

[CONTRIBUTION FROM THE GIBBS LABORATORY, HARVARD UNIVERSITY]

Polypeptides. XVII. A Study of the Kinetics of the Primary Amine-initiated Polymerization of N-Carboxy-anhydrides with Special Reference to Configurational and Stereochemical Effects

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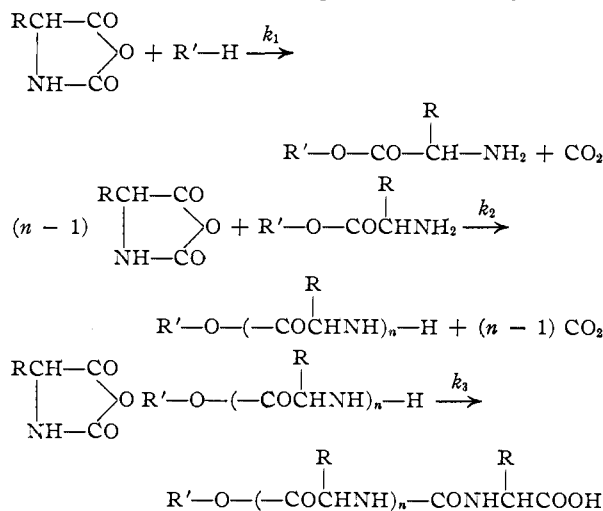
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The primary amine-initiated polymerization of N-carboxy-anhydride of γ -benzyl-L-glutamate in a number of solvents is shown to proceed at two successive rates following a relatively rapid initiation. The rate constant for the second propagation step is at least five times larger than that for the first and as a consequence those growing chains that first achieve the condition for the faster propagation then grow rapidly and, by lowering the anhydride concentration, reduce the rate of growth of the chains that have not attained the condition for fast growth. This is the origin of the very broad molecular weight distributions previously observed. In dioxane the transition from the slow to the fast propagation rate occurs when the average chain length has reached approximately 8 residues. Since this chain length lies in the range (7 to 13) which has been assigned to the critical length for stabilization of the α -helical configuration in this solvent it appears that the cause of the change in propagation constants is the configurational transition. It is shown that very minor HCl contamination of the N-carboxy-anhydride can substantially lower the second propagation constant and in the limit prevent its appearance altogether. Two alternative termination steps are identified and their combined effect limits the average degree of polymerization to about 500. In dimethylformamide only a single rate constant is observed and the molecular weight distribution of the resulting polypeptides is greatly reduced over that of the dioxane case. Thus the preparation of low molecular weight polypeptides having the narrow Poisson distribution of molecular weights appears possible. Investigation of the rate of polymerization of racemic and D,L-mixtures of N-carboxy-anhydride shows a pronounced lowering of the propagation rate relative to that of the pure L-anhydride. While the growing chain is non-helical the lowering is less than a factor of two and is thereby accounted for in terms of a preference of the chain to add the isomer corresponding to its terminal residue. Later, when the growing chain has reached the helical configuration, the lowering of the rate is greater than a factor of two and this indicates a preference exerted by residues lying behind the terminal one. When optical rotation as well as CO₂ evolution is used to follow polymerizations of L-, DL- and D-N-carboxy-anhydrides with preformed L-polypeptide (helical configuration) it is found that the screw sense of the initiating helix tends to be continued. In the D-anhydride case, the reversal of the helix sense occurs after about four residues have added; in the DL-anhydride case, reversal occurs later presumably as a result of the occasional addition of four D-residues in sequence.

The polymerization of N-carboxy- α -amino acid anhydrides has been generally thought to proceed by a repetitive, stepwise addition of the peptide units with the simultaneous expulsion of carbon dioxide.²⁻⁷ If initiation is not slow relative to propagation and side reactions do not occur, chain growth should proceed at a nearly uniform rate producing a Poisson distribution of molecular weights. Recently a termination step has been suggested^{8a} and the broadening of the molecular weight distribution which this would bring about has been analyzed.^{8b} Thus the polymerization is presumed to occur by the mechanism shown.

The purpose of this investigation has been to determine to what extent the formation of poly- γ -benzyl-L-glutamate from its N-carboxy-anhydride follows this kinetic scheme. This particular amino acid residue was chosen because it is one of the few precursors of naturally occurring amino acids whose anhydride and polypeptide are both soluble in liquids suitable for polymerization. Earlier investigations in this series^{9,10} had raised questions concerning the adequacy of the foregoing scheme even

when primary amines were used as initiators. The most obvious difficulty arose from finding that the molecular weight distribution of the resulting polymer was extraordinarily broad, that is, far broader than that which could be accounted for by any adjustment of the above mechanism. Consequently, we wished to search for the kinetic origin of this abnormally broad molecular weight distribution and eliminate it if possible. Finally, there



(1) United States Public Health Service Predoctorate Fellow, 1955-1957.

(2) F. Wessely and F. Sigmund, *Z. physiol. Chem.*, **159**, 102 (1926).

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

(4) W. G. Waley and J. Watson, *Proc. Roy. Soc. (London)*, **A199**, 499 (1949).

