[CONTRIBUTION FROM THE LABORATORY OF THE CHILDREN'S CANCER RESEARCH FOUNDATION, THE CHILDREN'S MEDICAL CENTER]

Polypeptides. III. The Synthesis of High Molecular Weight Poly-γ-benzyl-L-glutamates[1]

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The polymerization of γ-benzyl-N-carboxy-L-glutamate anhydride with initiators such as sodium hydroxide, sodium methoxide, sodium borohydride and triethylamine can lead to polypeptides of molecular weight over 500,000 (degree of polymerization > 2000). In the cases of the first three initiators the molecular weight is related to the anhydride–initiator ratio (A/I) but is always higher than calculated for a given A/I. With triethylamine initiation the molecular weight is essentially independent of the amount of initator over a twenty-fold range of A/I. Data are presented that show that increasing the anhydride concentration, or raising the temperature of polymerization, yields polymers of much lower molecular weight. The solvent in which the polymerization takes place also affects the molecular weight. Evidence is presented that the N-carboxy anhydride ring opens in two different ways in the presence of sodium methoxide. This may be an explanation of the obtaining of molecular weights higher than calculated from the A/I's.

Since the original synthesis of α-amino acid-N-carboxy anhydrides[3] these compounds have been used extensively for the preparation of poly-α-amino acids.[4] Water, alcohols and amines can serve as initiators or catalysts for the polymerization of N-carboxy-α-amino acid anhydrides, and most previous work has been performed using such materials as initiators. There are only a few studies on the molecular weights of the polypeptides obtained and few data, except the work of Stahmann,[5] on the effect of varying initiator concentration upon the molecular weight. Furthermore, there is no evidence that very high molecular weight polypeptides (molecular weight >100,000) could be prepared by the aforementioned means. In this communication[6] we describe the preparation of polypeptides derived from γ-benzyl-L-glutamate having molecular weights in the range of 15,000 to about 1,000,000. Data will be presented on the effects of various polymerization conditions such as type of initiator, concentration of anhydride, temperature of polymerization, and solvent upon the molecular weight obtained.

γ-Benzyl-N-carboxy-L-glutamate anhydride[7] was selected as starting material because polypeptides derived from it are soluble in many organic solvents and because such polypeptide polymers (and copolymers with other amino acids) can be converted into water-soluble polypeptides by removal of the benzyl groups. Early in the work it was concluded that to obtain maximum molecular weight (mol. wt.) and degree of polymerization (D.P.), it was desirable to use a solvent in which both the monomer (the N-carboxy anhydride) and the polymer (the peptide) were soluble. Dioxane fulfills this requirement for γ-benzyl-L-glutamate and most of the polymerizations were carried out in this solvent.

If the polymerization takes place after a rapid initiation step without side reactions or termination steps, the molecular weights obtained should be determined by the mole ratios of the anhydride (A) to the initiator (I)[8–11] as indicated in the scheme

$$
\begin{array}{l}
COOCH_2C_6H_5 \\
| \\
CH_2 \\
| \\
A \cdot CH_2 \\
| \qquad\qquad O \\
CH—C \diagup\!\!\diagdown \qquad\quad I \\
| \qquad\qquad O \qquad \xrightarrow{\text{initiator}} \\
NH—C \diagdown\!\!\diagup O
\end{array}
$$

γ-benzyl-N-carboxy-L-glutamate anhydride

$$
I \cdot \left[
\begin{array}{l}
COOCH_2C_6H_5 \\
| \\
CH_2 \\
| \\
CH_2 \\
| \\
—NH—CH—CO—
\end{array}
\right]_{A/I} + A \cdot CO_2
$$

poly-γ-benzyl-L-glutamate

When water is used as the initiator, it has been reported[12] that a tenfold variation in the anhydride–initiator ratio (A/I) did not significantly change the molecular weight of lysine polypeptides prepared in benzene solution. However, the use of anhydrous ammonia as initiator in dioxane solution yielded polypeptides of different molecular weights depending on the A/I,[5] but in this case there was no direct relationship between the A/I and the molecular weight. Although average molecular weights were determined by end group analysis for the α-amino nitrogen content of the ε-carbobenzoxylysine polypeptides,[5] this method of molecular weight determination is subject to criticism because of the possibility of the end amino group being involved in other reactions such as those recently postulated by Sela and Berger[13] and because its use is limited to materials of relatively low molecular weight.

In our work the determinations of molecular weight were made by viscometry, using the calibra-

(1) This work was supported by the Office of the Surgeon General, Department of the Army.

