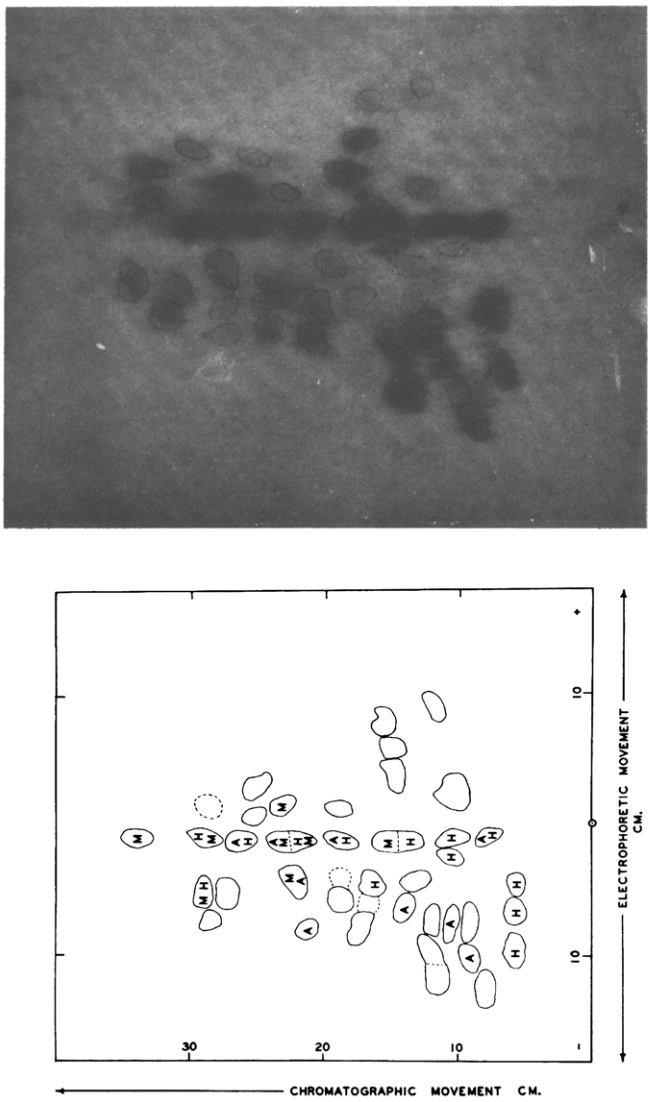

FIGURE 6: Picture and tracing of ninhydrin-positive spots and specific amino acid stained spots of a trypsin digest of the alkaline phosphatase of *B. licheniformis* after electrophoresis and chromatography. (A) Arginine; (H) histidine plus tyrosine; (M) methionine.

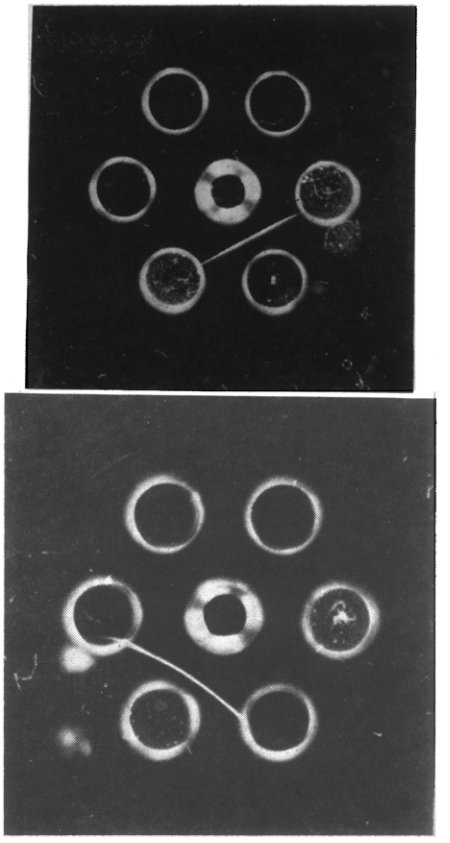

FIGURE 7: Immunodiffusion patterns of native alkaline phosphatase of *B. licheniformis* and the isolated 26,000 molecular weight subunit. (A) Center well contained antiserum against the pure alkaline phosphatase of *B. licheniformis*. Outer well contained the enzyme. (B) Center well contains antiserum against the pure enzyme; well 1 contains the native enzyme (121,000 molecular weight); well 2 contains the 26,000 molecular weight subunit. Note that a weak precipitin reaction can be observed with the subunit and the antiserum to the native enzyme.

from subunit studies that the 55,000 molecular weight speesic was not the smallest subunit size. This subunit component was isolated by preparative sucrose density centrifugation (Figure 5). Upon sedimentation equilibrium analysis the protein from the peak tubes (fractions 10–14) showed a homogeneous preparation with an approximate molecular weight of 26,000.