(5) E. Katchalski, *Advances in Protein Chem.*, **6**, 123 (1951).

(6) J. W. Breitenbach and K. Allinger, *Monatsh. Chem.*, **84**, 1103 (1953).

(7) C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York, N. Y., 1956; D. G. H. Ballard and C. H. Bamford, Special Pub. No. 2, Chem. Soc. (London), p. 25.

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

(9) E. R. Blout and R. H. Karlson, *ibid.*, **78**, 941 (1956).

(10) J. C. Mitchell, A. E. Woodward and P. Doty, *ibid.*, **79**, 3955 (1957).

was obvious interest in seeing if optical isomers had an effect in the kinetics and if particular preference was exhibited in the polymerization. This last point gained interest as evidence accumulated that the helical configuration had only one screw sense (probably right handed)^{11,12} and when it was

(11) J. T. Yang and P. Doty, *ibid.*, **78**, 498 (1956).

(12) W. Moffitt and J. T. Yang, *Proc. Natl. Acad. Sci.*, **42**, 596 (1956).

found that the helical configuration was substantially less stable when the polypeptide contained both D- and L-residues.¹³ It is these questions as well as those which arise in a conventional kinetic study that must be answered before this type of polymerization can be employed in making polypeptides that approach the specifications which are required of protein analogs.

Experimental Details

Measurement of CO₂ Evolution.—The rate of carbon dioxide evolution provides a convenient method for measuring the progress of this reaction. Since previous investigators¹⁴ have shown that the manometric techniques may lead to difficulties when the carbon dioxide pressure increases, a method similar to that of Breitenbach and Allinger⁶ was used. The gas was swept out by a stream of prepurified nitrogen and absorbed in a previously standardized solution of barium hydroxide. Although any strong base may be employed, barium hydroxide was used here because of the relatively large change in resistance resulting from precipitation. By measuring the decrease of conductivity of this solution with time, the amount of carbon dioxide evolved is measured and from this the amount of anhydride consumed is determined.

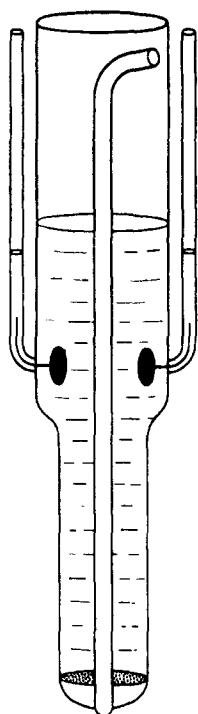


Fig. 1.—Carbon dioxide absorption cell. As shown in Fig. 1, a set of platinum electrodes were sealed into this cell so the conductivity of the solution could be obtained continuously.

An Industrial Instruments Conductivity Bridge (RC 16B) was used for this purpose. With a number of absorption cells an equivalent number of reactions could be run simultaneously using only one conductivity bridge.

In practice the amounts of gas measured were about 10⁻³ mole. In the interests of greater sensitivity a volume of the barium hydroxide solution was used such that W_t at the end of the reaction would be about $5 \times W_0$. The theory of this method has been described¹⁵ and can be expressed briefly as follows. If

- C_0 = initial concn. of Ba(OH)₂ soln. in equiv./l.
- C_t = concn. of Ba(OH)₂ soln. at time t
- W_0 = initial resistance of Ba(OH)₂ soln.
- W_t = resistance of Ba(OH)₂ soln. at time t
- K = conductivity cell constant of absorption cell
- $[CO_2]$ = moles of CO₂ absorbed.
- V = vol. of Ba(OH)₂ soln. in l.
- Λ = equivalent conductance of Ba(OH)₂

Then

$$2[CO_2] = V(C_0 - C_t) = V \left[\frac{1000K}{\Lambda W_0} - \frac{1000K}{\Lambda W_t} \right]$$

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

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

(15) S. Ivekovic and S. Asperger, *Arkiv. Kemi*, **22**, 238 (1950).

$$\begin{aligned} [CO_2] &= \frac{1000VK}{2\Lambda} \left[\frac{1}{W_0} - \frac{1}{W_t} \right] \\ &= \frac{VC_0}{2} \left[1 - \frac{W_0}{W_t} \right] \\ C_0 &= \frac{1000K}{W_0} \\ C_t &= \frac{1000K}{W_t} \end{aligned}$$

The presence of precipitated BaCO₃ had almost no effect on C_0 in this range.

Anhydride Preparation.—All the anhydrides used in this work were prepared by Drs. E. R. Blout, M. Idelson and G. Fasman and R. H. Karlson according to the procedure they have described.⁹ It is important to note that all possible steps in the preparation and purification of these anhydrides were done at -30° to preclude the reaction of water with anhydride. These anhydrides were recrystallized until the chloride content was less than 0.01 weight %. However, it was shown eventually that HCl even at this low level had a detectable effect on the reaction.

These anhydrides were then stored at either -30° or at Dry Ice temperatures. Under these conditions reproducible runs were obtained even after months of storage.

