moved with solid sodium thiosulfate and the solvent removed *in vacuo*. The residue was extracted twice with 50 ml. of ethyl acetate which was then washed successively with 25 ml. of 1 *N* hydrochloric acid, 25 ml. of 1 *N* sodium bicarbonate, 25 ml. of water and 25 ml. of saturated sodium chloride solution. After drying the ethyl acetate over sodium sulfate and concentrating *in vacuo*, the product was precipitated by the addition of petroleum ether to give 1.12 g., 75% yield, m.p. 84–85°.

Benzyloxycarbonylglycyl-L-phenylalanylglycine Ethyl Ester.—A. 1.85 g. (5 m*M*) of benzyloxycarbonylglycyl-L-phenylalanylhydrazide,[14] 520 mg. of freshly distilled glycine ethyl ester (5 m*M*) and 1.44 ml. (10 m*M*) of triethylamine were dissolved in 50 ml. of tetrahydrofuran; to the ice cold solution, 1.78 g. (10 m*M*) of solid NBS were added with swirling. After 5 minutes 200 ml. of water were added and an oil came out which solidified on standing. After filtration and drying, the crude product (1.7 g.; m.p. 106–111°) was crystallized from 85 ml. of absolute ethanol; 25 mg. of material were obtained after 30 minutes which melted at 119–131°. The solution was then kept at 5° overnight and 420 mg. of material which melted 116–117° were obtained. Upon concentration of the solution to 20 ml. and cooling an additional 950 mg. of material melting at 116–117° came out. This gave an overall yield of the optically pure compound of 62% with 1.1% yield of the racemate.

B. 1.85 g. (5 m*M*) of benzyloxycarbonylglycyl-L-phenylalanylhydrazide, 710 mg. (5 m*M*) of glycine ethyl ester hydrochloride and 3.6 ml. (25 mM) of triethylamine were dissolved in 20 ml. of dimethylacetamide–water, 1:1; to the ice cold solution 2.54 g. of iodine (10 m*M*) of I_2 in 10 ml. of dimethylacetamide were added with swirling. After 5 minutes excess iodine was removed with a few drops of sodium thiosulfate solution and the product precipitated with 150 ml. of water. After filtration and drying 1.85 g. of product which melted 108–118° were obtained. The crude product was crystallized from 92 ml. of absolute ethanol to give 20 mg. of material melting at 131–133°, 30 mg. melting at 118–129° and 200 mg. melting at 117°; after concentration of the alcohol 1.29 g. of substance which melted at 116–117° came out. This gave an over-all yield of 68% of the optically pure compound with 2.2% yield of the racemate.

Benzyloxycarbonylglycyl-L-seryl-L-alanylhydrazide.—2.28 g. (5 m*M*) of benzyloxycarbonylglycyl-L-seryl-L-alanine benzyl ester were dissolved in 20 ml. of ethanol and 0.5 ml. of hydrazine hydrate (10 m*M*) added. After standing overnight at room temperature, the crystalline product which came out was filtered, washed with ether and recrystallized from ethanol to give 1.7 g., 90% yield; m.p. 198–201°. A sample for analysis was recrystallized from ethanol with no change in melting point.

Anal. Calcd. for $C_{16}H_{23}N_5O_6$: N, 18.36. Found: 17.83.

(14) G. W. Kenner and R. J. Stedman, *J. Chem. Soc.*, 2069 (1952).

Glycyl-L-seryl-L-alanylhydrazide Dihydrobromide.—1.9 g. (5 m*M*) of benzyloxycarbonylglycyl-L-seryl-L-alanylhydrazide were dissolved in 15 ml. of glacial acetic acid and 10 ml. of 33% hydrogen bromide in glacial acetic acid were added. The product precipitated and after 10 minutes 50 ml. of ether were added and the product filtered off, washed with ether and crystallized from ethanol–water to give 1.9 g., 95% yield; m.p. 163–165°.

Anal. Calcd. for $C_8H_{19}N_5O_4Br_2$: N, 17.15; Br, 39.07. Found: N, 17.39; Br, 37.81.