Fingerprinting and Specific Staining of Tryptic Digests of Enzyme. Figure 6 shows a tracing and a picture of the ninhydrin-positive spots of a trypsin digest of alkaline phosphatase after electrophoresis and chromatography of the enzyme. Forty-two definite spots and five possible spots were noted. Amino acid analysis revealed that the enzyme contained 127 lysine and 33 arginine residues (Hulett-Cowling and Campbell, 1971). From these data one would expect 160–162 tryptic peptides. Approximately one-quarter of the number of expected peptides were observed. Specific stains for arginine (9 peptides), methionine (7 peptides), and histidine plus tyrosine (13 peptides), also revealed approximately one-fourth the number of spots expected for the whole enzyme on the basis of amino acid analysis (Hulett-Cowling and Campbell, 1971).

Immunological Results. The enzyme forms a tight precipitin band with antiserum prepared against the pure enzyme upon Ouchterlony double-diffusion analysis. The 26,000 molecular weight species (from the pooled fractions 10–14 of the experiment illustrated in Figure 5) is antigenically deficient with respect to the whole enzyme (Figure 7). Similar results were obtained with the 55,000 molecular weight species. The alkaline phosphatase of *B. licheniformis* did not cross-react (upon immunoelectrophoretic analysis) with antiserum against whole or subunit alkaline phosphatase of *E. coli* (Figure 8).

Discussion

Sedimentation equilibrium experiments gave a composite average molecular weight of 121,000 ± 3000 for the alkaline phosphatase of *B. licheniformis*. Treatment with dissociative agents converted the enzyme into two 55,000 molecular weight species and evidence exists for the possibility of four 26,000 species. It should be noted that these molecular weights are approximate since in a single experiment (one concentration of protein observed at one speed) heterogeneity and nonideality can compensate for each other and give the effect of an apparent monodisperse ideal system (Yphantis,

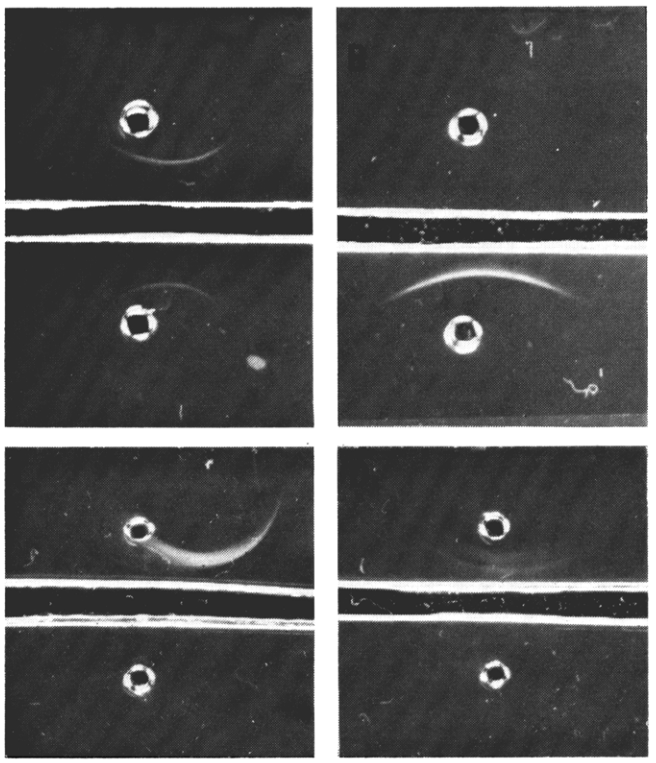

FIGURE 8: Immunoelectrophoresis patterns of the alkaline phosphatase of *B. licheniformis* and the alkaline phosphatase (native and subunits) of *E. coli*. (A) The wells contained alkaline phosphatase of *B. licheniformis;* the trough contained antiserum against *B. licheniformis* alkaline phosphatase. (B) The upper well contained *B. licheniformis* alkaline phosphatase; the lower well contained *E. coli* alkaline phosphatase (native); the trough contained antserium against *E. coli* alkaline phosphatase (native). (C) The upper well contained *B. licheniformis* alkaline phosphatase; the lower well contained *E. coli* alkaline phosphatase (native); the trough contained antiserum against *B. licheniformis* alkaline phosphatase. (D) The upper well contained subunits of *E. coli* alkaline phosphatase prepared by dialysis against 0.5 M HCl (the agarose contained 1 mM EDTA); the lower well contained *B. licheniformis* alkaline phosphatase; the trough contained antiserum against the subunits of *E. coli* alkaline phosphatase. The antisera against the *E. coli* alkaline phosphatase (native and subunits) were kindly supplied by Milton J. Schlesinger.

