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

Polypeptides. XVIII.[1] A Kinetic Study of the Polymerization of Amino Acid N-Carboxyanhydrides Initiated by Strong Bases

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Kinetic data have been obtained for the strong base initiated polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride under a variety of experimental conditions using both the previously described infrared absorption and carbon dioxide evolution methods. Strong base initiated polymerizations show a first-order rate following an initial autocatalytic period. Strong base initiated polymerizations thus differ from amine initiated polymerizations (which exhibit two successive pseudo-first-order rates) in the shape of the rate curve, in the rate, which is at least 100 times faster, and also in the production of much higher molecular weight polypeptides. Polymers isolated after the first 30% reaction show a non-proportional increase in molecular weight with increased extent of monomer conversion. Addition of monomer to a completed polymerization solution produces more polymer, and its molecular weight is the same as the original polymer. The solvent in which polymerization is performed affects the rate; thirty-fold differences in rate constant have been observed. Determination of activation energy by varying the temperature of the polymerization was inconclusive. The presence of small amounts of the optical isomer of an amino acid-N-carboxyanhydride markedly decreases its polymerization rate and lowers the molecular weight of the polypeptide formed. By using triphenylmethylsodium as an initiator for the polymerization in a proton-free solution, it has been shown that protons are not required for the production of high molecular weight polypeptide. The more electro-positive the cation of the strong base, the shorter is the autocatalytic period of the polymerization. It is suggested that the low molecular weight reaction product of strong base and amino acid-N-carboxyanhydrides are the effective initiators of the polymerization.

Since it was first shown[3,4] that sodium hydroxide initiated polymerizations of α-amino-acid-N-carboxyanhydrides (NCAs) yield linear polypeptides of very high molecular weight, this type of reaction has proved useful in the preparation of a considerable variety of such polymers. When sodium hydroxide initiated polymerizations were compared with primary amine initiated polymerizations at equal anhydride:initiator mole ratios (A/I), it was observed also that the hydroxide initiated reactions were completed in a much shorter time. Thus the preliminary investigations suggested different mechanisms might be involved in the polymerizations, and kinetic studies were undertaken in an attempt to elucidate the reaction mechanisms. Knowledge of the polymerization mechanism also could be of value in more complex systems, such as the copolymerization of two or more amino acid anhydrides. The kinetic results of primary amine initiated polymerizations have been reported.[5-11] This paper deals with kinetic studies of γ-benzyl glutamate NCA (L-, D- and DL-mixtures) initiated by strong bases; the preliminary results were reported previously.[12]

Experimental

Materials.—**γ-Benzyl-L-glutamate-N-carboxyanhydride** was prepared and purified as previously described.[4]

γ-Benzyl-D-glutamate-N-carboxyanhydride.—D-Glutamic acid, $[\alpha]^{20}\text{D}$ −32.3° (c 1, 6N HCl) was converted to γ-benzyl-D-glutamate, m.p. 173–173.5°, (immersed at 165°), $[\alpha]^{20}\text{D}$ −19.7 (c 7, acetic acid) and converted to the NCA in the manner previously described for the L-isomer[2]; m.p. 92–93° (sealed capillary, immersed at 82°, heated 2°/min.), $[\alpha]^{25}\text{D}$ 18.0° (c 3.78, ethyl acetate).

γ-Benzyl-DL-glutamate-N-carboxyanhydride was prepared from γ-benzyl-DL-glutamate, m.p. 161–162°, which was prepared from DL-glutamic acid by the same method as for the L- and D-isomers. The NCA preparation differed only in that the yield of pure product was lower (21%) because the solubility of the racemate is much greater in ethyl acetate–hexane than either the D- or L-isomers, m.p. 71–72° dec.

Initiators.—The alkali metal methoxides were prepared by dissolving the metal in anhydrous methanol and adding approximately three times the volume of benzene. The solutions should be clear at temperatures above 20°, but they separate into two phases if cooled. Each solution was standardized against benzoic acid with phenolphthalein indicator; the concentrations of the initiator solutions used were about 0.4 M.

Triphenylmethylsodium was prepared according to the method described in "Organic Syntheses."[13]

Solvents.—Dioxane and nitrobenzene were purified as previously described.[4] Methylene chloride was shaken with sulfuric acid until the acid remained colorless, was washed with water and was dried over anhydrous magnesium sulfate. It was then fractionally distilled, first from phosphorus pentoxide and then from sodium hydroxide pellets immediately before use; the fraction boiling at 41° was used for polymerization reactions.