The technique for handling the anhydride was as follows. The anhydride was transferred to small tared capped vials at -30° . These were stored at -30° or Dry Ice temperatures until used. A vial was then brought to room temperature in a desiccator and weighed. After the desired amount of initiator was added to the solvent by means of a Burroughs-Wellcome micrometer syringe, the vial containing the anhydride was uncapped and added to initiate the polymerization. Solution of the anhydride was complete in about 1–2 minutes. As a precaution against the presence of water, solvents were fractionally distilled immediately before using. All transfers of anhydride were conducted at -30° with the exception of the final one which took only about two seconds.

The carbon dioxide was driven out of the reaction vessel by a flow of prepurified nitrogen which was saturated with the reaction solvent. This was then absorbed in the Ba(OH)₂ solution. Resistance measurements were made at intervals until the reaction was 90% complete. However, as a check, the reaction was allowed to proceed until 98–100% of the theoretical amount of CO₂ was evolved.

Polymer Isolation.—After the reactions were at least 99% complete the polymers were isolated by lyophilization or precipitation into reagent grade isopropyl ether. The former technique was necessary for the low A/I material.

Solvents.—Dioxane was purified according to Fieser's method and then fractionally distilled from sodium before using. Alternatively, dioxane was purified by refluxing with sodium for 24 hours, fractionally distilling, storing over sodium and redistilling immediately before use. The dioxane prepared by these methods was identical in its behavior. The latter method was more generally used.

Nitrobenzene was treated with anhydrous P₂O₅, decanted and then fractionally distilled before use. Ethylene dichloride was fractionally distilled. Dimethylformamide was treated with NaHCO₃, decanted and fractionally distilled from phthalic acid at reduced pressure (40 mm., $T = 75^\circ$).

Dichloroacetic acid was fractionally distilled at reduced pressure.

Molecular Weight Determinations.—Intrinsic viscosities were measured in dichloroacetic acid using a modified Ubbelohde viscometer. Weight average molecular weights were obtained from these results by means of previously established relations.¹⁰

Titrations.—The method used in analyzing for amine groups was a conductimetric one.¹⁰ The polymer was dissolved in a phenol-ethanol medium and the change in conductivity was followed as 0.0100 M HClO₄ was added. The intersection of two straight lines gives the end-point.

The titration of N-carboxy-anhydride with sodium methoxide has been described.¹⁶ The titrations were conducted in dioxane solvent after bubbling prepurified nitrogen through the solution to drive out CO₂. The base (0.100 M)

(16) A. Berger, M. Sela and E. Katchalski, *Anal. Chem.*, **25**, 1534 (1953).

was added rapidly to prevent polymerization of the anhydride.⁹ The reproducibility of the method was $\pm 2\%$.

Polymerizations in Dioxane

Initiation and Propagation.—According to the reaction scheme given in the Introduction the consumption of anhydride can be represented as follows using [A], [I] and [P] for the molar concentrations of anhydride, initiator and polymer, respectively.

$$-\frac{d[A]}{dt} = k_1[A][I] + k_2[A][P] + k_3[A][P]$$

However, if $k_1 \geq k_2 \gg k_3$ and $[P] = [I] < [A]$ this can be represented adequately by a pseudo first-order rate equation

$$-\frac{d[A]}{dt} = k_2[A][P] = k'[A]$$

Consequently the conventional first-order plot of $\log([A_0]/[A])$ against time can be expected to be applicable. Our data are presented in this manner but the rate constants given are always bimolecular in units of liter mole⁻¹ sec.⁻¹.

Some typical results are shown in Fig. 2. These correspond to four polymerizations at different anhydride-initiator mole ratios: 4, 10, 20 and 40. The intercepts of the plots, extended back to zero time, are equivalent to the reaction of equimolar amounts of anhydride and initiator. This shows that the initiation by hexylamine is fast relative to the propagation step. From a study of the early part of the reaction the initiation rate constant can be estimated as 0.2 ± 0.1 liter mole⁻¹ sec.⁻¹

For the case where $[A]/[I]$ is 4 the reaction is seen to proceed in a completely linear fashion throughout. However, for the other three cases two successive linear regions are found. At first encounter this behavior may be thought to be simple autocatalysis. But the repeated and never failing display of two linear regions for all the purified anhydrides available prevents the acceptance of this interpretation. Consequently, we conclude that two successive propagation reactions do occur and that two propagation constants, k_{2a} and k_{2b} , can be used to describe the kinetics of the reaction. Values of these constants derived from the slopes of the two linear regions are listed in Table I for a number of

TABLE I
PROPAGATION CONSTANTS FOR AMINE-INITIATED POLYMERIZATIONS IN DIOXANE

Anhydride no.	$k_{2a} \times 10^3$				$k_{2b} \times 10^3$		
	4	20	40	80	20	40	80
442	5.2	4.6	4.4		28	32	
489	7.4	5.6	5.3		32	31	
		4.6			32		
		5.4			34		
498	8.1	9.3	6.6	9.4	28	31	30 ^a
			4.6			32	
531	7.1	8.1			34		
548	8.5	6.3	8.2		35	45	
		5.9			37		
550		6.9	4.6	5.5	33	30	27
			4.9			29	
			5.0			30	
			5.3			31	
GF 31A	4.8				28		
I 62C		4.9				41	
		5.8				42	