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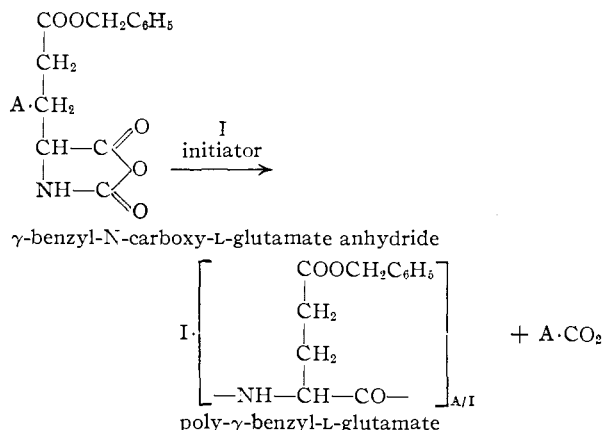(3) H. Leuchs, Ber., 39, 857 (1906); H. Leuchs and W. Geiger, ibid., 41, 1721 (1908).

(4) E. Katchalski, "Advances in Protein Chemistry," Vol. VI, Academic Press, Inc., New York, N. Y., 1951, pp. 123–185.

(5) R. R. Becker and M. A. Stahmann, THIS JOURNAL, 74, 38 (1952).

(6) For a preliminary Note see E. R. Blout, R. H. Karlson, P. Doty and B. Hargitay, ibid., 76, 4492 (1954).

(7) W. E. Hanby, S. G. Waley and J. Watson, J. Chem. Soc., 3009 (1950).

(8) R. B. Woodward and C. H. Schramm, THIS JOURNAL, 69, 1551 (1947).

(9) S. G. Waley and J. Watson, ibid., 70, 2299 (1948).

(10) D. Coleman and A. C. Farthing, J. Chem. Soc., 3218 (1950).

(11) D. Coleman, ibid., 3222 (1950).

(12) M. A. Stahmann, L. H. Graf, E. L. Patterson, J. C. Walker and D. W. Watson, J. Biol. Chem., 189, 45 (1951).

(13) M. Sela and A. Berger, THIS JOURNAL, 75, 6350 (1953); 77, 1893 (1955).

tion provided by the light scattering and viscosity measurements of Professor Paul Doty, Drs. A. M. Holtzer and J. H. Bradbury,[14,15] whose help in this difficult phase of the work we gratefully acknowledge. For the most part the viscosity measurements reported here were made with dichloroacetic acid solutions in which poly-γ-benzyl-L-glutamate (PBLG) has been shown to exist in a non-associated random coil configuration.[14,15] A few measurements were made in dimethyl formamide solution in which PBLG exists as an intramolecularly H-bonded rigid rod with the dimensions of the α-helix postulated by Pauling and Corey.[16]

Results

Since primary amines had been used previously as initiators,[4] n-hexylamine was first investigated as an initiator. It was soon apparent that the products of this reaction did not give average molecular weights, as indicated by viscosity, which corresponded to those predicted from the anhydride–initiator ratios. Some typical data are shown in Fig. 1 for A/I's from 50 to 1000. In the figures A/I is plotted against D.P. and mol. wt., and the dashed diagonal line represents the "theoretical" A/I–D.P. relationship. From this figure in which the diameter of the circles represents the maximum error in the measurement ($\pm 10\%$), it appears that the molecular weight obtained is approaching a maximum at or slightly under 100,000 (D.P. $\sim$450). The results of many experiments show that in no case was a mol. wt. over 100,000 obtained using primary amines as initiators.
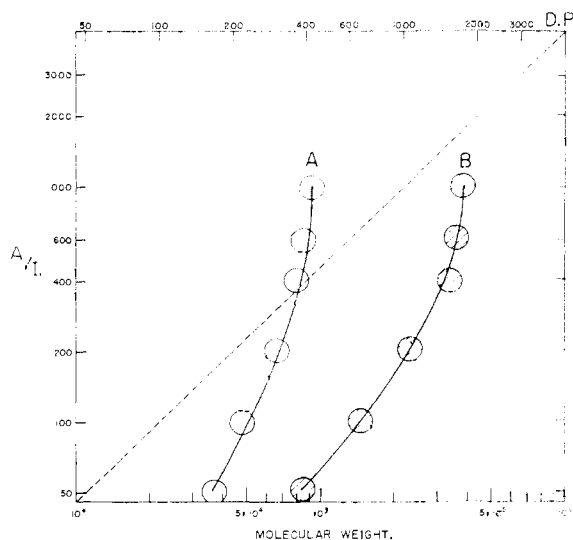

Fig. 1.—Poly-γ-benzyl-L-glutamates from polymerizations in dioxane solution; with n-hexylamine initiator, A; with sodium hydroxide initiator, B; degree of polymerization (D.P.) as a function of anhydride–initiator ratio (A/I). The molecular weights were obtained from reduced specific viscosities at concentrations of 0.2% in dichloroacetic acid.

(14) P. Doty, A. M. Holtzer, J. R. Bradbury and E. R. Blout, THIS JOURNAL, 76, 4493 (1954).

(15) P. Doty, A. M. Holtzer and J. H. Bradbury, ibid., 78, 947 (1956).

(16) L. Pauling, R. B. Corey and H. R. Branson, Proc. Nat. Acad. Sci., 37, 205 (1951), L. Pauling and R. B. Corey, ibid., 37, 235 (1951).