Kinetic Methods.—The rate of reaction was measured both by infrared[11] and carbon dioxide evolution methods[10] previously described. The polymers were isolated by precipitation into 95% ethanol, and their weight average molecular weight (MW_w) was determined viscometrically in dichloroacetic acid.[4]

Post-polymerization Addition of γ-Benzyl-L-glutamate NCA to a Completed γ-Benzyl-L-glutamate Polymerization Solution.—A gram of γ-benzyl-L-glutamate in 25 ml. of dioxane was initiated to A/I 20 with sodium methoxide initiator (1:3 methanol–benzene). When this polymerization was complete after 1.5 hr., 5 ml. of the solution was removed and precipitated into 95% ethanol. To the remaining 20 ml. of solution, 0.8 g. of NCA was added and was allowed to polymerize for another 1.5 hr. Again 5 ml. of the viscous solution was removed and precipitated into ethanol; 0.6 g. of NCA was added to the remaining 15 ml. of solution. The procedure was repeated until 0.2 g. of NCA was added and allowed to polymerize in the last 5 ml. of very viscous solution, in which the final A/I was 100. The viscosities of

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[1] For the last papers in this series see XVII, R. D. Lundberg and P. Doty. THIS JOURNAL, **79**, 3961 (1957).

[2] Chemical Research Laboratory, Polaroid Corporation, Cambridge 39, Mass.

[3] E. R. Blout, R. H. Karlson, P. Doty and B. Hargitay, THIS JOURNAL, **76**, 4492 (1954).

[4] E. R. Blout and R. H. Karlson, *ibid.*, **78**, 941 (1956).

[5] S. G. Waley and J. Watson, *Proc. Roy. Soc.* (*London*), **A199**, 499 (1949).

[6] W. C. Hanby, S. G. Waley and J. Watson, *J. Chem. Soc.*, 3239 (1950).

[7] D. G. H. Ballard and C. H. Bamford, *Proc. Roy. Soc.* (*London*), **A223**, 495 (1954).

[8] E. Katchalski, Y. Shalitin and M. Gehatia, THIS JOURNAL, **77**, 1925 (1955); M. Sela and A. Berger, *ibid.*, **77**, 1893 (1955).

[9] D. G. H. Ballard and C. H. Bamford, The Chemical Society, Special Publication No. 2, 25 (1955).

[10] P. Doty and R. D. Lundberg, THIS JOURNAL, **78**, 4810 (1956); R. D. Lundberg and P. Doty, *ibid.*, **79**, 3961 (1957).

[11] M. Idelson and E. R. Blout, *ibid.*, **79**, 3948 (1957).

[12] E. R. Blout and M. Idelson, *ibid.*, **78**, 3857 (1956).

[13] W. B. Renfrow, Jr., and C. R. Hauser, "Organic Syntheses," Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 607.

these five products were measured in dichloroacetic acid; these results (experiment 2) and those of other less extensive but similar experiments are given in Table II.

When polymerizations were studied kinetically the same procedure as above was followed, but in addition samples of the reaction mixtures were transferred to an infrared cell in which the consumption of NCA was followed. The MW_w for some of these experiments are given in Table II and a rate curve is shown in Fig. 2.

Results and Discussion

Sodium Methoxide Initiated Polymerization.—The infrared method[11] was used for obtaining most of the kinetic data reported here; the rest of the data were obtained by the carbon dioxide evolution method.[10] The tables of results indicate which method was used. Typical plots of monomer concentration as a function of time for sodium methoxide initiated polymerizations of γ-benzyl-L-glutamate NCA are shown in Fig. 1. These results, when compared with primary amine initiated reactions, show marked differences. Firstly, the rate of NCA consumption with sodium methoxide initiation is very much faster. Secondly, methoxide initiated polymerizations differ from the primary amine initiated polymerizations in the shape of the kinetic curves. In the latter, immediately after the rapid reaction of amine with NCA a slow pseudo-first order rate (k_{2a}) is observed concurrent with the production of low molecular weight (β_L) polymer.[10,11] From the examination of many kinetic curves of strong base initiated polymerizations, we have concluded that the initial slow reaction (which follows the fast reaction of base with NCA) is not first order in NCA concentration, although some of the kinetic curves may appear qualitatively similar to the k_{2a} portion seen in amine initiated reactions, but rather it is an autocatalytic reaction. Furthermore, during this autocatalytic portion of the strong base initiated polymerizations no isolable β-polymer is produced—only α-polymer is observed. The strong base initiated reactions finally attain a pseudo-first order reaction rate which, from measurement of both the 1860 and 1790 cm.$^{-1}$ infrared absorption bands of the NCA,[11,12] extends to at least 95% of the monomer consumption. This rapid polymerization corresponds with the faster polymerization rate of NCAs with amine initiation (k_{2b}) which produces "α" polypeptides. Thirdly, sodium methoxide initiated polymerizations yield polypeptides whose molecular weight (MW) and degree of polymerization (DP) are always higher than theoretical at A/I's as high as 1000[4] (*cf.* Table I). This means that only a fraction of the sodium methoxide is utilized in the production of high molecular weight polymer.