^a A/I = 100

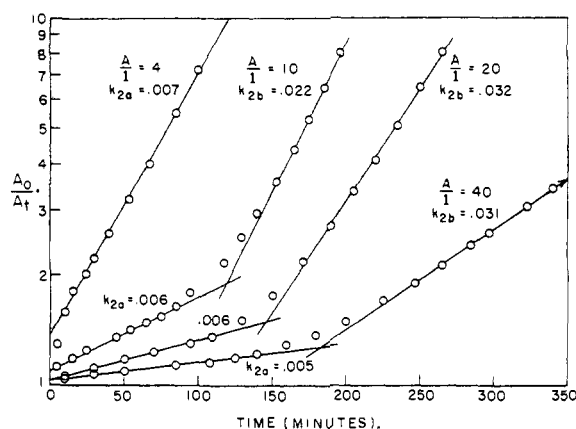


Fig. 2.—Log of ratio of original anhydride concentration to that at time t versus time for four different anhydride-initiator ratios; $[A_0] = 0.152$.

experiments. It is seen that k_{2a} is about 0.006 and k_{2b} is about 0.033 liter mole⁻¹ sec.⁻¹. The somewhat higher values k_{2b} are associated with anhydrides of exceptional purity. In general the reproducibility of k_{2a} for a given anhydride was within $\pm 20\%$; that for k_{2b} was within $\pm 5\%$.

The other conclusion worth drawing from Fig. 2 concerns the location of the transition between the two linear regions of the kinetic plot. It is seen that in terms of fraction of anhydride consumption it occurs earlier in the reaction as the ratio $[A]/[I]$ increases. The other polymerizations listed in Table I showed similar behavior. An eventual explanation of the two successive propagation steps must therefore also elucidate dependence of the transition on the $[A]/[I]$ ratio and its absence at very low values of this ratio.

Initiation by Preformed Polymer.—It is of interest to determine whether the second propagation rate is continued in the presence of more anhydride. Therefore a preformed polymer was prepared in dioxane by initiating to $[A]/[I]$ of 20 and followed kinetically to 98% completion ($k_{2a} = 0.0063$, $k_{2b} = 0.035$). Sufficient anhydride was added to give an additional $[A]/[I] = 12$, and more solvent added to maintain a concentration of 4%. The rate curve obtained (Fig. 3) is linear except for the very early stages of the reaction. In this region one must allow for complete solution of the anhydride in the viscous medium. However, some of the curvature may be real, caused by generation of more of the faster reacting species. The rate constant obtained for the linear portion of the curve is $k_2 = 0.038$ liter mole⁻¹ sec.⁻¹, which is in good agreement with k_{2b} .

Molecular Weights of Polypeptides Prepared in Dioxane.—The number and weight average molecular weights of poly- γ -benzyl-L-glutamate samples prepared in dioxane by hexylamine initiation have been investigated by Mitchell, Woodward and Doty.¹⁰ Their relevant results together with supporting observations of our own are summarized here.

The number average degree of polymerization, according to the proposed reaction scheme, should be equal to the $[A]/[I]$ ratio. The proof that this is the case could be obtained by showing that the

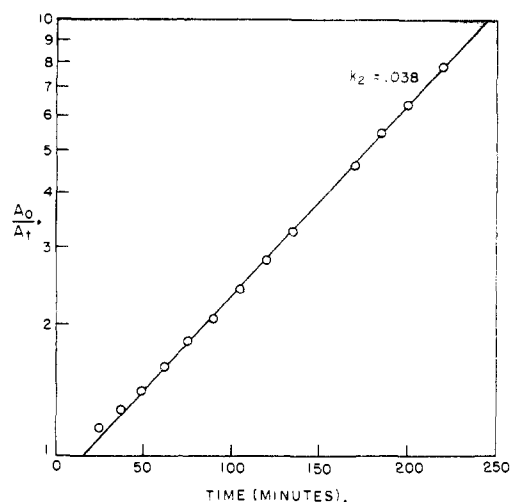


Fig. 3.—Kinetic curve for polymerization initiated by preformed polymer: $[A_0] = 0.063$, $[I] = 0.0046$.

moles of amine groups in the product is the same as the moles of initiator added. The following experiment offers a test of this point. A polymerization at $[A]/[I] = 20$ was allowed to proceed for 10 hours whereupon it would be expected to have reached 99.8% of completion. It was then lyophilized to remove the dioxane over a period of another 10 hours. When this product was dissolved and titrated it was found to contain 85% of the amine groups added as initiator. This indicates that the number has not substantially altered during this long polymerization. Since the reaction of the initiator was found to be very rapid and since any that might have remained would have been lost during lyophilization, it appears that amine groups are conserved during the ordinary polymerization. Therefore each initiator appears to have given rise to one polypeptide chain and the number average degree of polymerization, DP_n , can be taken as equal to the ratio, $[A]/[I]$.