Poly-(glycyl-L-seryl-L-alanyl)$_n$.—1.25 g. of glycyl-L-seryl-L-alanylhydrazide dihydrobromide and 2.6 ml. of triethylamine were dissolved in 10 ml. of dimethylacetamide. To the ice cold solution, 1.6 grams of iodine in 10 ml. of dimethylacetamide were added. After 10 minutes excess iodine was removed with a drop of a concentrated solution of sodium thiosulfate. Upon addition of a large volume of ether an oil came out, the oil was dissolved in 20 ml. of water, placed in a cellophane sac and dialyzed for 4 hr. against running tap water and 10 hr. against distilled water. The contents of the sac was lyophilized to give 250 mg. of product, 50% yield.

Anal. Calcd. for $(C_8H_{13}N_3O_4)_n$: C, 44.64; H, 6.09; N, 19.52. Found: C, 43.96; H, 6.32; N, 18.99.

Benzyloxycarbonylamino Acid Hydrazides.—0.1 mole of benzyloxycarbonylamino acid was suspended in 75 ml. of methanol and dry HCl gas passed in for a few minutes until the temperature raised to about 60° (or 10 g. of conc. sulfuric acid was added). After standing at room temperature for 15 minutes, 500 ml. of water were added and the oil which came out was washed twice with 200 ml. of water and dissolved in 150 ml. of ether. The organic layer was washed twice with 100 ml. of 1 *N* sodium bicarbonate, 100 ml. of water, dried over sodium sulfate and the ether was removed *in vacuo*. The benzyloxycarbonylamino acid ester was dissolved in 100 ml. of methanol, and 5 g. (0.1 mole) of hydrazine hydrate were added. After standing overnight the methanol was removed *in vacuo* and the oil which was obtained was dried over sulfuric acid at 0.1 mm. Hg and was crystallized from methanol–ether to give the benzyloxycarbonylamino acid hydrazide in 70–90% over all yield.

Acknowledgments.—We would like to express our thanks to Professor E. Katchalski for his interest and helpful suggestions during this investigation. This work was supported by grants A-3083, H-4762, A-2965, H-3838 and M-2562 of the National Institutes of Health, United States Public Health Service and by grant NSF-G-13957 of the National Science Foundation.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA, IOWA CITY, IOWA, AND THE DEPARTMENT OF BIOCHEMISTRY, DUKE UNIVERSITY MEDICAL CENTER, DURHAM, N. C.]

Dissociation of Catalase into Subunits[1,2]

BY CHARLES TANFORD AND REX LOVRIEN[3]

RECEIVED OCTOBER 30, 1961

It is shown that commercial *lyophilized* catalase, which has a much lower specific activity than *crystalline* catalase, is partially dissociated to half and quarter molecules. Acid and base denaturation, leading to complete inactivation, produce complete dissociation to quarter molecules.

The purpose of this paper is to show that one of the manifestations of the denaturation of catalase is a dissociation of the molecule into subunits one-half and one-quarter as heavy as the native molecule. Most of the studies involved comparison between two commercial preparations of beef liver catalase,[4] one of these being a recrystallized aqueous suspension, the other a lyophilized powder. The specific enzymatic activity

(1) Presented in part at the 135th National Meeting of the American Chemical Society, Boston, Mass., 1959.

(2) The experimental work described here was carried out with the technical assistance of Susan Fordemwalt.

(3) Department of Biochemistry, Indiana University Medical School, Indianapolis, Ind.

(4) Both purchased from the Worthington Biochemical Corp., Freehold, N. J.

of the lyophilized powder is much less than that of the crystalline product, presumably as a result of the denaturation which catalase undergoes when frozen in an aqueous medium.[5] A few experiments on acid- and base-denatured catalase are also described.

Experimental

The crystalline beef liver catalase (Lot 5460) used in this work was described as having been prepared by the method of Tauber and Petit.[6] Its spectral properties and enzymatic activity, shown in Table I, agreed substantially with values reported by Tauber and Petit. Catalase preparations by other methods from other sources may possess a wide range of activities and spectral properties, which have been summarized by Maehly and Chance.[7] The most active preparations from most sources have k greater than 3.0×10^7 liters/mole-sec.