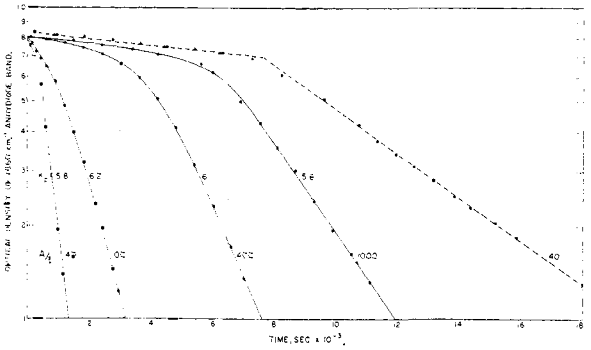

Fig. 1.—Plots of optical density of 1860 cm.$^{-1}$ anhydride C=O band measured as a function of time during sodium methoxide initiated polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride in dioxane solution (4 g./100 ml.) at A/I 40. 100. 400. 1000. The dashed line is a rate curve for an A/I 40 *n*-hexylamine initiated polymerization. The polymerization rate constants (k_p) are shown in a horizontal line with the symbol.

TABLE I

RESULTS OF SODIUM METHOXIDE INITIATED POLYMERIZATIONS[a] OF γ-BENZYL-L-GLUTAMATE-N-CARBOXYANHYDRIDE

A/I	DP_w[c]	k_p[b]	DP_w	k_p	DP_w	k_p	DP_w	k_p
40	525	5.8						
	475	5.7						
Av.	**500**	**5.7**						
100	1200	6.5	1000	6.2				
	1200	7.5	900	6.0				
	1200	6.1						
	1250	8.1						
Av.	**1210**	**7.0**	**950**	**6.1**				
400	1800	6.1	1750	5.3	2300	4.8		
	1800	7.2	2300	6.7	2500	5.3		
					2300	5.0		
Av.	**1800**	**6.6**	**2020**	**6.0**	**2340**	**5.4**		
1000	2600	5.6	2450	4.9	3200	3.2	2800	3.0
	2300	5.6	2050	3.3	2500	2.7	3300	2.8
	2050	4.6	2600	4.7			2800	3.1
Av.	**2320**	**5.3**	**2360**	**4.3**	**2070**	**3.0**	**2950**	**3.1**
[MeOH][d]	0.066		0.026		0.0066		0.0026	

[a] Kinetics determined by infrared method[11] at 34 ± 1°, at 4% (g./100 cc.) NCA in dioxane solution. [b] Liters mole^{-1} sec^{-1}. [c] The weight average degree of polymerization (DP_w) as noted in this and succeeding tables were obtained from reduced specific viscosities at concentrations of 0.2% in dichloroacetic acid as described in reference 4. [d] The initiator solution of sodium methoxide contains methanol and benzene in a volume of ratio of about 1:3. The methanol concentration given (mole liter^{-1}) arises from the initiator solution and a methanol–benzene solution used to give comparable conditions at different A/Is.

To calculate the polymerization rate constant the growing chain concentration should be used, but we have been unable to measure this concentration. However, since the ratio of DP_w/DP_n is known to be less than 2,[14] the weight average molecular weight (DP_w) gives a fair approximation of the total number of high molecular weight chains formed at the *end of the polymerization*. *Assuming* that all chains grow throughout the polymerization, a polymerization rate constant, k_p, can be calculated.[11] Under these circumstances

$$[I] \cong \frac{[A_0]}{DP_w}$$

and

$$k_p = \frac{\ln \frac{OD_2}{OD_1} \times DP_w}{(t_2 - t_1) \times [A_0]}$$

The values of k_p are shown in Table I. Using this approximation one obtains a constant value of k_p at each methanol concentration indicated in the last row of the table.

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(14) P. Doty and J. T. Yang, unpublished results.

Postpolymerization Addition of NCA.—Because the DP_w of the polymers produced by strong base initiation of NCA is greater than the A/I, not all of the sodium methoxide initiator is utilized for the production of high molecular weight polymer. The sodium methoxide undoubtedly has reacted with NCA since this reaction is very fast, but that portion which has not been utilized in the production of high polymer could be present either as an inert species or an active species capable of producing high polymer. Evidence that there is present an active species has been obtained by adding additional NCA to a polymerization solution in which the original NCA has been consumed. The added NCA polymerizes at the same rate observed in the straight line portion of the first polymerization, and the polymer obtained at the end of the polymerization of the added NCA has the same mol. wt. as samples of polymers removed at the end of the polymerization of the initial NCA. Some of these data are shown in Table II, and rate curves are shown in Fig. 2.

TABLE II

POSTPOLYMERIZATION ADDITION OF γ-BENZYL-L-GLUTAMATE-N-CARBOXYANHYDRIDE TO POLY-γ-BENZYL-L-GLUTAMATE REACTION MIXTURES[a]

Expt.	Total A/I[b]	DP_w
1	20	650
	40	750
2	20	550
	40	650
	60	650
	80	650
	100	700
3	40	800
	80	850
4	40	
	80	850
5	100	1100
	200	1100
6	100	
	200	1200
7	400	1350
	800	1550
8	400	2150
	800	2250
9	1000	3350
	2000	3500

[a] Initiated by sodium methoxide using 4% NCA in dioxane solution. [b] In each case the initial A/I is shown first. The figures below it are the A/I's after addition of NCA to the completed initial polymerization. The additional NCA was added as solid.

