using solvent system 4 indicated that XXVIII was dephosphorylated to 6-benzamido-9-β-D-ribofuranosylpurine which gave a spot with R_{f_4} 0.82.

Adenosine-5′ Phenyl Hydrogen Phosphate (XXIX).—To a solution of 100 mg. of crude XXVIII in 3.5 ml. of absolute methanol was added 1.5 ml. of methanolic 1 *N* sodium methoxide. After refluxing the mixture for 70 minutes, the *p*H was adjusted to *ca.* 2 by addition of Dowex-50 (H^+). The acidic solution was concentrated *in vacuo* to dryness and trace amounts of water were removed by co-distillation with ethanol *in vacuo*. On addition of acetone and subsequent removal of the solvent *in vacuo*, the residue afforded a crude desired product as a yellowish powder; yield 70 mg. (88%). On paper chromatography, the product gave a main spot of R_{f_1} 0.66 with a trace amount of slowly traveling unidentified contaminant; ultraviolet absorption $\lambda_{max}^{H_2O}$ 230. When the crude XXIX was treated with 4 ml. of water, the almost colorless pure product, m.p. 221–222° dec., was obtained as an insoluble precipitate: yield 24 mg. (30%). This compound showed a single spot (R_{f_1} 0.66) on paper chromatogram. A suspension of this product in a small amount of water was neutralized with 1 *N* sodium hydroxide to give a clear solution which was adjusted to *p*H *ca.* 4 by portionwise addition of Dowex-50 (H^+). To the solution separated from resin, was added several grains of Dowex-50 (H^+) to precipitate colorless crystals (m.p. 222° dec.), which were collected and analyzed; ultraviolet absorption: $\lambda_{max}^{pH\ 8}$ 260 (14700), $\lambda_{min}^{pH\ 8}$ 228 (3100).

Anal. Calcd. for $C_{16}H_{18}O_7N_5P \cdot 1/2H_2O$: C, 44.45; H, 4.43; N, 16.20; P, 7.16. Found: C, 44.21; H, 4.57; N, 16.42; P, 7.42.

The sodium salt of XXIX was treated with crude venom as in the case of XXVIII. Paper chromatograms obtained with three different solvent system revealed that XXIX was completely hydrolyzed to adenosine and orthophosphate. Aqueous extract of the spots, which gave the R_f value of R_{f_1} 0.83, R_{f_4} 0.36 and R_{f_6} 0.79, showed identical ultraviolet spectrum with that of adenosine.

Adenosine-5′ Phosphate (XXX).—To a suspension of 70 mg. of crude powdery XXIX in 3 ml. of water was added 1 *N* sodium hydroxide to adjust the *p*H to *ca.* 9. After the addition of 0.5 ml. of 0.005 *M* magnesium acetate, 3.5 ml. of ammonia–ammonium chloride buffer (*p*H 9.0) and 0.3 ml. of enzyme solution (venom phosphodiesterase of Russel's viper),[36,49] the solution was incubated at 36–38° for 23 hours. Paper chromatography revealed that over 95% of XXIX was converted to adenosine-5′ phosphate. The mixture was decationized with 0.2 ml. of Dowex-50 (H^+) and concentrated *in vacuo* to *ca.* 3 ml. After the *p*H was adjusted to 9.5 with saturated aqueous barium hydroxide solution, a small amount of insoluble material was removed by centrifugation. To the supernatant was added three volumes of ethanol and the resulting precipitate was collected. The crude barium salt of XXX thus obtained was decationized with 0.1 ml. of Dowex-50 (H^+) in 5 ml. of water. The aqueous solution was concentrated *in vacuo* to *ca.* 0.5 ml. to which acetone was added to turbidity. The oily precipitate was seeded with a trace of adenosine-5′ phosphate. Upon scratching, colorless crystals appeared, which were collected to give 24 mg. (42%) of adenosine-5′ phosphate. The melting point of the product was 187–188° (turned brown and bubbled). The standard sample[37] of adenosine-5′ phosphate as well as a mixture of the latter and the product similarly turned brown and bubbled on melting at 187–188°. The each single spot observed for the product on paper chromatograms gave respective R_f values of R_{f_1} 0.08, R_{f_2} 0.57, R_{f_3} 0.56, which was identical with that for authentic adenosine-5′ phosphate. The ultraviolet spectrum in water or 1% sodium bicarbonate was also identical with that of the standard sample.