TABLE I

SPECTRAL PROPERTIES AND CATALYTIC ACTIVITY

	Molar absorbancy (1 cm. light path), $\epsilon \times 10^{-5}$ 276 mμ	406 mμ	Activity $k \times 10^{-7}$ (moles/l.)$^{-1}$ sec.$^{-1}$
Crystalline beef liver catalase			
Tauber and Petit[6]	4.0	3.1	1.9
Lot 5460	4.1	3.0	1.9
Lyophilized powder			
Lot 5457	3.2	2.0	0.11
Lot 5458	3.4	0.73	0.23

Only freshly prepared solutions of the crystals were employed for study. Such solutions do not lose activity nor undergo other changes when allowed to stand at room temperature for 12 hours. There was some evidence, however, that crystalline catalase loses some of its activity if kept in solution for extended periods.

Two preparations of lyophilized powder (Lots 5457 and 5458) were used. As Table I shows, they differed from each other as well as from the crystalline preparation.

Both kinds of protein preparations were dissolved in water to make stock solutions, the concentrations of which were determined by drying at 107°. The solubility of the crystalline material was low and weighings were made on a micro-balance, using miniature weighing bottles weighing about 1 g.

Solutions for measurement were made from such stock solution by addition of reagent grade KCl, KOH, HCl and water. All measurements were made at 25°. All those reported in this paper (except the spectra shown in Fig. 4) were at an ionic strength of 0.10 or 0.15.

Enzymatic activity was measured at 25° by a modification of the method of Beers and Sizer.[8] Results are reported in terms of the second-order rate constant k in the rate equation

$$-\mathrm{d}(H_2O_2)/\mathrm{d}t = k(H_2O_2)(\text{catalase})$$

where parentheses represent molar concentrations. The molecular weight of catalase was taken as 250,000 in making this calculation. Activity determinations of other workers quoted in this paper, when reported as "*K*at.f." values, were converted to k values, and, where necessary, to 25°, by the procedure of Maehly and Chance.[7]

Viscosity measurements were made in capillary viscometers, using procedures previously described.[9,10] Sedimentation velocity was measured on a Spinco model E ultracentrifuge. Spectral data were determined either on a Cary model XI or a Beckman model DU spectrophotometer.

Results

Viscosity.—Measurements of sedimentation and diffusion coefficients have demonstrated that active crystalline catalase is a typical globular protein, compact and sparingly solvated.[11,12] The same conclusion is reached from the radius of gyration of 39.8 Å. measured by Malmon[13] and from the intrinsic viscosity of 3.9 cc./g. of a fairly active sample ($k = 1.4 \times 10^7$ liter/mole-sec.) determined by Shirakawa.[14]

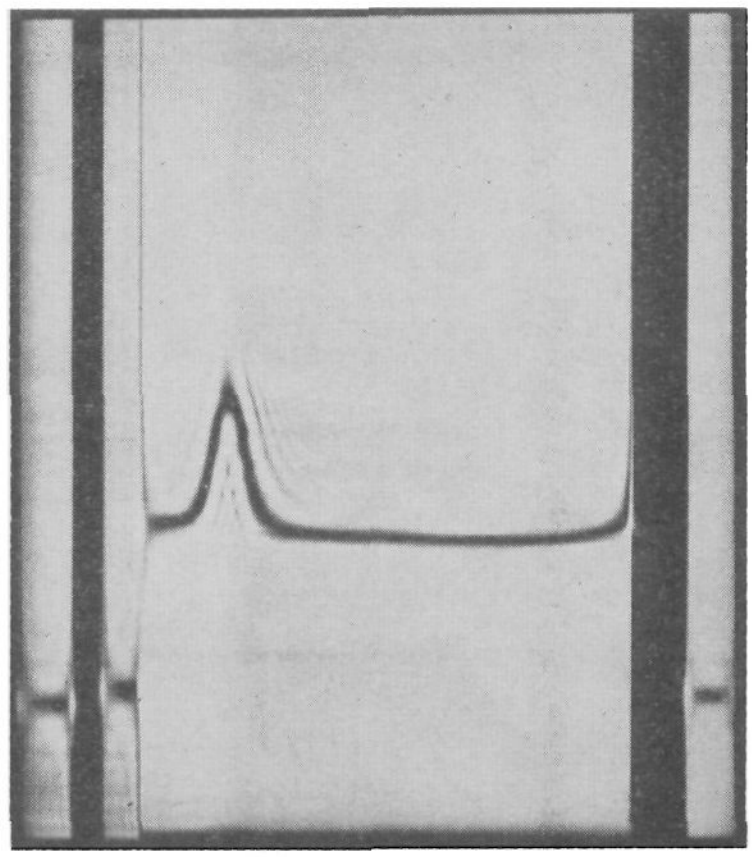

Fig. 1.—Sedimentation pattern for crystalline catalase (Lot 5460); speed 42,040 r.p.m.; picture taken *ca.* 30 minutes after full speed was reached.