Because of these results, other possible initiators for this reaction were considered. It was found that sodium hydroxide (in water or methyl alcohol) not only initiated, but also that the reaction was considerably faster[17] than that resulting from primary amine initiation and that higher mol. wt.'s were obtained for a given A/I. Some typical results are shown in Fig. 1. An indication of the rate of the reaction is given by the fact that the A/I 200 reaction is more than 95% completed in 8 hours, producing a polymer of D.P. $\sim$1000 and mol. wt. > 200,000. It is noteworthy that the D.P.'s obtained with this initiator converge around 2000, and there is again evidence of an approach to a maximum D.P.

The results with sodium hydroxide initiation stimulated the investigation of other alkali initiators. In Fig. 2 the results obtained with sodium methoxide are shown, the results with sodium borohydride being similar. With each of these initiators the reaction was also very fast and high mol. wt.'s were obtained. With both initiators the maximum weight average molecular weights were about 600,000 (D.P. $\sim$2700), and there is a slightly flatter slope to the A/I–D.P. curve which may indicate that any maximum D.P., if it exists, has not yet been reached.
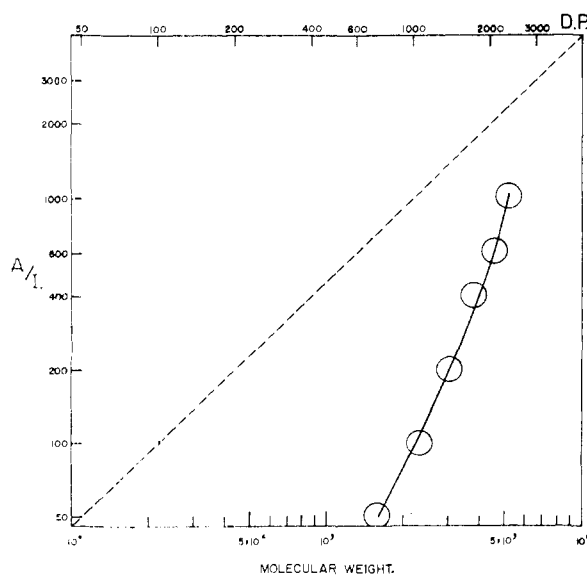

Fig. 2.—Poly-γ-benzyl-L-glutamates from polymerizations in dioxane solution with sodium methoxide initiator; degree of polymerization (D.P.) as a function of anhydride–initiator ratio (A/I). The molecular weights were obtained from intrinsic viscosities in dimethylformamide.

Less extensive experiments have been performed using phenyllithium and sodium acetate as initiators. The D.P.'s obtained with such initiators are of the same order of magnitude as those shown in Figs. 1 and 2.

An investigation was then made of initiation by secondary and tertiary amines, especially since a secondary amine-amide, sarcosine dimethyl amide, has been used extensively as an initiator for prepar-

(17) E. R. Blout, P. Doty, M. Idelson and R. Lundberg, unpublished work.

ative and kinetic work.[17,18] The surprising results obtained with diethylamine are shown in Fig. 3. From these data it appears that initiation with A/I 50 to 1000 produces polypeptides whose D.P. (~1000) is essentially independent, over this twenty-fold range of A/I, of the amount of initiator used.
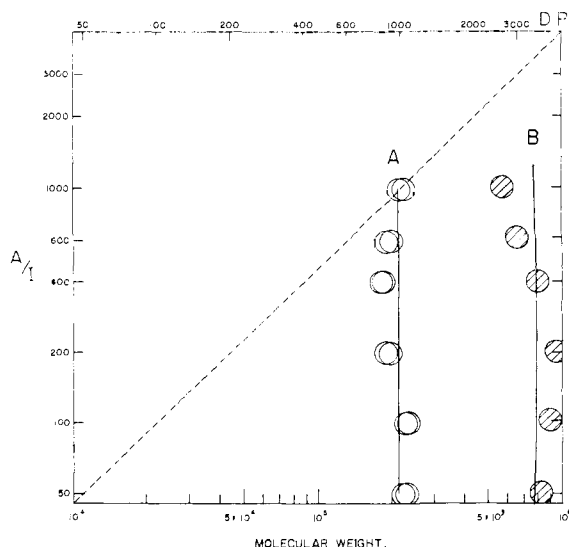

Fig. 3.—Poly-γ-benzyl-L-glutamates from polymerizations in dioxane solution: with diethylamine initiator, A; with triethylamine initiator, B; degree of polymerization (D.P.) as a function of anhydride–initiator ratio (A/I). The molecular weights were obtained from reduced specific viscosities at concentrations of 0.2% in dichloroacetic acid.

Initiation with triethylamine gave results similar to diethylamine (Fig. 6) in the relationship between A/I and D.P. The D.P.'s obtained (2500 to 4500) were higher than with any other initiator.