Molecular Weights during the Polymerization.—It was of interest to investigate the molecular weights of polymer removed at various stages of the polymerization reactions. These data are shown in Table III, where it can be seen that DP reaches at least 65% of its ultimate value after 25% of the NCA has been polymerized. In other words the molecular weight of the polymer produced does not change proportionately to the extent of NCA consumption during the portion of the polymerization which obeys a first-order kinetic law.

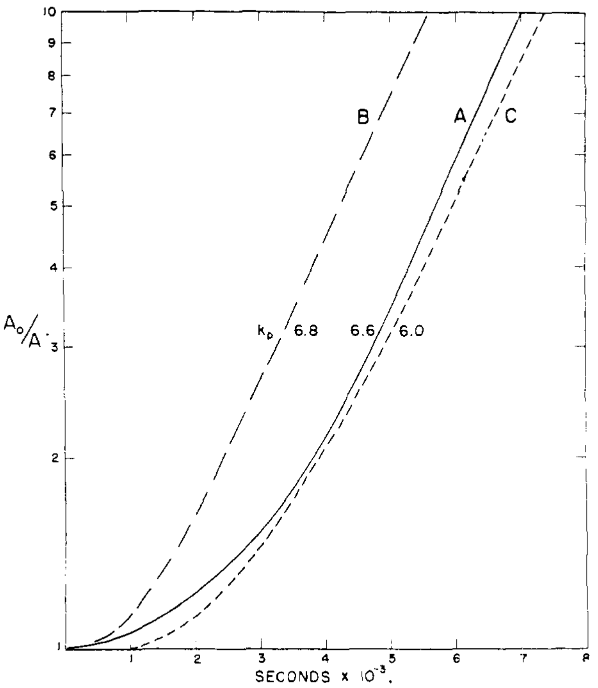

Fig. 2.—Curve A ———, plot of anhydride consumption (A_0/A) measured by CO_2 evolution method as a function of time for an A/I 400 sodium methoxide initiated polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride in dioxane solution (4 g./100 ml.). Curve B — — —. the rate curve obtained upon the addition of an equal quantity of L-NCA to a completed L-polymerization. Curve C -- - - -. the rate curve obtained upon the addition of an equal quantity of D-NCA to a completed L-polymerization. The k_p values are shown in a horizontal line with the symbol.

The yield of the high molecular weight polymer produced in the early part of the reaction (25–50% NCA reacted) corresponds to the amount of NCA consumed. Therefore, with the exception of the small amount of NCA that reacts immediately with the sodium methoxide, all of the NCA consumed is utilized in production of *high* molecular weight polymer. But, since the molecular weight of the polymer produced in the later part of the polymerization does not increase in proportion to the amount of NCA consumed, new chains must be produced while the already formed chains stop growing when they reach a certain molecular weight. We are led to conclude that there may be present a steady state concentration of growing chains during the straight line portion of the reaction.

In the calculation of k_p above, it was assumed that all the chains grew throughout the polymerization, but if there is a steady state between termination and initiation, the concentration of growing chains at any time would be *less than* the total number of chains present at the end of the reaction. Thus k_p is a polymerization constant whose value may be considerably less than the value which would be calculated from the concentration of growing chains if this concentration were known.

Solvent Effects.—Because dioxane is a good solvent for both γ-benzyl glutamate NCA and the poly-γ-benzyl glutamates and because it is trans-

TABLE III

DEGREE OF POLYMERIZATION OF POLY-γ-BENZYL-L-GLUTAMATE MEASURED DURING POLYMERIZATION

A/I	NCA Consumed, %	DP_w
100	25	1100
	50	1250
	75	1250
	100	1250
200	31	800
	40	1000
	53	1050
	62	1050
	72	1100
	79	1100
	82	1100
	86	1100
	89	1100
	93	1100
	95	1100
	100	1200
400	40	1650
	51	1750
	73	2100
	100	2400
1000	27	2550
	51	3050
	76	3450
	100	3350

parent in the infrared between 1500 and 1900 cm.$^{-1}$, most of the kinetic work has been performed in this solvent (*cf.* Table I). Certain other solvents were investigated briefly and the results are shown below. The shapes of the kinetic curves in nitrobenzene, methylene chloride and benzene were qualitatively similar to those observed in dioxane; that is, an initial autocatalytic portion followed by a straight line portion. In dimethylformamide, however, no straight line portion was observed, and therefore it was not possible to calculate a first-order rate constant. It is perhaps significant that the solvents having the highest dielectric constant, dimethylformamide and nitrobenzene, yielded the lowest DP polymers. Benzene, whose dielectric constant is almost identical with that of dioxane, produces polymers of comparable DP but at a much faster rate (*cf.* Tables I and IV).

Since it is necessary with sodium methoxide initiation of NCAs to use methanol to obtain homogeneous initiator solutions, we have investigated the effect of methanol concentration on the polymerization rate and the ultimate DP of the product. Some results are seen in Table I, where the data indicate that with increasing methanol concentration, the polymerization rate is increased and the DP is decreased. However, although the effect is real, the magnitude is small since a twenty-five fold increase in methanol concentration only doubled the k_p.