We were unable to obtain a sufficiently high concentration of the crystalline catalase to make accurate viscosity measurements. The result of Shirakawa was therefore not tested. However, we measured the intrinsic viscosity of both samples of lyophilized catalase, at ionic strength 0.15 near neutrality. The result, for both samples, was 3.9 cc./g. Moreover, the constant K of the Huggins equation[15]

$$\eta_{sp}/c = [\eta] + K[\eta]^2 c$$

had a value near 2.0 in each case. Both these results indicate[16] that the catalase molecules in the lyophilized samples are still compact and sparingly hydrated. Inactivation in this instance is clearly not a process involving unfolding of the polypeptide chains.

Sedimentation.—Figures 1 to 3 show sedimentation patterns obtained for crystalline catalase and for the two lyophilized samples. The latter are seen to consist of three major components, whereas the crystalline sample is homogeneous. The two lyophilized samples differ considerably in the relative amount of each component. The relative

(5) K. Shikama and I. Yamazaki, *Nature*, **190**, 83 (1961).

(6) H. Tauber and E. L. Petit, *J. Biol. Chem.*, **195**, 133 (1952).

(7) A. C. Maehly and B. Chance, in "Methods of Biochemical Analysis," D. Glick, ed., Vol. 1, Interscience Publishers, Inc., New York, N. Y., 1954, p. 352.

(8) R. F. Beers and I. W. Sizer, *J. Biol. Chem.*, **195**, 133 (1952).

(9) C. Tanford and J. G. Buzzell, *J. Phys. Chem.*, **60**, 225 (1956).

(10) C. Tanford, *ibid.*, **59**, 798 (1955).

(11) J. B. Sumner and N. Gralen, *J. Biol. Chem.*, **125**, 33 (1938).

(12) J. B. Sumner, A. L. Dounce and V. L. Frampton, *ibid.*, **136**, 343 (1940).

(13) A. G. Malmon, *Biochim. et Biophys. Acta*, **26**, 233 (1957).

(14) M. Shirakawa, *J. Faculty Agr. Kyushu Univ. (Japan)*, **9**, 173 (1949).

(15) M. L. Huggins, *J. Am. Chem. Soc.*, **64**, 2716 (1942).

(16) C. Tanford, "Physical Chemistry of Macromolecules," John Wiley and Sons, Inc., New York, N. Y., 1961, section 23.

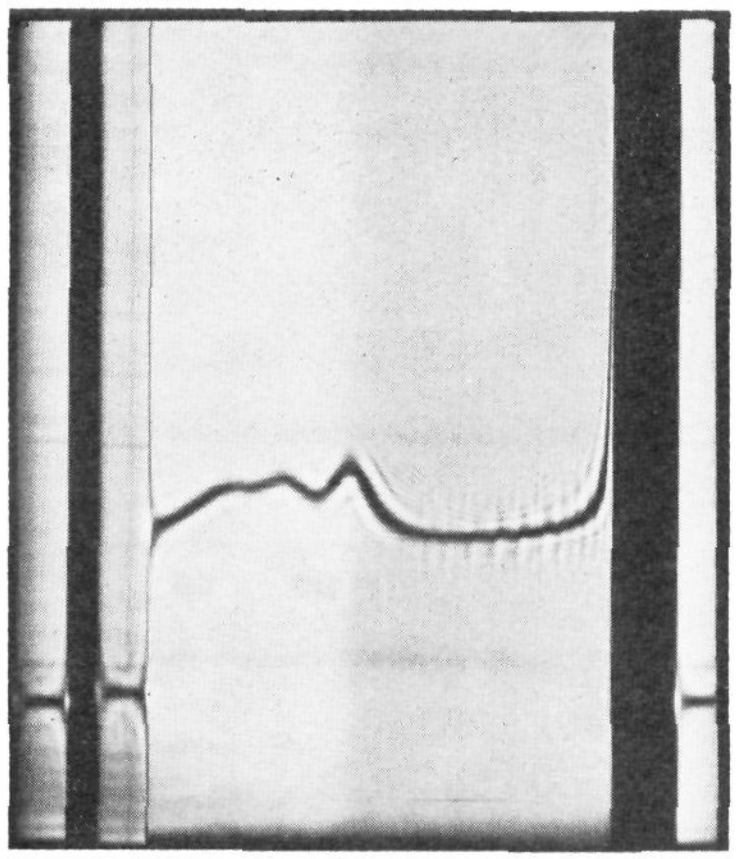

Fig. 2.—Sedimentation pattern for lyophilized catalase (Lot 5458); speed 59,780 r.p.m.; picture taken *ca.* 30 minutes after full speed was reached.