Examination of the effect of anhydride concentration upon molecular weight yielded the results shown in Fig. 4, namely, that anhydride concentrations up to 5% (with constant A/I) appears to have no effect on the weight average molecular weight obtained, but at higher anhydride concentrations the molecular weights drop sharply.
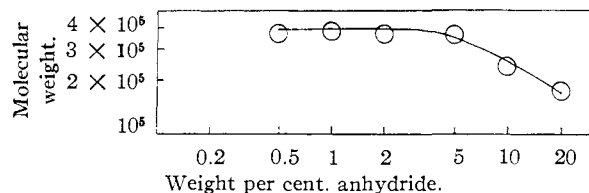

Fig. 4.—Plot of obtained molecular weight of poly-γ-benzyl-L-glutamates as a function of anhydride concentration. Polymerizations run in dioxane solution with sodium methoxide initiation and anhydride–initiator ratio of 200.

A series of experiments designed to show the effect of the polymerization temperature upon the molecular weight is shown in Fig. 5. From these data it is apparent that to obtain maximum molec-

(18) C. H. Bamford, W. E. Hanby and F. Happey, Proc. Roy. Soc. (London), **A206**, 407 (1951).

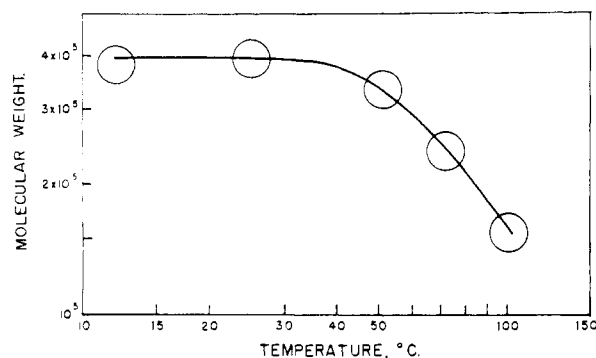ular weight the reaction preferably should not be carried out above 30°.

Fig. 5.—Plot of obtained molecular weight of poly-γ-benzyl-L-glutamates as a function of the temperature of polymerization. The polymerizations were run in dioxane solution with sodium methoxide initiation and anhydride–initiator ratio of 200.

Finally in Table I, there are shown the molecular weights obtained by initiation of γ-benzyl-N-carboxy-L-glutamate anhydride with sodium methoxide and A/I 200 in a variety of solvents.

TABLE I

Solvent	Anhydride concn., g./100 cc.	$(\eta_{sp}/c)_{c=0.2}$ in dichloro-acetic acid	Molecular weight
Dioxane	2	2.07	365,000
Anisole	2.5	1.96	345,000
Benzene	1	1.93	340,000
Dioxane + 0.01% n-hexaldehyde	5	1.78	310,000
Chloroform	2.5	1.68[c]	290,000
		1.37[d]	230,000
Chlorobenzene	2.5	1.04	170,000
Dioxane + 0.1% n-hexaldehyde	5	0.85	135,000
Ethyl acetate[a]	2.5	.84	134,000
Nitrobenzene	2.5	.77	124,000
Acetonitrile[b]	2.5	.73	115,000
Dimethylformamide	5	.55	83,000
Nitromethane[e]	2.5	.41	59,000
Methanol[b]	2.5	.07	12,000
Dioxane + 1% n-hexaldehyde[e]	5		

[a] A precipitate appeared within two hours. [b] After addition of initiator, precipitation was evident. [c] U.S.P. grade. [d] Chloroform purified as described in Experimental section. [e] The product was not precipitable by ethanol and was obviously of low molecular weight.

It can be seen that not only does the polymerization solvent affect the ultimate molecular weight because of precipitation of the product before the reaction goes to completion, e.g., the reaction in methanol, but also some liquids which are good solvents for both monomer and polymer give low D.P.'s. In Fig. 6 the results for polymerization in dimethylformamide with sodium methoxide initiation are given (cf. Fig. 3).

A good indication of the important role the initiator plays in the polymerization of N-carboxy anhydrides is evident from Table I. The results obtained with a variety of initiators and γ-benzyl-N-
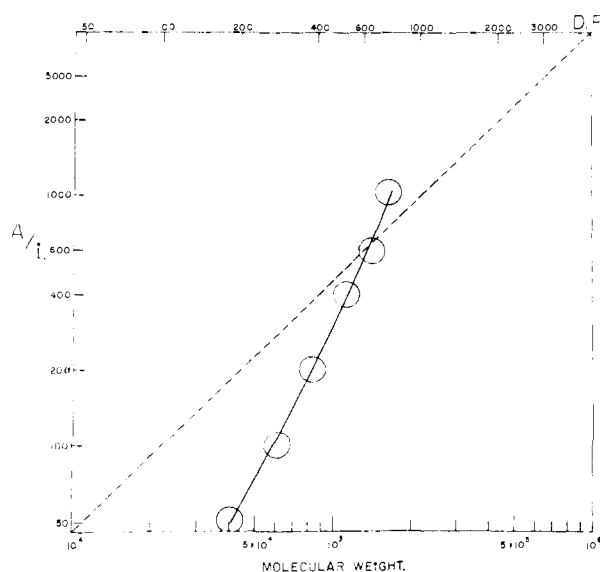
Fig. 6.—Poly-γ-benzyl-L-glutamates from polymerizations in dimethylformamide solution with sodium methoxide initiator; degree of polymerization (D.P.) as a function of anhydride–initiator ratio (A/I). The molecular weights were obtained from reduced specific viscosities at concentrations of 0.2% in dichloroacetic acid.

carboxy-L-glutamate anhydride using A/I 5 in dioxane solutions are given below.