Effect of Temperature.—In an attempt to determine the activation energy of the polymerization reaction, polymerizations have been performed at 15, 25 and 40° and A/I 40, 100, 400 and 1000. The results, presented in Table V, indicate that k_p is independent of temperature.

TABLE IV

EFFECT OF SOLVENT ON THE SODIUM METHOXIDE INITIATED POLYMERIZATION OF γ-BENZYL-L-GLUTAMATE-N-CARBOXYANHYDRIDE

Solvent	[NCA] (g./100 cc.)	A/I	DP_w	k_p, l. mole^{-1} sec.$^{-1}$
Nitrobenzene[a]	4	100	300	1.1
Methylene chloride	4	100	600	4.2
		100	600	4.0
		400	850	1.1
Benzene	1	100	1050	29.5
		100	1300	38.0
		400	2400	18.0
		400	2950	20.0
Dimethylformamide	4	100	200	[b]
		400	300	[b]

[a] Using carbon dioxide evolution kinetic method. All other polymerizations were measured with the infrared kinetic method. [b] The reaction was not first order.

TABLE V

EFFECT OF TEMPERATURE ON THE POLYMERIZATION OF γ-BENZYL-L-GLUTAMATE-N-CARBOXYANHYDRIDE[a]

A/I	Temp., °C.	DP_w	k_p (l. mole^{-1} sec.$^{-1}$)
40	15	1000	5.4
	25	730	7.4
100	15	1550	6.3
	25	1050	6.0
	40	750	5.1
400	15	2400	5.3
	25	1500	5.8
	40		
1000	25	2050	4.4
	40	1620	6.4

[a] Kinetics determined by carbon dioxide evolution method at 4% (g./100 cc.) NCA in dioxane solution.

Optical Isomers. Effect on Rate and DP.—In order to determine the effects of the optical configuration of NCAs on the polymerization, we have examined the reaction mixtures of D-, L- and DL-γ-benzyl-glutamate NCA using sodium methoxide initiation in dioxane solution. The effect of optical isomer incorporation on the polymerization rate constant (k_p) and the DP_w is shown graphically in Fig. 3 and 4, respectively. As may be expected the pure D-NCA has the same polymerization rate as the pure L-NCA. However, when even as little as 5 mole % of L-NCA is polymerized with 95 mole % D-NCA (or *vice versa*) the polymerization rate k_p is reduced to $^{1}/_{3}$ the value obtained for each pure optical isomer. At equimolar proportions of D and L k_p is about $^{1}/_{17}$ that of the pure isomers. The effect of optical isomers on the DP_w is not as large as the effect on the polymerization rate constant, but at equimolar proportions of D- and L-isomers, the DP_w is about $^{1}/_{5}$ that of the pure isomeric polymers. These high molecular weight DL-copolymers have been used to estimate the specific rotation of an α-helix free of any residue contribution and to demonstrate the lower configurational stability of DL-helices.[15]

Effect of Initiators.—To obtain insight into what might be occurring during the autocatalytic period

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[15] E. R. Blout, P. Doty and J. T. Yang, THIS JOURNAL, **79**, 749 (1957).

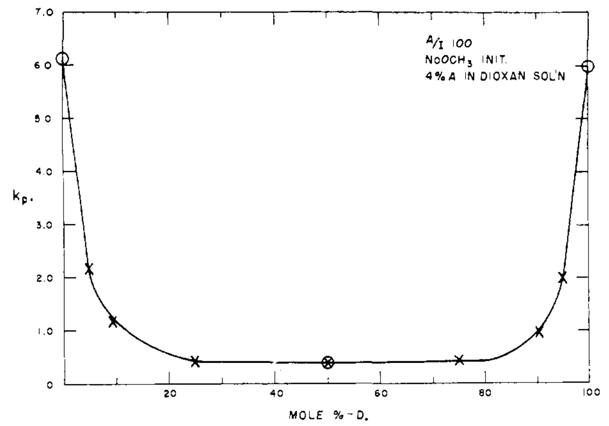

Fig. 3.—The polymerization constants k_p, for sodium methoxide initiated polymerizations of γ-benzyl-L-glutamate-N-carboxyanhydride in dioxane solution (4 g./100 ml.) as a function of mole per cent. D-anhydride isomer: X, polymers made with DL-NCA and optically active NCA; O, polymers made with mixtures of pure D- and pure L-NCA.