The weight average degree of polymerization, DP_w , is derivable from intrinsic viscosity measurements in dichloroacetic acid as a result of recent studies.¹⁰ (The average value actually falls slightly below the weight average for samples having a broad molecular weight distribution but this need not concern us here.) Consequently the ratio DP_w/DP_n can be evaluated and used as an indication of the breadth of the molecular weight distribution. Some typical results are shown in Table II where they are compared with the result expected for a Poisson distribution, that is, the distribution expected for the mechanism described earlier provided that the termination reaction is negligible. If the termination reaction is not negligible the value of the ratio, DP_w/DP_n , will be increased toward a limiting value of 2. The observation is, however, that the ratio is much greater than this. Its magnitude exceeds that generally encountered in any kind of polymerization and consequently its explanation should require a very substantial alteration in the mechanism proposed.

It is relevant to note that if trace amounts of water or other possible initiator had interfered in these polymerizations they would have increased the

TABLE II
THE POLYDISPERSITY OF TYPICAL POLYMERS PREPARED IN DIOXANE

$[A]/[I]$	$[\eta]$	DP_w	DP_w/DP_n Poisson	DP_w/DP_n Found
4	0.08	13	1.25	3.3
20	.27	170	1.05	8.5
40	.34	225	1.03	5.5

number of chains produced and hence have lowered the molecular weight over that expected. Consequently an explanation cannot be expected from this source.

Termination Steps.—Evidence for a termination step of the kind indicated in the Introduction was provided by titration data¹⁰ which showed that carboxyl groups were present. Since the concentration of these groups (about one for each 300 residues) was independent of the $[A]/[I]$ ratio and hence of the time required for the polymerization, and since the production of carboxyl groups by debenzoylation was not detectable under polymerization conditions, these results were interpreted as favoring the termination step mentioned. If this were the only termination step involved, a check could be made on this suggestion by carrying out a polymerization at a $[A]/[I]$ ratio that exceeded 300. If the proposed termination step were valid the rate of polymerization should gradually slow and come to a stop before all of the anhydride was consumed.

A polymerization of this kind was carried out and the kinetic plot is shown in Fig. 4. The anhydride concentration was determined in this case directly by titrating with sodium methoxide.¹⁶ The solid circles correspond to a control experiment under identical conditions with no added initiator. The qualitative behavior of the polymerization is clearly of the type anticipated above. A quantitative consideration of this experiment, however, leads to an inquiry into other aspects of the polymerization.

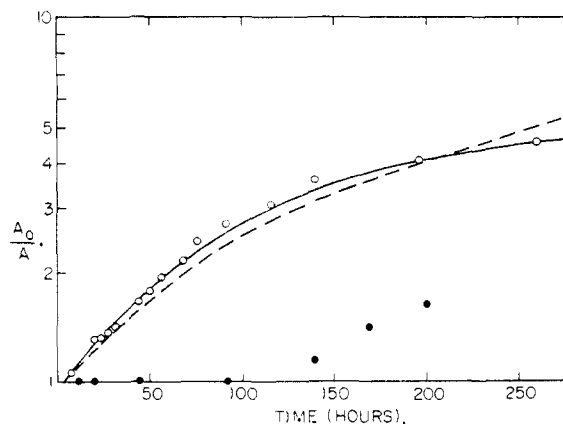


Fig. 4.—Kinetic curve for polymerization initiated by *n*-hexylamine at very high $[A]/[I]$. The dashed line is the calculated curve for Sela-Berger termination reaction. Solid line is calculated assuming termination through cyclization. The solid points are those for a control with no initiator added.

The calculated curve for $k_{2b} = 0.032$ and $k_3 = k_{2b}/300$ gives very poor agreement with the experi-

mental data. Indeed the value of k_{2b} calculated from the initial slope of the data in Fig. 4 is only 0.015. On the basis of a subsequent discussion on the effect of residual HCl in the anhydride it is evident that the most likely explanation of the low apparent value of k_{2b} is that some of the initiator was inactivated by reaction with HCl. The amount of HCl required to remove half of the initiator and therefore restore the value of k_{2b} to that usually found is 1.3×10^{-4} g. per gram of anhydride. Since the residual chloride content is at just this level (0.01%)⁹ this explanation seems tenable. If this is accepted, the $[A]/[I]$ in this case was actually about 1000. If the anhydride consumption is recomputed on this basis a fairly satisfactory fit of data can be obtained if $k_3 \sim k_{2b}/1000$ (dashed line in Fig. 4). This result indicates that only one-third as many chains are terminated with carboxyl groups as titration results had indicated.¹⁰ Thus the majority of the carboxyl groups found by titration may have been the result of debenzylolation.