(49) One milliliter of the enzyme solution contained 40γ of the enzyme protein.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, THE WEIZMANN INSTITUTE OF SCIENCE, REHOVOTH, ISRAEL, AND FROM THE DEPARTMENT OF BIOCHEMISTRY AND THE UNIT FOR RESEARCH IN AGEING, THE ALBERT EINSTEIN COLLEGE OF MEDICINE, YESHIVA UNIVERSITY, BRONX 61, NEW YORK]

Peptide Synthesis *via* Oxidative Activation of Acid Hydrazides[1,2]

BY Y. WOLMAN,[3] P. M. GALLOP, A. PATCHORNIK AND A. BERGER

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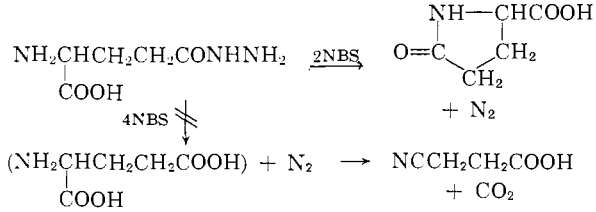
A new method for the synthesis of peptides has been developed based on the oxidative activation of N-acyl-α-amino acid or peptide hydrazides. The method is simple, rapid and gives yields which compare favorably with and occasionally exceed those found with methods in current use. It was found to give little racemization and accordingly, could be applied to the synthesis of peptides containing side chains which are not sensitive to the oxidative conditions employed. Oxidation of a tripeptide hydrazide led to the synthesis of a poly-α-amino-acid with a known sequence.

Curtius[4] first showed that upon oxidation of acyl hydrazides by iodine bis-diacyl hydrazides were obtained. A few years ago Carpino found that

$$2RCONHNH_2 + 2I_2 \longrightarrow RCONHNHOCR + N_2 + 4HI$$

under highly acidic conditions acyl hydrazides may be oxidized by chlorine to the corresponding acyl chlorides.[5] Recently it was found that various acyl hydrazides including benzyloxycarbonylamino acid hydrazides were oxidized by 2 moles of N-bromosuccinimide (NBS) in dilute aqueous solution to the corresponding carboxylic acids with evolution of nitrogen. However, the γ hydrazide of glutamic acid was not oxidized through glutamic acid to β-cyano propionic acid as expected but pyrrolidone carboxylic acid was obtained in quantitative yield.[6] These data suggested the possibility that the intermediate which resulted from acyl hydrazide oxidation was a powerful acylating agent and that this intermediate could be formed



(1) Presented in part before the 28th meeting of the Israel Chemical Society, Rehovoth 1961 (Y. Wolman and P. M. Gallop, *Bull. Research Council Israel*, **10A**, 43 (1961)). A preliminary communication of this work has been reported (Y. Wolman, P. M. Gallop and A. Patchornik, *J. Am. Chem. Soc.*, **83**, 1263 (1961)).

(2) Part of a thesis submitted by Y. Wolman in partial fulfillment of the requirements for the degree of Doctor of Philosophy to the Hebrew University, Jerusalem, May 1961.

(3) On leave of absence from the Weizmann Institute of Science, present address, the Albert Einstein College of Medicine.

(4) T. Curtius, *J. Prak. Chem.*, **50**, 281 (1894).

(5) L. A. Carpino, *J. Am. Chem. Soc.*, **79**, 96 (1957).

(6) P. M. Gallop, S. Seifter and C. Franzblau, unpublished results.

in the presence of a free amino group. Therefore, a direct reaction to form a peptide bond was anticipated when the oxidative activation of a benzyloxycarbonyl amino acid hydrazide takes place in the presence of an amino acid ester.