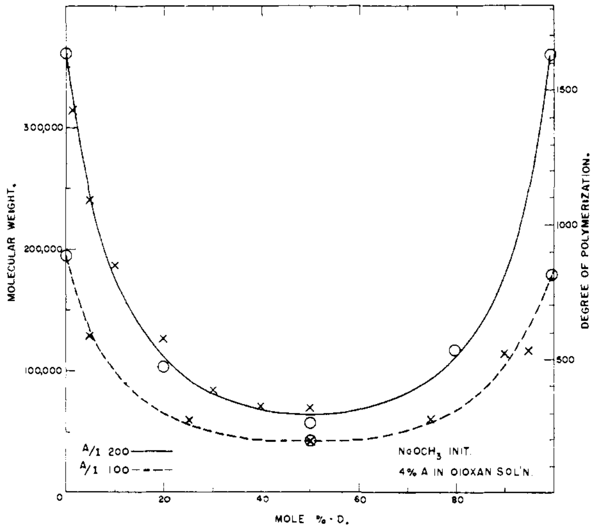

Fig. 4.—The degree of polymerization and molecular weight obtained with sodium methoxide initiated polymerizations of γ-benzyl-L-glutamate-N-carboxyanhydride in dioxane solution (4 g./100 ml.) as a function of mole per cent. D-anhydride isomer: X, polymers made with DL-NCA and optically active NCA; O, polymers made with mixtures of pure D- and pure L-NCA.

of the polymerization, the strong base initiator was changed. Polymerizations had been performed previously using sodium hydroxide, sodium borohydride and phenyllithium in addition to sodium methoxide[4] as initiators. To test the generality of strong base initiation and to conduct a reaction in a proton free system, triphenylmethylsodium in ether was prepared and used as an initiator. At A/I 100 with triphenylmethylsodium initiator and γ-benzyl-L-glutamate NCA, a kinetic curve was obtained similar to those with sodium methoxide initiated polymerizations; the value of k_p was 2.0 and DP of the polypeptide was 650. It is interesting that extrapolation of the k_p values in Table I gives a value of approximately two at zero methanol concentration. The polymerization mechanism is apparently not affected by the anionic portion of the initiator, and the reaction can take place in the absence of protons.

The cationic component of the initiator was studied next. The methoxides of the alkali metals, lithium, sodium, potassium and cesium were used as initiators at A/I 400; the results are presented in Table VI and the rate curves in Fig. 5.

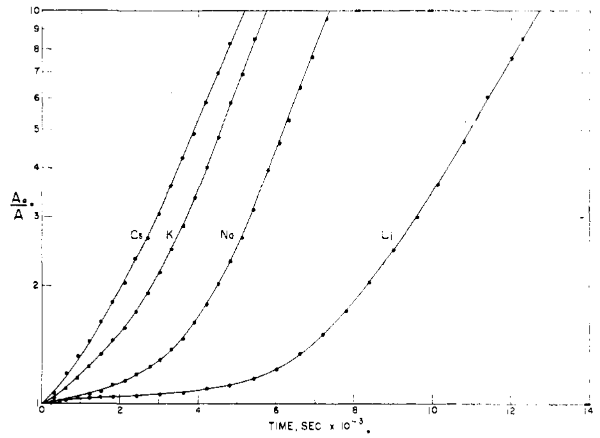

Fig. 5.—Plot of anhydride consumption (A_0/A) measured by CO_2 evolution method as a function of time for A/I 400 methoxide initiated polymerizations of γ-benzyl-L-glutamate-N-carboxyanhydride in dioxane solution (4 g./100 ml.), showing the effect of various strong base initiator cations.

With the exception of lithium methoxide, all the initiators produced polymers of essentially the same DP, at the same first-order rate. The principal differences occurred in the autocatalytic part of the reaction; these differences are reflected in the time for 90% completion of the reaction, (shown in Table VI) where it may be seen that the duration of the autocatalytic period decreases as the electronegativity of the cation decreases.

TABLE VI

RATE CONSTANTS AND DEGREE OF POLYMERIZATION OF ALKALI METAL METHOXIDE INITIATED REACTIONS AT A/I 400

Alkali ion	Time for 90% NCA consumption, sec.	k_p	DP_w
Li	12,700	1.6	620
Na	7,000	5.8	1500
K	5,700	7.9	1950
Cs	5,200	6.9	1900

Reaction Mechanism.—If the compounds I and II, the products of reaction between NCA and sodium methoxide,[4,16] are considered to be ion pairs in dioxane solution, the degree of dissociation will vary with the cationic component.[17] Also the alkali metal salts of I and II (Chart I) should show increasing ionic character, as do inorganic alkali salts, as the atomic weight of the cation increases from lithium to cesium. This then leads to postulating a higher electron density on the anion of the