Having gone into this matter in so much detail it becomes necessary to pursue the possible effects of another termination step that is peculiar to this particular polypeptide, that is, the loss of amine end groups through a cyclization reaction with the elimination of benzyl alcohol.^{9,17} The experiment described in the last section shows this reaction proceeds at a detectable rate (about 0.5–1.0% amine loss per hour).⁹ Over the period of time taken for polymerization of $[A]/[I]$ of 40, for example, it produces termination of about 5% of the chains. But during the much longer reaction time of the polymerization for $[A]/[I]$ of 510 (or 1000) this cyclization reaction would have very pronounced effects. Indeed the assumption of 1% loss per hour of amine groups by a unimolecular mechanism can account for nearly all of the decay in rate observed (solid line in Fig. 4 calculated on this basis). It is clear however, that the role of this termination will be negligible in the other polymerization discussed in this paper because they were completed in much shorter times.

The result of these considerations is to show that a termination is readily demonstrated at very high anhydride-initiator ratios, and therefore a ceiling does exist for the molecular weight. The present evidence does not enable us to delineate the relative importance of the two termination steps. However, the effect of these termination reactions is not large enough to be revealed kinetically for $[A]/[I]$ of 40 and less. For example, in a polymerization of $[A]/[I] = 20$ carried to 95% completion (5 hours) one would estimate that less than 7% of the chains would be terminated.

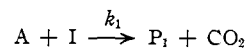
Helix Formation as the Cause of the Change in the Propagation Constant and the Consequent Broadening of the Molecular Weight Distribution.—The termination steps discussed in the previous section are obviously of minor importance in the kinetics of the polymerization in the anhydride-initiator ratios dealt with in this investigation

(17) The rate of disappearance of amine groups in the lyophilized solid sample is about 0.5% per hour at 25°. Consequently titrations must be carried out promptly or the material must be stored at -30° or lower. Other experiments¹⁰ also support this conclusion.

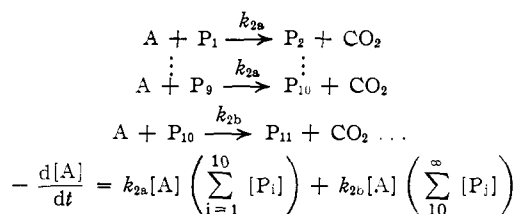
$[A]/[I]$ 4 to 40. Consequently, no explanation can be found here for the extreme breadth of the molecular weight distribution shown by the molecular weight studies. The origin of this effect would appear to lie in the nature of the kinetics of the polymerization as shown in Fig. 2. This is evident by recognizing that inasmuch as the amine groups are essentially preserved during the reaction, the individual chains can be expected to grow throughout the reaction. Yet the rate constant governing their growth appears to increase sharply at a certain point. Regardless of the cause of this change its effect is clearly the endowment of chains with a much faster rate of growth once some condition has been met. As a consequence, those chains that qualify for this faster growth can be expected to undergo a rapid increase in chain length. Those chains that may, for statistical reasons, be somewhat delayed in attaining the conditions for vast growth will be further delayed by the rapid consumption of anhydride by the fast growing ones. Therefore, many of the chains may never actually reach the condition for rapid growth and as a consequence a very broad molecular weight distribution would result. Thus the extreme broadening of the molecular weight distribution is seen to be the direct result of the phenomenon of two successive propagation constants for chain growth.

The cause of the shift from a slow propagation constant to a fast one is of obvious interest. The mid-point of the transition from one rate to another is seen (Fig. 2) to occur at a point where the anhydride molecules consumed per initiator is in the region of 7 to 12 for an $[A]/[I]$ 20. That is, the transition occurs when a certain chain length is reached and is not obviously related to the anhydride-initiator ratio. Since previous studies¹⁰ have shown that the minimum chain length required for formation of the α -helical configuration is about 10, it appears that this configurational change is the cause of the dramatic shift in propagation rates. Convincing evidence for this conclusion and proof of the transfer of chains from this β to the α configurations through growth has been provided by an infrared spectral study of the reaction by Idelson and Blout.¹⁸ They have shown that the β L-polypeptide (non-helical) is produced in the region corresponding to the lower rate, and simultaneously with the incidence of the higher rate the formation of the α -polypeptide (helical) is observed. Through the use of deuterium labeled initiator, these investigators have shown that the β L-form is indeed the precursor of the α -polypeptide. Since the energy of activation for both rate constants is found to be low (<10 kcal.) the change in rate accompanying the configurational change is apparently due to a lowering of the entropy of activation resulting from an increased accommodation provided for the anhydride molecule at the end of a helix.

We may express the above interpretation through the equations



(18) M. Idelson and E. R. Blout, *THIS JOURNAL*, **79**, 3948 (1957).



where $[P_i]$ represents the concentration of the polymer chain containing i peptide units.