TABLE II

| Initiator | Resulting poly-γ-benzyl-L-glutamate | |
	(η_{sp}/c) $c = 0.2$ in dichloroacetic acid	Weight average mol. wt.
n-Hexylamine	0.12	15,000
Diethylamine	0.55	83,000
Triethylamine	1.62	280,000
Sodium hydroxide	0.23	31,000
Sodium methoxide	.22	29,500
Sodium borohydride	.24	32,500

Experimental

γ-Benzyl-L-glutamate.—Six hundred cc. of 48% hydrobromic acid and 330 g. of L-glutamic acid ($[\alpha]^{25}$D +31.8° (c 1.09 in 6 N HCl)) were added to 2200 cc. of benzyl alcohol. This mixture was heated on the steam-bath with frequent stirring until all the glutamic acid was in solution (ca. 1.5 hours). If an oily phase (benzyl bromide) separates before the glutamic acid is in solution, the heating should be stopped. At this point the reaction mixture was cooled to 30–40° as rapidly as possible, then added with stirring to a solution of 660 cc. of pyridine in 4400 cc. of 95% ethanol. Precipitation occurred upon cooling to 20° and the precipitation was allowed to continue at 3° for 12 hours. The precipitate was then isolated by filtration, washed with ethanol, then with ethyl ether and air-dried. The product was recrystallized from 10 liters of 5% ethanol (maximum temperature <70°), sufficient sodium bicarbonate being added to keep the pH at 7. The product should be kept at high temperatures for the shortest possible time in order to minimize decomposition into benzyl alcohol and glutamic acid. The hot solution was filtered, cooled as rapidly as possible to 3°, and left at this temperature for 12 hours. The precipitate was then isolated by filtration, washed with water (adjusted to pH 7 with sodium bicarbonate), washed with distilled water, slurried with ethanol, filtered, washed with ethyl ether and air-dried; yield of white plates of γ-benzyl-L-glutamate 151 g., 29.5%, m.p. 174°, $[\alpha]^{25}$D +19.5° (c 7.16 in acetic acid); reported[7] m.p. 169–170°. This ester should be stored at 0° or lower to minimize decomposition.

γ-Benzyl-N-carboxy-L-glutamate Anhydride.—117 g. of freshly recrystallized γ-benzyl-L-glutamate was suspended in 600 cc. of purified and sodium-dried dioxane[19] and phosgene bubbled through at 60–65°. After two hours a clear, pale yellow solution was obtained. This solution was concentrated in vacuo, at a maximum temperature of 50°, to a volume of ca. 200 cc. The oil was diluted with 100 cc. of chloroform, and n-hexane added slowly until crystallization begins. Crystallization was complete in 12 hours at 3°. The precipitate was isolated by filtration, washed thoroughly with n-hexane to remove excess phosgene and crystallized from about 800 cc. of chloroform by dissolving at room temperature and cooling to −30°, and then brought to room temperature. The precipitate was dissolved in the minimum amount of dry ethyl acetate at room temperature, filtered, and diluted with one volume of n-hexane. After 24 hours at −30°, the solid is isolated by filtration and dried at 27° (1 mm.) for 6 hours; yield 89 g. or 67.5%, m.p. 93–94°, $[\alpha]^{25}$D −17.6°, −18.2° (c 3.78 in ethyl acetate). Anal. Calcd. for $C_{13}H_{13}O_5N$: C, 59.3; H, 5.0; N, 5.3. Found: C, 59.4; H, 5.3; N, 5.3.

In a previous publication[6] it was reported that chloride determinations were run on γ-benzyl-N-carboxy-L-glutamate anhydride. The observed chloride content was assumed to be present as hydrochloric acid, and a sufficient amount of initiator was added to neutralize this acid over and above the amount used as initiator. The procedure of Volhard was used to determine Cl⁻.

It has been found since that efficient recrystallization as described above reduces the chloride content to a negligible value; if any is found in the anhydride, it is recrystallized until the chloride is less than 0.01%. No compensation for chloride has been used in the experiments reported herein.