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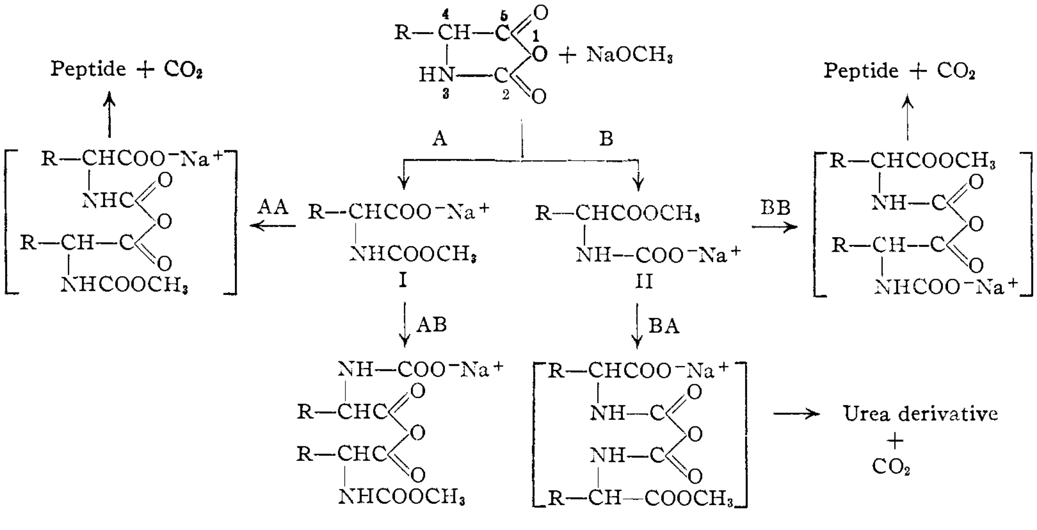
(16) A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1554 (1953).

(17) It has been shown by conductance measurements in our Laboratories that I and II in dioxane–benzene–methanol solution behave like weakly ionized salts. Measurements were impossible in pure dioxane because the salts were insoluble.

Chart I



cesium salt of II than on the anion of the lithium salt. In other words the same steady-state condition should be attained with each alkali metal (except lithium); it merely requires less time for the cesium methoxide initiated polymerization to reach the steady state because the degree of dissociation of the cesium salt of I or II is greater than that of the corresponding sodium salt. Inspection of Fig. 5 and Table VI indicates that this is the case. From these experiments it is clear that the duration of the autocatalytic portion of the polymerization reaction is a function of the electronegativity of the cation of the initiator. Furthermore, it is suggested that the autocatalytic period is a result of the partial dissociation of the primary product of the reaction between initiation and NCA.

Compounds I and II may react with additional NCA to produce either peptides or another type of link, depending on the mode of reaction. Chart I shows the paths by which NCA reacts to produce polymeric products. If reaction always occurs at the same carbonyl group of the NCA, *e.g.*, anhydride C_2 in path A, then a mixed carbamic–carboxylic acid anhydride forms; these anhydrides are unstable and spontaneously lose carbon dioxide to form peptide bonds.[18] The same situation occurs if reaction always follows path B, reaction at C_5. If, however, an NCA molecule reacts by path B with a chain formed by all A reactions, the result is an ordinary carboxylic acid anhydride which does not decarboxylate under the reaction conditions. Similarly, if an NCA molecule reacts by path A with a peptide chain which formed by path B, the product is a carbamic acid anhydride, which decarboxylates to a urea derivative. The reaction sequences may be summarized as follows.

1. Continuous ring opening by either path A or B leads to polypeptides.
2. Ring opening by path A followed by ring opening by path B leads to carboxylic acid anhydride formation.
3. Ring opening by path B followed by ring opening by path A leads to urea formation.

All the products may react further with additional NCA to produce peptides, but only like additions (sequences under (1)) yield purely peptide linked molecules. The addition of NCA to a completed polymerization results in its polymerization to new polymer chains. This indicates that, after all the NCA has been consumed in the production of polymer, I and II still exist in the reaction solution. Since it has been shown that polypeptides produced by strong base initiation are uninterrupted α-helices,[19] sequences such as (1) are by far the predominant ones in the polymerization reaction.[20]

It is possible that reaction sequences (2) and (3) are involved in termination steps. The identification of terminal groups in strong base initiated polypeptides is extremely difficult because, even at low A/I's, the DP is very high. The DP has been shown to be related to A/I, but it is not equal to A/I[4]. Since the methoxides of Na, K and Cs all lead to polymers of approximately the same DP, it appears that those cations are not directly concerned in a termination reaction. (However, experiments using mixtures of sodium methoxide and lithium bromide for initiation have shown that the presence of lithium bromide lowers the DP of the polymer compared to that obtained in its absence.[21])

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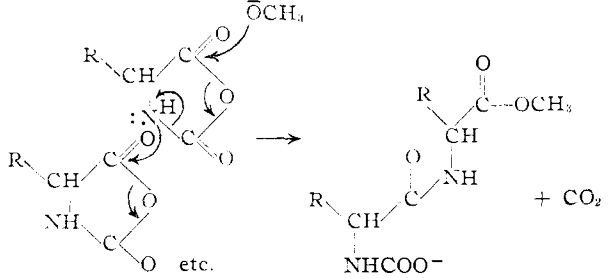
[18] See for example W. Dieckmann and F. Breest, *Ber.*, **39**, 3052 (1906).

[19] P. Doty, J. H. Bradbury and A. M. Holtzer, This Journal, **78**, 947 (1956).

[20] Since we have shown that protons are unnecessary for strong base initiated polymerizations of NCAs, an attractive possibility is a purely electronic mechanism. If the strong base attacks the NCA as shown then polymerization simply involves intermolecular electron transfer through the N atom and simultaneous elimination of carbon dioxide as indicated.