The easily isolatable benzyloxycarbonylglycylglycine *p*-nitrobenzyl ester was selected as a standard product in order to find optimal conditions for the peptide synthesis. It was found that best yields were obtained when two moles of NBS as the oxidant were added to a mixture containing one mole of benzyloxycarbonylglycylhydrazide, one mole of glycine *p*-nitrobenzyl ester and two moles of base usually triethylamine (TEA). After four to five minutes the dipeptide was precipitated from the reaction mixture by adding four to five volumes of water. When the oxidizing agent was added first to the benzyloxycarbonylglycylhydrazide and the glycine *p*-nitrobenzyl ester was added later, only the bis dibenzyloxycarbonylglycylhydrazide ($C_7H_7OCONHCH_2CONHNHOCCH_2NHOCOC_7H_7$) was obtained in low yield. The dipeptide was obtained in 80–86% yield using NBS as oxidizing agent in various solvents (tetrahydrofuran, dioxane, dimethylacetamide and acetonitrile). Dimethylacetamide was chosen as the solvent for the peptide synthesis as it has the advantage of dissolving most amino acid and peptide ester hydrohalides.

Various oxidizing agents have been tested to ascertain whether they could serve as oxidants in this method of peptide synthesis (see Table I).

TABLE I

YIELDS OF BENZYLOXYCARBONYLGLYCYLGLYCINE *p*-NITROBENZYL ESTER OBTAINED BY USING VARIOUS OXIDANTS IN DIMETHYLACETAMIDE AS SOLVENT WITH TRIETHYLAMINE AS BASE

Oxidant	Yield, %	M.p., °C.
N-Bromosuccinimide	83	107–108
Iodine	86	107–108
Iodine[a]	89	107–108
Iodine[b]	84	107–108
Hydrogen peroxide[c]	35	107–108
Hydrogen peroxide and potassium iodide[d]	56	107
Hydrogen peroxide and potassium iodide[e]	83	107–108
N-Bromoacetamide	46	105
N-Chloroacetamide	0	...
N-Chlorosuccinimide	51	104
tert-Butylhypochlorite	28	101
Potassium ferricyanide[b]	48	105
Silver oxide[f]	76	106–109

[a] Dimethylacetamide–water 1:1 mixture as solvent. [b] Dimethylacetamide–water 1:1 mixture as solvent using sodium carbonate as base. [c] Reaction time 18 hr. at room temp., no base present. [d] Reaction time 5 hr. at room temp., no base present, 0.02 moles of KI per mole of hydrazide, using dimethylacetamide–water 4:1 mixture as solvent. [e] Reaction time 30 minutes at room temp. no base present, 4 moles of KI per mole of hydrazide, using dimethylacetamide–water 3:1 mixture as solvent. [f] Reaction time 18 hr. at 4°, no base present.

Iodine ($2I_2$) seems to give the dipeptide in good yield in a very pure form and any excess of unreacted iodine could be removed with sodium thiosulfate.

Although in most cases small amounts of bis-dibenzyloxycarbonylglycylhydrazide were obtained with the crude product of the desired dipeptide, it was shown that the bis compound was not an intermediate in the peptide synthesis. Bis-dibenzyloxycarbonylglycylhydrazide was prepared by reacting the mixed anhydride of benzyloxycarbonylglycine and isobutylchloroformate[7] with benzyloxycarbonylglycylhydrazide. Oxidation of this compound with various oxidizing agents in the presence of glycine *p*-nitrobenzyl ester did not give the desired dipeptide.

The amount of racemization occurring in this method of synthesis was tested using the procedure of Anderson, *et al.*,[8] which can distinguish benzyloxycarbonylglycyl-L-phenylalanylglycine ethyl ester from benzyloxycarbonylglycyl-DL-phenylalanylglycine ethyl ester. Upon oxidation of benzyloxycarbonylglycyl-L-phenylalanyl hydrazide with NBS in the presence of glycine ethyl ester employing tetrahydrofuran as a solvent, the tripeptide benzyloxycarbonylglycyl-L-phenylalanylglycine ethyl ester in 62% yield and the racemic mixture in 1.1% yield were obtained. Oxidation with iodine using mixture of dimethylacetamide–water 2:1 as solvent gave the pure optical isomer in 68% yield and the racemic mixture in 2.2% yield.