Solvents.—The solvents were purified as follows: Dioxane was purified according to the method of Fieser[19] and fractionally distilled from sodium borohydride; b.p. 100.8–101.0°. It is necessary to redistil from sodium immediately before use to obtain maximum molecular weights in the polymerizations. The solvents were dried and distilled as follows: ethyl acetate, dried over CaH_2, 77°; benzene, 80°; methanol, dried over sodium, 64.3°; nitromethane, 100–101°; chlorobenzene, dried over P_2O_5, 130°; acetonitrile, dried over P_2O_5; nitrobenzene, dried over P_2O_5, 99–100° (26 mm.); anisole, dried over P_2O_5 then sodium, 151°; chloroform, dried over $CaCl_2$ and CaH_2, 60.2–61.2°; dimethylformamide, 47° (14 mm.); acetone, 56°; n-hexaldehyde, 129–131°.

Initiators.—The sodium hydroxide initiator was prepared by dissolving 4 g. of sodium hydroxide pellets (U.S.P.) in 250 cc. of absolute methanol. The solution was standardized against 0.1 N hydrochloric acid, concn. 0.322 N; sodium hydroxide in water initiator had concn. 0.697 N.

The sodium methoxide initiator was made by dissolving 3 g. of sodium (cut under petroleum ether) in 250 cc. of absolute methanol. When 750 cc. of benzene was added, a clear solution obtained, which was standardized against benzoic acid using bromthymol blue[20]; concn. 0.381 M in 3:1 benzene:methanol.

The sodium borohydride initiator was prepared by slurrying 4 g. of sodium borohydride in dried tetrahydrofuran, filtering the solution and then standardizing against 0.1 N hydrochloric acid; concn. 0.0604 M.

n-Hexylamine was refluxed and then distilled from calcium hydride, b.p. 130.0°, and dissolved in dry dioxane; concn. 38.2 mg./cc.

Diethylamine was fractionally distilled from calcium hydride, b.p. 55.4–55.5°, and dissolved in dry dioxane; concn. 21.3 mg./cc.

Triethylamine was refluxed with sodium for 8 hours, distilled from sodium, b.p. 89°, and dissolved in dried dioxane; concn. 36.2 mg./cc.

Polymerizations.—A typical polymerization was carried out as follows: Into a 50-cc. erlenmeyer flask was placed 20 cc. of dioxane (freshly distilled from sodium) and the calculated amount of initiator solution (25 to 500 c.mm.) using an ultramicroburet. The γ-benzyl-N-carboxy-L-glutamate anhydride (1.0 g.) was added at −30° and dissolved immediately.

(19) L. F. Fieser, "Experiments in Organic Chemistry," 2nd ed., D. C. Heath & Co., Boston, Mass., 1941, p. 361.

(20) A. Berger, M. Sela and E. Katchalski, Anal. Chem., 25, 1554 (1953).

TABLE III

Anhydride-initiator mole ratio (A/I)	n-Hexylamine	Sodium hydroxide	Sodium methoxide	Sodium borohydride	Diethylamine[b]		Triethylamine		Sodium methoxide
	Dioxane	Dioxane	Dioxane	Dioxane	Dioxane		Dioxane		Dimethylformamide
	$(\eta_{sp}/c)_{c\ 0.2}$	$(\eta_{sp}/c)_{c\ 0.2}$	$[\eta]$	$(\eta_{sp}/c)_{c\ 0.2}$	$(\eta_{sp}/c)_{c\ 0.2}$ Series		$[\eta](\eta_{sp}/c)_{c\ 0.2}$		$(\eta_{sp}/c)_{c\ 0.2}$
					A	B			
50	0.26	0.55	2.14	0.66	1.54	1.35	19.8	4.27	0.29
100	.34	0.91	4.18	1.17	1.39	1.38	25.9	4.58	.42
200	.45	1.36	6.59	1.55	1.14	1.20	26.3	4.75	.55
400	.53	1.83	9.7	2.26	1.11	1.10	16.6	4.11	.72
600	.56	1.99	14.0	3.11	1.20	1.14	12.8	3.48	.88
1000	.60	2.14	16.4	4.10	1.30	1.34	12.4	2.83	.99

[a] The reduced specific viscosities (η_{sp}/c) were determined in dichloroacetic acid solution, the intrinsic viscosities $[\eta]$ in dimethyl formamide solution. [b] Because of the unusual results with diethylamine initiation two series of experiments were run.

The flask was stoppered and allowed to stand at room temperature until titration of the residual anhydride[20] indicated the reaction was essentially complete (>95%). The time varied from 1 to 72 hours, depending on the initiator and the A/I. The titration was performed using a 2-cc. sample of the (usually viscous) solution, which was diluted with 10 cc. of dioxane, the carbon dioxide removed with nitrogen and titrated under nitrogen against sodium methoxide solution by the method of Berger, Sela and Katchalski.[20] When titration values indicated the reaction was more than 95% complete, the solution was slowly poured with vigorous stirring into 400 cc. of 95% ethanol. After 20 minutes the fibrous polymer was isolated by filtration, washed with ethanol and dried at 50° (1 mm.) for 6–25 hours. The yields ranged from 70 to 99%, increasing with the molecular weight obtained. The high molecular weight polymers (mol. wt. > 100,000) gave solutions of low mobility and were extremely fibrous when precipitated. For a polymer made with A/I 200 using sodium methoxide initiator, yield 99%, $[\alpha]^{25}$D 15.0° (chloroform), $[\alpha]^{25}$D −14° (dichloroacetic acid). Anal. Calcd. for $(C_{12}H_{13}O_3N)_x$: C, 65.8; H, 6.0; N, 6.4. Found: C, 65.5; H, 6.1; N, 6.4.