1964). It is also impossible to distinguish between an association equilibrium and a population of polymers not in equilibrium in certain cases. Acknowledging these limitations it is possible to analyze our data.

The molecular weight analysis of the performic acid oxidized enzyme, the formic acid dialyzed enzyme and the absence of an effect of reducing agents on subunit formation suggests that cystine does not play a role in the quarternary structure of the enzyme. These observations are reinforced by the absence of cysteic acid in the amino acid analysis of the performic acid oxidized enzyme (Hulett-Cowling and Campbell, 1971).

Evidence for four repeating polypeptide chains comes from tryptic peptide analysis and dialysis of the 0.01 M HCl-treated enzyme back to pH 7.3. The isolated lighter component had a molecular weight of approximately 26,000. Tryptic peptide maps showed approximately one-fourth the number of ninhydrin-positive spots and specific amino acids upon staining as predicted from amino acid analysis. This suggests that the protein is made up of four repeating polypeptide chains.

These data indicate that the enzyme is likely held together by noncovalent bonds. From our experiments, it can not be determined if the bonds are ionic, hydrogen, hydrophobic, or a combination of the three. Further experiments are in progress to assess this question.

Upon immunodiffusion and immunoelectrophoresis the enzyme formed tight precipitin lines with antibody against the whole enzyme. The patterns with the 26,000 and 55,000 molecular weight species indicated that both species were antigenically deficient as compared to the whole enzyme. It is probable, therefore, that antigenic sites are lost on dissociation of the enzyme.

The fact that the 26,000 molecular weight species does react with the whole enzyme antibody in *B. licheniformis* suggests that the immunological response between the enzyme antibody and the subunits is not similar to that observed with the alkaline phosphatase of *E. coli* (Schlesinger, 1965, 1967). Antibody formed against native enzyme from *E. coli* gives a weak precipitin band with the alkaline phosphatase subunit of *E. coli*, although it is able to coprecipitate the enzyme subunits when the enzyme–antibody precipitate is formed. It, therefore, appears that the dissociation of *B. licheniformis* alkaline phosphatase may not result in as drastic a conformational change as that reported to take place in *E. coli* alkaline phosphatase (Schlesinger, 1965, 1967).

Acknowledgment

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References

Garen, A., and Levinthal, C. (1960), *Biochim. Biophys. Acta 38*, 470.

Gottlieb, A. J., and Sussman, H. H. (1968), *Biochim. Biophys. Acta 160*, 167.

Hirs, C. H. W. (1967), *Methods Enzymol. 11*, 59.

Hulett-Cowling, F. M., and Campbell, L. L. (1971), *Biochemistry 10*, 1364.

Ingram, V. M. (1963), *Methods Enzymol. 6*, 831.

Kadner, R. J., Nyc, J. F., and Brown, D. M. (1968), *J. Biol. Chem. 243*, 3076.

Kawahara, K., and Tanford, C. (1966), *J. Biol. Chem. 241*, 3228.

Kimmel, J. R., Kato, G. K., Paiva, A. C. M., and Smith, E. L. (1962), *J. Biol. Chem. 237*, 2525.

Reynolds, J. A., and Schlesinger, M. J. (1967), *Biochemistry 6*, 3552.

Rothman, F., and Bryne, R. (1963), *J. Mol. Biol. 6*, 330.

Schlesinger, M. J. (1965), *J. Biol. Chem. 240*, 4293.

Schlesinger, M. J. (1967), *J. Biol. Chem. 242*, 1599.

Schlesinger, M. J., and Barrett, K. (1965), *J. Biol. Chem. 240*, 4284.

Svedberg, T., and Pederson, K. O. (1940), The Ultracentrifuge, Johnson Reprint Corporation, New York, N. Y., p 478.

Torriani, A. (1968), *J. Bacteriol. 96*, 1200.

Yphantis, D. A. (1964), *Biochemistry 3*, 297.