It appears likely that the acyl hydrazide undergoes preferential oxidation in the presence of an amine to either an acyl diazonium salt or an acyl azo intermediate which then reacts with the nucleophilic amine to form a peptide bond with evolution of nitrogen. In the absence of the amine and to a small extent in its presence, the intermediate reacts with the unreacted hydrazide to form the bis-diacylhydrazide.

Using this procedure some di- and tripeptides were prepared (see table II).

Benzyloxycarbonylamino acid hydrazides were made from the corresponding esters, the esters were obtained by esterification of the benzyloxycarbonylamino acids using acidic catalysis.[9]

Since oxidative activation of the acyl function in the presence of amino groups was demonstrated, the formation of sequence polymer from a free tripeptide hydrazide was investigated.

A tripeptide hydrazide, glycyl-L-seryl-L-alanylhydrazide dihydrobromide was prepared as follows: Benzyloxycarbonylglycyl-L-seryl-L-alanine benzyl ester was reacted with hydrazide to form the benzyloxycarbonyl tripeptide hydrazide. The benzyloxycarbonyl group was removed with hydrogen bromide in glacial acetic acid. After iodine oxidation of the peptide hydrazide in dimethylacetamide, ether was added and the crude product was dissolved in water and dialysed against running tap water for 4 hr. and then against distilled water overnight. Lyophilization of the contents of the dialysis bag gave a 50% yield of the sequence polymer. After acid hydrolysis of the product, amino acids analysis[10] gave ratios for glycine,

(7) J. R. Vaughan and R. L. Osato, *J. Am. Chem. Soc.*, **74**, 676 (1952).

(8) G. W. Anderson and F. M. Callahan, *ibid.*, **80**, 2902 (1958).

(9) Simmonds, *et al.* (S. Simmonds, J. I. Harris and J. S. Fruton, *J. Biol. Chem.*, **188**, 251 (1951)) used a similar procedure and isolated the ester after 36-hr. (for isolation of the esters and subsequent reaction with hydrazine, see Experimental).

TABLE II

SYNTHESIS OF PEPTIDES *via* OXIDATIVE ACTIVATION OF AMINO ACID HYDRAZIDES

Peptide	Oxidant	Solvent[a]	Yield, %	M.p., C°	Crystallized from	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Z.Gly+GlyOEt	I_2	[b]	75	83–84[c]	Water						
Z.Gly+NHBz	I_2	B	78	120–121	Methanol	68.43	68.50	6.08	6.28	9.39	9.30
Z.DL-Ala+GlyOBz(NO_2)	NBS	A	75	116–117	Ethanol–water	57.83	58.09	5.10	5.29	10.11	9.81
Z.DL-Ala+GlyOBz(NO_2)	I_2	B	73	116–117	Ethanol–water						
Z_2.L-Lys+GlyOBz(NO_2)	NBS	A	99	94	Ethanol–water	61.37	61.73	5.65	5.82	9.24	9.19
Z_2.L-Lys+GlyOBz(NO_2)	I_2	B	90	94–95	Ethanol–water						
Z_2.L-Lys+L-Glu(OEt)OBz(NO_2)	NBS	A	82	105–106	Ethanol–water	61.18	61.14	5.99	6.12	7.93	8.26
Z_2.L-Lys+L-Glu(OEt)OBz(NO_2)	I_2	B	69	106	Ethanol–water						
Z_2.L-Lys+L-PheOEt	I_2	B	58[d]	120–121	Ethanol–water	67.21	67.28	6.66	6.64	7.12	7.44
Z.Gly-L+PheOEt	NBS	B	76	Oil[e]							
Z.Gly-L-Phe+GlyOEt	NBS	A	62	116–117[f]	Ethanol						
Z.Gly-L-Phe+GlyOEt	I_2	C	68	116–117	Ethanol						
Z.L-Pro+Gly-GlyOBz(NO_2)	I_2	C	61	152–153	Ethylacetate	57.82	57.64	5.26	5.42	11.24	11.56
Z.L-Hypro+Gly-GlyOBz(NO_2)	I_2	C	42[g]	139–140	Ethylacetate	56.02	55.87	5.09	5.17	10.89	11.05
Z.Gly-L-Ser+L-AlaOBz	I_2	B	50	132–133	Ethylacetate	60.38	60.09	5.95	5.88	9.18	9.25