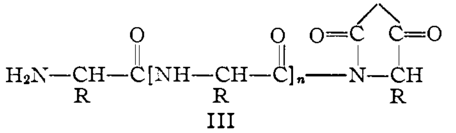


[21] E. R. Blout and R. H. Karlson, unpublished results.

If NCA reacted with a growing chain in the AB or BA fashion discussed above, the α-helix would be disrupted, the symmetry of the chain would be destroyed and thus the reaction could be materially slowed down or even stopped.

A method for studying the effect of the introduction of residues which interrupt the normal structure of an α-helix is the inclusion of the D-isomer of γ-benzyl-glutamate NCA in the polymerization of L-NCA. Any difference in either the polymer produced or the kinetics of polymerization can only be attributed to the steric effect of one optical isomer on a growing chain of the other, unless each species is completely selective in its reactivity toward the two isomers. Thus, if a growing chain of L-residues reacts only with L-NCA and growing D-chains only with D-NCA, the rate of polymerizature of a 1:1 D,L-NCA mixture would be reduced by one-half. On the other hand if there is no or some selectivity, the rate could change by either more or less than one-half. Figures 3 and 4 illustrate the profound influence that optical configuration has on both the rate and degree of polymerization of NCAs. If a growing chain of L-residues meets a D-NCA, the D-residue will not fit into the L-α-helix as well as an L-residue would; therefore the observed large decrease in the rate of polymerization can be attributed to the difficulty a growing chain encounters in trying to incorporate the opposite optical isomer into itself. The decrease in the degree of polymerization obtained with D,L-mixtures compared to that obtained with the pure optical isomers may be ascribed to (a) the decreased rate of polymerization which allows more time for chains to be initiated, and/or (b) an increase in the frequency of a terminating reaction. Termination reactions in D,L-systems may be facilitated by the enantiomorph forcing the end of the growing polypeptide chain end out of the helical configuration. In other words the reaction sequences AB and BA in Chart I, which lead to interruptions in the helical structure, may be regarded as termination reactions.

From the data presented in this paper it may be concluded that strong base initiated polymerizations of NCAs differ fundamentally from primary amine initiated reactions. Ballard and Bamford have pointed out that salt catalyzed reactions also differ from primary amine initiated polymerizations. In addition, they have published mechanisms for salt catalyzed reactions[9,22] and have indicated that these reactions play a significant part in strong base initiated polymerizations. Their mechanism for salt catalyzed polymerizations involves (1) amine end groups in the growing species and (2) a reactive NCA on the other end of the growing chains III.



III

We do not believe that postulated intermediates such as III are involved in strong base initiated polymerizations of NCAs for the following reasons: Firstly, very high molecular weight poly-L-proline has been obtained by strong base initiated reactions,[23] and there is no opportunity in this case for the enolization or the subsequent reactions which are proposed in their mechanism. Secondly, no amine groups can be formed in triphenylmethylsodium initiated polymerizations. Finally, the postulated addition to both "amine" ends and "NCA" ends of growing chains would lead to a broader molecular weight distribution than has been observed.[14] It appears from this evidence that the mechanism suggested by Ballard and Bamford is not involved in polymerizations initiated by strong bases.

Tertiary amine initiated polymerizations[4,24] differ from the strong base type in that the MW of the polymers produced by tertiary amines is essentially independent of the anhydride/initiator ratio (A/I), while the MW of strong base initiated polymers is dependent upon the A/I^4. For this reason we believe that somewhat different mechanisms apply to the tertiary amine and strong base initiated polymerizations.

To summarize now, we can postulate the following reactions as being involved in strong base initiation of NCAs.

(1) The initial reaction of the strong base is to form compounds of types I and II. This reaction is very fast.

(2) The second reaction involves the dissociation of I and/or II (or perhaps a very low DP polymer of I and/or II); its extent depends on the electronegativity of the cation.

(3) The anions formed in reaction (2) then react very quickly with NCA to yield polypeptide. Since reaction (2) is an equilibrium reaction, an autocatalytic rate curve such as observed in the nonlinear portion of the reaction is found. Reaction (2) and termination reactions (4) occurring simultaneously may lead to a steady-state concentration of growing chains, with a first-order rate curve observed. This is the major portion of the reaction.

(4) Finally a termination reaction almost certainly is involved because (a) the MW does not increase proportionately with monomer consumption and (b) addition of more anhydride to a finished polymerization solution does not increase the molecular weight of the polymer, although new polymer is formed. The nature of the termination reaction is not known but an attractive hypothesis involves the addition of NCA in a fashion which yields substituted ureas or substituted linear carboxylic acid anhydrides which force the polypeptide out of its normal α-helical configuration.

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(22) D. G. H. Ballard, A. Elliott and W. C. Hanby, "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956, p. 91.

(23) G. D. Fasman and E. R. Blout, to be published.

(24) D. G. H. Ballard and C. H. Bamford, *J. Chem. Soc.*, 381 (1956).