Optical Rotation.—0.5596 g. of PBLG was refluxed under nitrogen with 10 cc. of 48% hydrobromic acid for 6 hours. Five ml. of water was added, the solution extracted twice with 10-ml. portions of benzene and nitrogen bubbled through the aqueous layer to remove the residual benzene. The solution was made up to 25 cc. with water and its optical rotation determined in a 4-dm. cell.

Poly-γ-benzyl-L-glutamate	(α)	$[\alpha]^{25}$D Based on glutamic acid assuming complete hydrolysis, °C.
NaOCH₃ initiated	1.30°	+21.6°
n-Hexylamine init.	1.36	+22.6
Sodium hydroxide init.	1.48	+22.6[a]
L-Glutamic acid control	1.74	+28.9

[a] 8.5 hours reflux.

Viscosity Measurements.—The relative viscosity, η_{rel}, was measured by the ratio of flow time of solution to flow time of solvent in viscometers for which the latter was never less than 80 seconds. The reduced specific viscosity, $(\eta_{rel}-1)/c$, was then employed to determine the molecular weight by either of two procedures. In the one case this quantity was plotted against concentration (in g./100 cc.) and the intrinsic viscosity, $[\eta]$, determined by extrapolation to zero concentration. This quantity is related to the molecular weight in a one-to-one fashion as shown by Doty, Bradbury and Holtzer.[15] Alternatively, a relation is obtained between the molecular weight and the reduced specific viscosity at a concentration of 0.2 g./100 cc. from the data used to obtain the intrinsic viscosity for the published work just mentioned. In this way a single measurement of relative viscosity could be used to obtain the molecular weight. This later procedure is, of course, not quite as accurate as the determination of the intrinsic viscosity itself because of the greater uncertainty involved in a single measurement and

because the concentration dependence of the reduced specific viscosity in the calibrating samples may not be quite the same for the samples being measured. Experience shows, however, that this concentration dependence is reproducible and that the over-all error associated with molecular weights determined in the second manner is generally no more than double that involving the determination of the intrinsic viscosity. The uncertainty of ±10% used here is more than adequate to allow for these errors. The viscosities of the PBLG preparations shown in Figs. 1, 2, 3 and 6 are listed in Table III.

Reaction of γ-Benzyl-N-carboxy-L-glutamate Anhydride with Sodium Methoxide.—5.0 g. of the N-carboxy anhydride (0.019 mole) was dissolved at −30° in 25 cc. of dry dioxane and slowly poured into a solution of 50 cc. of 0.381 M sodium methoxide in 3:1 benzene:methanol (0.019 mole) in 25 cc. of dioxane. The solution was brought to room temperature, and after ten minutes there was added 100 cc. of dry ether saturated with hydrogen chloride. The solution was then evaporated to about half its volume in vacuo, another 100 cc. of dry ether saturated with hydrochloric acid was added, and the solution evaporated to dryness in vacuo at 30°. The semi-solid residue was dried at 30° (1 mm.) for two hours, extracted three times with ether, the ether extracts combined and the ether removed at 30°; yield 5.44 g. It was then diluted with 25 cc. of ether, and extracted twice with 25-cc. portions of 5% sodium carbonate. The aqueous layer was then extracted with 25 cc. of ethyl ether, the ethereal solutions combined, dried over magnesium sulfate and the ether removed in vacuo; yield of product insoluble in sodium carbonate solution was 2.56 g.

The aqueous alkaline solution was brought to pH 1 with 6 N hydrochloric acid and extracted three times with 25-ml. portions of ether, the extracts combined, dried over magnesium sulfate and the ether removed in vacuo. The yield of viscous oil was 1.67 g. Anal. Calcd. for N-carbomethoxy-γ-benzyl-L-glutamate: N, 4.75; neut. equiv., 295. Found: N, 5.1; neut. equiv., 320, 307, 301.

Discussion

The results presented here indicate that it is possible to prepare polypeptides of very high molecular weight (D.P. > 1000) by initiation with inorganic bases and with secondary and tertiary amines. With the inorganic bases there appears to be some relationship between the A/I and the D.P. obtained, in that the D.P. increases with A/I up to A/I 1000. Since it is known from the light scattering data that these polypeptides have the dimensions of rods with diameters of 15 Å., it is apparent that branching side reactions are small. Thus we need be concerned only with the normal polymerization of N-carboxy anhydrides.