[a] The solvents employed were A: tetrahydrofuran; B: dimethylacetamide; C: dimethylacetamide–water 2:1. [b] The reaction was carried in dioxane–water (3:1) and the dipeptide was extracted with ethyl acetate after removing the solvent *in vacuo*. [c] G. W. Anderson and R. W. Young, *J. Am. Chem. Soc.*, **74**, 5307 (1952), gave m.p. of 84–85°. [d] Bis-dibenzyloxycarbonyl-ε-benzyloxycarbonyllysylhydrazide was obtained in 38% yield. [e] G. W. Kenner, R. J. Stedman, *J. Chem. Soc.*, 2069 (1952). [f] See ref. 8. [g] The tripeptide was obtained in 12% yield by coupling benzyloxycarbonyl hydroxyproline and glycylglycine *p*-nitrobenzyl ester by the mixed anhydride procedure, in 18% yield using the dicyclohexylcarbodiimide procedure and in 51% yield employing the azide method (Y. Wolman and A. Berger, unpublished results).

serine and alanine of 1.00, 1.03, 0.92, respectively.

Van-Slyke amino nitrogen analysis gave an equivalent weight for the polymer of 3,400. Bound hydrazine (presumably carboxy terminal since it was sensitive to further iodine oxidation) was measured colorimetrically[11] and an equivalent weight of 2,500 was found.

The molecular weight was measured by sedimentation and diffusion using the synthetic boundary cell.[12] The s^0_{20} was 0.73 svedberg and the diffusion constant was 24.7×10^{-7} cm.^{2}/sec. Assuming a partial specific volume of 0.73 a molecular weight of 2,800 was calculated.

These results are in fairly good agreement and indicate that a relatively sharp distribution of polymer size with about 12 tripeptide repeating units exist after dialysis.

Experimental

Bis-dibenzyloxycarbonylglycylhydrazide.—In 20 ml. of dimethylformamide, 2.1 g. (10 mM) of benzyloxycarbonylglycine were dissolved and placed in an ice–salt bath; to the mixed anhydride formed by the addition 1.44 ml. (10 mM) of triethylamine and 1.33 ml. (10 m*M*) of isobutylchloroformate, 2.2 g. (10 m*M*) of benzyloxycarbonylglycylhydrazide were added. After standing at room temperature overnight, seven volumes of water were added and the crude product filtered off and washed successively with 100 ml. of 1 *N* hydrochloric acid, 100 ml. of 1 *N* sodium bicarbonate, 100 ml. of water and air dried. The crude compound was crystallized from acetic acid–water to give 3.1 grams; 75% yield; m.p. 211–212°. Sample for analysis was crystallized from glacial acetic acid without change in melting point.[13]

Anal. Calcd. for $C_{20}H_{22}N_4O_6$: C, 57.96; H, 5.35; N, 13.52. Found: C, 57.90; H, 5.32; N, 13.46.

Benzyloxycarbonylglycylglycine *p*-Nitrobenzyl Ester.—A. 1.45 g. (5 m*M*) of glycine *p*-nitrobenzyl ester hydrobromide, 1.12 g. (5 m*M*) of benzyloxycarbonylglycylhydrazide and 2.16 ml. (15 m*M*) of triethylamine were dissolved in 20 ml. of dimethylacetamide. 1.78 g. (10 m*M*) of solid N-bromosuccimide were added to the ice cold solution. After 5 minutes the product was precipitated by the addition of 5 volumes of cold water, filtered and crystallized from ethanol–water to give 1.72 g. of product, 86% yield; m.p. 107–108°. Sample for analysis was crystallized from dioxane–water without change in melting point.