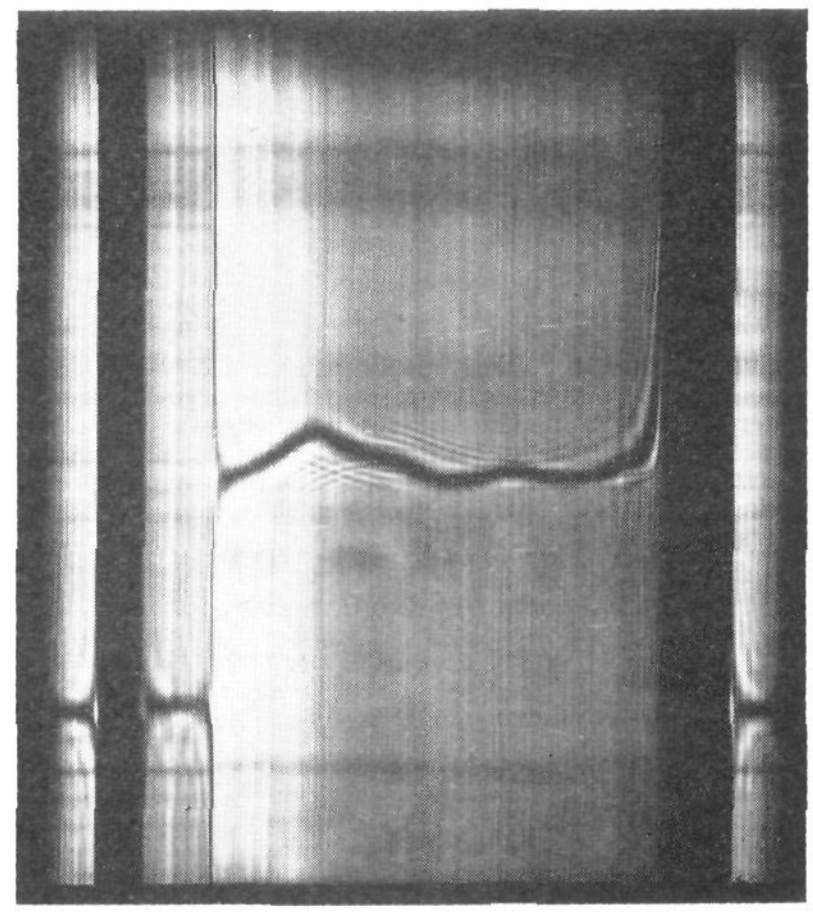

Fig. 3.—Sedimentation pattern for lyophilized catalase (Lot 5457); speed 59,780 r.p.m.; picture taken *ca.* 40 minutes after full speed was reached.

areas under the three peaks, in order of decreasing sedimentation rate, are 12, 24 and 64% for Lot 5457, and 35, 30 and 35% for Lot 5458.

Sedimentation coefficients were determined from the positions of the peaks of the refractive index gradient patterns, as a function of time. Because of the overlap between the two slowest peaks of the lyophilized preparations, there is introduced an error which is likely to make the sedimentation rate for the second peak somewhat too high and that for the slowest peak somewhat too low. The peaks became better separated at times later than those shown in Figs. 2 and 3, but were then too broad to permit accurate measurement of their positions. The measurements were all made at 25°, and corrected to 20° by assuming that the viscosity ratio for the solutions was the same as the viscosity ratio for the solvent. No correction for density of the solutions was made as the experimental precision (0.15 to 0.2 S.) did not warrant it. All measurements were at an ionic strength of 0.1 or 0.15, over a wide range of *p*H. No detectable influence of *p*H was observed between *p*H 4.5 and *p*H 10, and all results obtained within this range have been averaged together. Results outside this range of *p*H will be discussed later.