Thus in the early stages of the polymerization, the anhydride consumption should be first order. This is the situation that is observed at an $[A]/[I]$ of 4, and in the early regions at higher $[A]/[I]$. However, if additional anhydride is available to increase the chain length sufficiently to form an α -helix, this configuration may react at a new and higher rate (k_{2b}). The flux of polymer chains through the region of critical chain length will result in an autocatalytic reaction since

$$\frac{d \sum_{j=10}^{\infty} [P_j]}{dt} = k_{2b}[A][P_{10}]$$

This conversion is dependent on the concentration of chains just below the critical size and the anhydride concentration. When both have been lowered sufficiently the reaction will again be pseudo first order. This is the effect observed in Fig. 2. Consequently, a relatively large number of the more slowly propagating chains will remain as such because insufficient anhydride is available for them to reach the critical size. Because of this, the rate constant obtained for the second propagation rate will be an underestimate of its true value, perhaps by as much by a factor of 2—see also footnote 13 in reference 18. Ostensibly, at sufficiently high $[A]/[I]$ all of the chains should be able to pass through this critical size region before the anhydride is consumed. A trend of higher rate constants for k_{2b} should therefore be observed with increasing $[A]/[I]$. This is the observed behavior in Fig. 2 at $[A]/[I]$ of 10 and 20 but not beyond. A subsequent discussion on the role of impurities in this polymerization demonstrates that a small amount of HCl is sufficient to suppress this trend at higher $[A]/[I]$. However, Idelson and Blout do observe an increase in k_{2b} with $[A]/[I]$ with a highly purified anhydride sample.¹⁸ Thus the kinetic behavior and the molecular weight distribution of the products are compatible with the concept that a configuration change under the conditions of this polymerization has effected a change in reaction rate.

Effect of Impurities.—It has been observed that some samples of γ -benzyl-L-glutamate anhydride gave a rate constant for k_{2b} which differed from each other more than could be explained by probable experimental error. To investigate the origin of these occasional variations, a sample of the N-carboxy anhydride was polymerized directly after its first crystallization. At an $[A]/[I]$ of 20, a linear pseudo first-order behavior was observed throughout the polymerization (case A, Fig. 5) in marked contrast to the two successive growth stages previously observed. However, after one

recrystallization of this anhydride from methylene dichloride, two successive propagation rates became clearly evident (B in Fig. 5). In both cases the initial propagation rates of the polymerization, k_{2a} , were about equal.

In addition to the difference in kinetic behavior, there was a striking difference in the reaction mixtures when the polymerizations neared completion. The solution of impure anhydride was a quite rigid gel, whereas the product of the recrystallized anhydride was a viscous solution. The α -helical content estimated from infrared spectra was about 40% in case A and about 75% for case B. Such marked differences in kinetic behavior and reaction products must therefore be the result of different growth mechanisms.

One of the impurities to be removed by recrystallization of the anhydride is HCl. Analysis of the unpurified anhydride showed the HCl content to be 1.0 mole %. Since this could react with the initiator, *n*-hexylamine, to produce a salt which in turn may affect the course of the polymerization, a series of reactions were carried out on a highly purified anhydride at an A/I of 20 (case C, Fig. 6) to which 5.0, 0.67, and 0.23 mole % of *n*-hexylamine hydrochloride had been added (D, E and F, respectively, in Fig. 6). The results show that this adduct completely eliminates the second propagation rate in cases D and E.

Further evidence that the propagation mechanism has been altered drastically is offered by the molecular weights of the products. The weight average DP was determined in the ultracentrifuge by the Archibald approach-to-equilibrium method for case C and E, and found to be 170 and 46, respectively. The DP_w expected in absence of any complicating factors would be 21. In concordance with the evidence introduced earlier it is seen that the absence of the second propagation step has reduced the weight average DP and therefore narrowed the molecular weight distribution. (It can be argued that since alkali halides⁶ do initiate the polymerization, then the presence of this added amine salt could lower the molecular weight. Actually, the amount added corresponds to only 13% of the initiator present on a molar basis. Also, the initial propagation rate is not affected significantly; this indicates that the salt is no more active as an initiator than the amine itself.)

The kinetic behavior in the presence of only 0.23 mole % of amine salt (case F of Fig. 6) deserves further comment. Under these conditions the second propagation rate is just beginning to appear. Taken by itself this behavior might be falsely interpreted as an autocatalytic reaction. However, the behavior observed with the more highly purified anhydride (case C) clearly indicates that this is just the emergence of the second propagation rate. The explanation for the variation in k_{2b} for different anhydride samples is now evident. Since the lower limit of chloride detectability is about 0.08 mole %, it is apparent that amounts of HCl beyond detection can have a large effect on the rate. The difficulty in obtaining exactly reproducible results from one anhydride preparation to another can be ascribed to variations in HCl content all of which

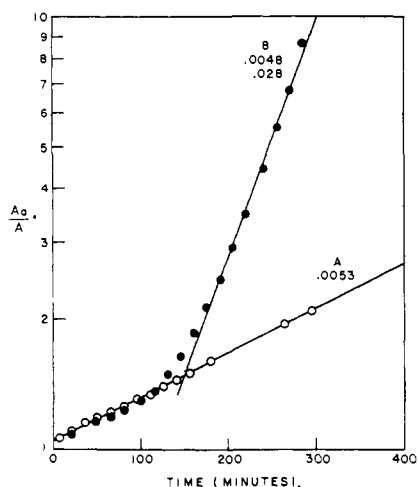


Fig. 5.—Initiation with *n*-hexylamine to $[A]/[I] = 20$ in dioxane. Case A corresponds to impure anhydride and Case B to the same anhydride after one recrystallization; $[A]_0 = 0.152$.