Anal. Calcd. for $C_{19}H_{19}N_3O_7$: C, 56.85; H, 4.77; N, 10.47. Found: C, 56.77; H, 4.73; N, 10.51.

B. 1.45 g. (5 mM) of glycine *p*-nitrobenzyl ester hydrobromide, 1.12 g. (5 mM) of benzyloxycarbonylglycylhydrazide and 3.6 ml. (25 mM) of triethylamine were dissolved in 20 ml. of dimethylacetamide; 2.54 g. (10 mM I_2) of iodine in 10 ml. of dimethylacetamide were added to the ice cold solution. After 5 minutes dilute sodium thiosulfate solution was added to remove the unreacted iodine, followed by the addition of 5 volumes of water. After 30 minutes the crude product was filtered off and crystallized from ethanol–water to give 1.70 g., 85% yield; m.p. 107–108°.

C. 1.45 g. (5 m*M*) of glycine *p*-nitrobenzyl ester hydrobromide, 1.12 g. (5 m*M*) of benzyloxycarbonylglycylhydrazide and 0.72 ml. of triethylamine (5 m*M*) were dissolved in 20 ml. of dimethylacetamide; 1.5 of a 30% solution of hydrogen peroxide were added and the reaction left for 18 hr. at room temperature. After addition of 5 volumes of water the crude product was filtered off and crystallized from ethanol–water to give 700 mg., 36% yield; m.p. 107–108°.

D. 1.45 g. (5 m*M*) of glycine *p*-nitrobenzyl ester hydrobromide, 1.12 g. (5 mM) of benzyloxycarbonylglycylhydrazide and 0.72 ml. of triethylamine were dissolved in 20 ml. of dimethylacetamide; to the cold solution 25 ml. of water containing 6.56 g. (20 mM) of potassium ferricyanide and 2.96 g. (20 m*M*) of potassium carbonate were added and the reaction mixture stirred vigorously. After 10 minutes 5 volumes of water were added and the crude product filtered off and crystallized from ethanol–water to give 960 mg.; 48%, m.p. 105°.

Benzyloxycarbonylglycylglycine Ethyl Ester.—1.12 g. (5 m*M*) of benzyloxycarbonylglycylhydrazide, 720 mg. (5 mM) of glycine ethyl ester hydrochloride and 3.6 ml. (25 mM) of triethylamine were dissolved in 18 ml. of dioxane and 7 ml. of water; to the ice cold solution 2.54 g. (10 m*M* I_2) of solid iodine in powdered form were added with vigorous mixing. After 5 minutes, excess iodine was re-

(10) D. H. Spackman, W. H. Stein and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(11) S. Seifter, P. M. Gallop, S. Michaels and E. Meilman, *J. Biol. Chem.*, **235**, 2613 (1960).

(12) E. G. Pickels, W. F. Harrington and H. K. Schachman, *Proc. Natl. Acad. Sci. U. S.*, **38**, 943 (1952).

(13) Simmonds, *et al.* (S. Simmonds, J. I. Harris and J. S. Fruton, *J. Biol. Chem.*, **188**, 252 (1951)) claimed that dibenzyloxycarbonylglycylhydrazide was obtained as a by-product during the synthesis of benzyloxycarbonylglycylleucine methyl ester using the azide method. Their compound had a melting point of 138° and was crystallized from aqueous ethanol. Shortly afterwards Fruton, *et al.* (J. S. Fruton, R. B. Johnston and M. Fried, *ibid.*, **190**, 39 (1951)), described the synthesis of benzylcarbonylglycinylamide and this compound had the same melting point, solubility properties and analysis as the compound they described earlier as the dibenzyloxycarbonylglycylhydrazide. Since there is only one hydrogen difference between these two compounds, elementary analysis might not differentiate between them. This is an example where some amide is formed as a by-product using the azide method is peptide synthesis; other cases are known.

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.

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[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]

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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.