Only one of the lyophilized samples (Lot 5458) was used for detailed measurements. The three sedimentation coefficients observed in a single run with Lot 5457 have, however, roughly the same magnitude as those observed for Lot 5458. Exact values cannot be computed as the temperature control unit of the ultracentrifuge was not operating at the time that the measurements on Lot 5457 were made.

Results are summarized in Table II. The value of 11.6 S. for the crystalline sample is in good agreement with other determinations. The only really precise value in the literature is that of Deutsch,[17] who used horse erythrocyte catalase. He obtained a value of 11.6 S. at 1% concentration, and estimated 11.8 S. as the best value for $s^{\circ}_{20,\,w}$.

TABLE II

SEDIMENTATION COEFFICIENTS[a]

At 20°, in dilute KCl solution, *p*H 4.5 to 10

Crystalline catalase, <0.5% concn.	*11.6* S.
Lyophilized powder (Lot 5458), 1 to 2% concn.	
First peak	11.3 S.
Second peak	7.6 S.
Third peak	4.15 S.

[a] All results are averages of five to ten determinations. The standard deviation within each set was 0.15 to 0.2 S. For Lot 5458 the results were generally smaller at 2% total protein than at 1%, but the difference lay within the standard deviation.

The different sedimentation rates of the three peaks of Lot 5458 must reflect differences in molecular weight, rather than differences in frictional ratio, since the intrinsic viscosity of this sample is identical with that of crystalline catalase. The first peak has a slightly slower sedimentation rate than the single peak of the crystalline preparation, but the difference is accounted for by the fact that this rate was measured at a higher protein concentration. Thus this peak presumably represents molecules which are indistinguishable (by sedimentation) from native catalase.

For molecules which differ in molecular weight but not in frictional properties, the sedimentation coefficient varies as $M^{2/3}$. From this relation we obtain the ratios 4.0:2.2:0.90 for the molecular weights corresponding to the three peaks of Lot 5458. Taking into consideration the fact that the method of measurement is likely to give too high

(17) H. F. Deutsch, *Acta Chem. Scand.*, **6**, 1516 (1952).

a sedimentation coefficient for the second peak and too low a value for the slowest peak, as pointed out above, these figures indicate that the second and third peaks correspond to one-half and one-quarter molecules, respectively. It is improbable that the low value for the third peak represents some degree of unfolding for no effect of *p*H on its sedimentation rate was found: a partially unfolded molecule would be expected to expand with increasing charge.

It may be noted that a molecular weight can be calculated from our data by combination of Perrin's and Simha's equations, in the form given by Scheraga and Mandelkern.[18] Using for their β-factor a value of 2.20×10^6, for the partial specific volume a value of 0.74,[17] and the sedimentation coefficient and intrinsic viscosity reported above, we get a molecular weight of 260,000 for the crystalline sample. With $\beta = 2.15 \times 10^6$ we would obtain a molecular weight of 270,000. Deutsch[17] obtained a value of 269,000 from sedimentation and diffusion coefficients of horse erythrocyte catalase.

Denaturation at High and Low *p*H.—It has been stated that the catalytic activity, spectra and sedimentation behavior of catalase samples examined by us remain unaltered between *p*H 4.5 and *p*H 10. The true stability range of the protein (at 25°) is probably wider, however, extending from *p*H 3.5 to *p*H 11. We did observe that catalase solutions examined between *p*H 3.5 and 4.5 and between *p*H 10 and 11 were slightly less active and had slightly lower Soret absorption than neutral solutions. An appearance of (or small increment in) species sedimenting at lower rates was also observed. These changes, however, always occurred during the addition of acid or base, and the properties of the solutions remained unaltered even after several hours of standing once the final *p*H was attained. It is probable that the changes observed were the result of brief exposure during the mixing process to *p*H values below *p*H 3.5 or above *p*H 11, and that the native protein is entirely stable between *p*H 3.5 and 11.

True denaturation occurs below *p*H 3.5 and above *p*H 11, both for crystalline and lyophilized samples. The process is quite slow at *p*H 3.5 or *p*H 11, but increases in rate as the *p*H becomes more extreme. All activity is lost, and, at a similar rate, the absorption at the Soret peak (406 mμ) is decreased. A typical experiment showing the latter effect is given in Fig. 4.