If it is assumed that the polymerization reaction involves the three steps of initiation, propagation and termination, then there are several possible

explanations of the results. First, let us assume the initiation is slow and propagation is very fast. This would explain obtaining D.P.'s higher than A/I's. It seems unlikely, however, that strong bases such as sodium hydroxide and sodium methoxide react slowly with N-carboxy anhydrides. If we assume that the reaction of anhydrides with bases (initiation) is fast, we must then postulate the possibility of other competing reactions which may affect the amount of effective initiation. If, for example, sodium methoxide reacts with γ-benzyl-N-carboxy-L-glutamate to give two products I and II shown below, then it is possible that only one of them is an effective initiator or they react with additional anhydride at different rates.[21]
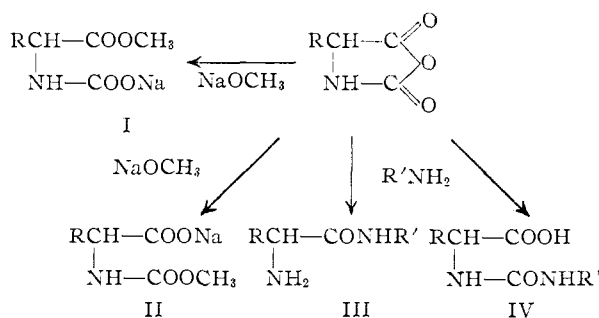
That N-carboxy anhydrides can and do react with sodium methoxide to give two different openings of the anhydride ring has been shown by previous work[20] and several experiments of the type referred to above. Through reaction of γ-benzyl-N-carboxy-L-glutamate anhydride and one mole of sodium methoxide we obtained a product in about 30% yield whose properties corresponded to II (or its acid form). Our recent work with sodium acetate (vide supra) indicates that sodium acetate is also an initiator, and therefore it would appear logical that a compound such as II (and its higher isomers) might be intermediates in the polymerization reaction. This type of reaction product is quite different from the previously assumed products of initiator and anhydride such as III.

If our suggestion is correct, then, the higher than theoretical molecular weights we obtained (based on A/I) could be explained by the fact that only part of the initial reaction product is used in the subsequent polymerization. Secondly, it is possible that the fast rate of polymerization observed with sodium hydroxide and sodium methoxide initiation minimizes side reactions (including termination reactions) and thus yields high molecular weight products. This situation is being investigated and kinetic experiments will be reported in subsequent papers.[17]

Initiation with secondary and tertiary amines appears to be quite anomalous (cf. Tables II and III as well as Fig. 3), and is certainly worthy of further study. There can be no doubt, however, that tri-

ethylamine initiation produces very high molecular weight polypeptides.

From the results of the polymerization reaction in various solvents as shown in Table I, it is obvious that lower molecular weights are obtained from solvents in which precipitation occurs during the polymerization. In addition lower molecular weight products result from using purified chloroform as compared with chloroform which contains 0.75% ethyl alcohol (U.S.P. grade). This may be due to traces of phosgene, in the "purified" chloroform, which may react with an amine-containing intermediate. Dimethylformamide gives lower molecular weights, perhaps because of partial hydrolysis in the presence of sodium methoxide. Of particular interest are the results with dioxane containing different amounts of hexaldehyde. With only 1% hexaldehyde in the dioxane no polymer was isolated, and 0.1% hexaldehyde showed a marked depression in the molecular weight of the resulting polymer as compared with polymers prepared in the absence of hexaldehyde. It appears that aldehydes interfere strikingly in the reaction, perhaps due to the formation of azomethines, and this effect is being investigated further. In this connection a few experiments have been performed adding acetone to the polymerization solvent. It was interesting to note that even the addition of 5.8% acetone (molal equivalent of 10% hexaldehyde) to the dioxane did not decrease the molecular weight of the resulting poly-γ-benzyl-L-glutamate compared to that obtained in pure dioxane.

The effects of increasing temperature and concentration of the anhydride upon the ultimate molecular weight (Figs. 4 and 5) certainly indicate that much of the previous work upon related anhydrides,[22] especially when the anhydride was polymerized in the solid state,[23] was done under conditions that lead to lower molecular weights. In fact, extrapolation of the data in Fig. 4 to 100% anhydride concentration gives an intercept around mol. wt. 60,000, which, coupled with the effect of high temperature upon this polymerization, explains in part why lower molecular weight products were obtained by some previous investigators. It is now quite apparent that in order to obtain maximum molecular weights, polymerizations of N-carboxy-α-amino acid anhydrides should be carried out at temperatures below 40° and at concentrations of anhydride of 5% or lower.

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(21) For a discussion of recent work on the mechanism of the polymerization of amino acid N-carboxy anhydrides cf. D. G. H. Ballard and C. H. Bamford, "Symposium on Peptide Chemistry," Special Pub. No. 2, Chem. Soc. (London) 1955, pp. 25–48.

(22) See ref. 4 above for references to several previous investigations.

(23) E.g., see A. Patchornik, M. Sela and E. Katchalski, THIS JOURNAL, 76, 299 (1954).