We have not studied the denaturation process in detail, but we have examined the sedimentation behavior of several samples of crystalline catalase denatured in this way. In every case a single symmetrical peak was observed, with sedimentation coefficient at 20° equal to 4.6 S. or below. The inference is that complete dissociation to quarter molecules has occurred. On the alkaline side a progressive decrease in sedimentation coefficient with increasing *p*H strongly suggests that unfolding with a resulting increase in frictional coefficient is also occurring.

(18) H. A. Scheraga and L. Mandelkern, *J. Am. Chem. Soc.*, **75**, 179 (1953).

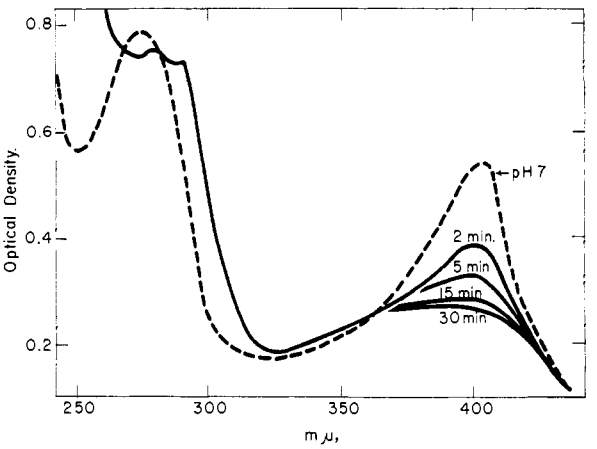

Fig. 4.—The ultraviolet absorption spectrum of crystalline catalase (0.51 mg./cc.) at *p*H 11.5, as a function of time after mixing. The dashed line shows the spectrum of a similar solution (0.53 mg./cc.) at *p*H 7. The temperature was 25°, the ionic strength was 0.02.

Discussion

We have shown that the reduction in enzymatic activity which occurs during lyophilization of catalase is accompanied by dissociation of much of the catalase to half and quarter molecules. We have also found that denaturation by acid or base, with complete loss of activity, is accompanied by complete dissociation to quarter molecules. For alkaline denaturation this result has been confirmed by Osbahr and Eichhorn.[19] It has been shown by Samejima and Shibita[20] that another method of denaturation, by use of urea or formamide, also leads to dissociation into subunits.

It cannot, however, be concluded from these data that the state of association of a catalase sample is a unique measure of its activity, for our two lyophilized samples contained apparently (from uncorrected areas of sedimentation diagrams) 35 and 12%, respectively, of undissociated material. The corresponding activities were 12 and 6%, respectively, of the activity of our crystalline sample. Another indication that retention of the 250,000 molecular weight alone does not suffice to ensure maximal activity is found in the work of Deutsch[17] with erythrocyte catalase. He prepared several samples, all of which behaved identically in the ultracentrifuge, yet differed in activity by as much as 65%.

It should also be noted that the absorption intensity of the Soret band has frequently been related to enzymatic activity,[7] and it is certainly true that all really active catalase samples have a high Soret absorbance. Again, however, a one-to-one correspondence cannot be valid. The Soret absorption of our Lot 5458 was much below that of Lot 5457 (Table I), but its specific activity was twice as large. Once more the same conclusion can be drawn from Deutsch's studies with erythrocyte catalase.[17] There was a 65% difference in activity between his most active and least active

(19) A. J. Osbahr and G. L. Eichhorn, in press.

(20) T. Samejima and K. Shibita, *Arch. Biochem. Biophys.*, **93**, 407 (1961).

samples. The maximum variation in Soret absorption was 7%, and there was no correlation between the small differences in absorption and the differences in activity.

As a final complication, we should note that there was a difference in the distribution of *visible* light absorption between the three peaks of Lots 5457 and 5458, as observed in the sedimentation diagrams. In Lot 5458 absorption was associated with the leading peak only, whereas all three species absorbed light in Lot 5457.

Acknowledgments.—This work was supported by research grants from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, and from the National Science Foundation.

[CONTRIBUTION FROM ANALYTICAL RESEARCH DEPARTMENT, ELI LILLY AND CO., INDIANAPOLIS 6, IND.]

Spectropolarimetric Studies on Proteins. Bovine Plasma Albumin and Insulin

BY MAX M. MARSH

RECEIVED MAY 18, 1961

Optical rotatory dispersion curves from which the contributions of the sulfur-containing amino-acids were subtracted have been obtained for insulin and bovine plasma albumin. Drude equation and Moffitt equation constants derived from these curves have been calculated. Similar data on these proteins after cleavage of disulfide bonds provide a comparison of effects of the cleavage itself on optical rotatory properties of the proteins; application of two different methods of cleavage provides a comparison of effects of specific procedures, as well. Measurements on all materials were made in two different solvent systems. The selected solvents represent different extremes in terms of the conformational behavior of protein molecules. The presence of zinc in the insulin molecule is shown to have an appreciable effect on its optical rotatory properties.

Considerable emphasis is presently being placed on the measurement and interpretation of optical rotatory dispersion curves of proteins as an aid in the evaluation of the secondary and tertiary structural features of their configurations. In particular, the work of Moffitt,[1-3] Doty,[4-6] Yang[2,4,5], Blout,[7-9] and others involving optical rotatory dispersion measurements has shown interesting correlations with the apparent helical nature of polypeptides and proteins in solution.

A complicating feature of the analysis of optical rotatory dispersion curves is the necessity for separation of the contributions to the optical rotatory dispersion of the ordered secondary structure itself from those of the amino acid constituents of the protein. A working concept of these contributions is one which consists of a consideration of the anomalous character of the dispersion curve as being due to the ordered structure while that of the intrinsic rotatory contributions of the amino acid residues is thought of as being simple dispersion. A difficulty in utilizing this concept was pointed out by Turner, Bottle and Haurowitz,[10] who demonstrated the effect of oxidation of cystine bridges in albumin on the optical rotation values at the D-line (5890 Å.). In this case the anomalous rotatory behavior of certain of the amino acid constituents themselves namely cystine, can be interpreted as contributing to the over-all anomalous rotatory characteristic of this protein.

Since the studies mentioned above were carried out in a solvent (88% formic acid) in which helix formation is *not* believed to be favored, the results might be misleading in the sense that the implication is that the oxidation of cystine bridges does not alter the helical content of the protein. Of course, one expects the helical content to be virtually zero either before or after oxidation of *albumin* in 88% HCOOH; however, a more rigid structure such as that found in insulin might exhibit significant changes in secondary order on cleavage of -S-S- bonds even in formic acid. The problem also arises regarding results of such oxidative procedures in solvents wherein the formation of a helix *is* favored.

It is also possible that the *manner* in which the disulfide bonds are cleaved could have some effect on the optical rotatory dispersion, particularly if the reaction conditions could permit interaction of some groups other than -S-S-; if the hypothesis of Turner, *et al.*, is supported, however, the manner of cleavage should not cause significant alteration of rotatory properties *if the disulfide bond contributions and those of their conversion products are first subtracted* (assuming no other changes in the molecule).

In order to get a more complete picture of the optical rotatory changes which take place, the proteins in this study—bovine plasma albumin and insulin—were examined spectropolarimetrically; thus the continuous optical rotatory dispersion curves from 300 to 589 mμ were obtained, rather than $[\alpha]_D$ values alone. Likewise to make some interpretation of the effect of the manner of cleavage on the rotatory changes seen, the -S-S- bonds of these proteins were cleaved reductively with sulfite as well as oxidatively with performic acid in separate experiments.

The oxidation of -S-S- bonds with performic acid

(1) W. Moffitt, *J. Chem. Phys.*, **25**, 467 (1956).
(2) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.*, **42**, 596 (1956).
(3) W. Moffitt, *ibid.*, **42**, 736 (1956).
(4) P. Doty and J. T. Yang, *J. Am. Chem. Soc.*, **78**, 498 (1956).
(5) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957).
(6) P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.*, **43**, 213 (1957).
(7) E. R. Blout and R. H. Karlson, *J. Am. Chem. Soc.*, **80**, 1259 (1958).
(8) E. R. Blout and L. Stryer, *Proc. Natl. Acad. Sci.*, **45**, 1591 (1959).
(9) G. D. Fasman, M. Idelson and E. R. Blout, *J. Am. Chem. Soc.*, **83**, 709 (1961).
(10) J. E. Turner, R. T. Bottle and F. Haurowitz, *ibid.*, **80**, 4117 (1958).