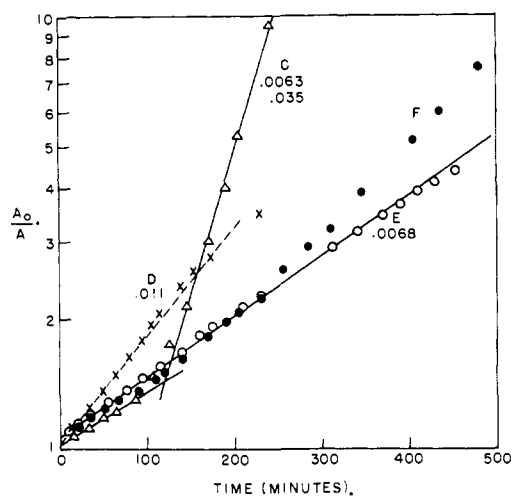


Fig. 6.—Polymerizations under same conditions as Fig. 5. Case C is a highly purified anhydride (HCl < 0.1 mole %). Case D, E and F correspond to the addition of *n*-hexylamine hydrochloride to the extent of 5.0, 0.67 and 0.23 mole %, respectively.

are below the limits of detection. This emphasizes the importance of sufficient recrystallizations to eliminate this impurity. We are indebted to Dr. E. R. Blout for suggesting the examination of the anhydride after its first crystallization and of the role of hexylamine hydrochloride.

The Occurrence and Elimination of the Two-stage Propagation in Other N-Carboxy-anhydride-Solvent Systems

L-Leucine N-Carboxy-anhydride.—The kinetic behavior of γ -benzyl-L-glutamate anhydride has been correlated with the molecular weight distribution of the products, and the origin of the behavior traced to the coil-helix transition during chain growth. However, since this anhydride cannot be purified through sublimation, the possibility remains that some impurity not removable through recrystallizations is responsible for the observed be-

havior. Therefore, a study of the polymerization of the anhydride of L-leucine purified in the same way was carried out. These polymerizations were conducted in nitrobenzene solution at an $[A]/[I]$ of 20 and are shown in Fig. 7. The polymer formed does

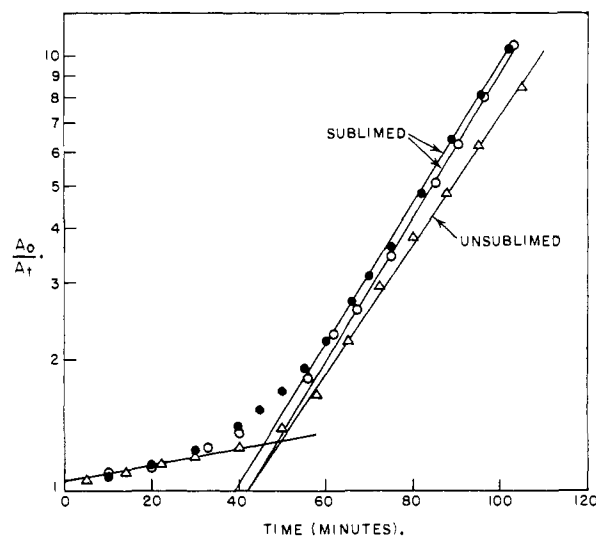


Fig. 7.—Polymerization of L-leucine N-carboxy-anhydride with initiation by *n*-hexylamine; $[A]_0 = 0.134$, $[I] = 0.0066$; ($k_{2a} = 0.013$, $k_{2b} = 0.090$ l. mole⁻¹ sec.⁻¹).

precipitate under these conditions after the reaction is about 25% complete. As a result the quantitative significance of the rate constant obtained is questionable, but the demonstration of a large increase in rate similar to the γ -benzyl-L-glutamate case is apparent. (Precipitation of the polymer would be expected to decrease the rate so that this does not affect the argument.) When this anhydride was sublimed its polymerization was found to be essentially the same as for the unsublimed case. These observations therefore support the argument that the analogous behavior exhibited by γ -benzyl-L-glutamate is not due to the method of anhydride purification and indicate that two-stage propagation may be a general feature in the formation of polypeptides by this reaction. Similar behavior has also been found with L-phenylalanine anhydride.

Polymerization of γ -Benzyl-L-glutamate Anhydride in Other Solvents.—The phenomenon of two apparent rate constants in the polymerization of γ -benzyl-L-glutamate in dioxane has been observed in a number of solvents. The rate constants obtained are listed in Table III.

TABLE III
PROPAGATION RATE CONSTANTS IN VARIOUS SOLVENTS
 $T = 25^\circ$, $[A]/[I] = 20$

Solvent	Dielectric constant	k_{2a}	k_{2b}
Nitrobenzene	36	0.021	0.11
Ethylene chloride	10.3	.015	.14
Chloroform	5.1	.045	.30
Benzene	2.3	.062	.31
Dioxane	2.3	.006	.032

There is seen to be a surprising lack of correlation of rate with dielectric constant of the solvent.

Bamford,⁷ *et al.*, have noted that the propagation rate of the polymerization of several racemic anhydrides is very dependent on solvent polarity. They have observed that the polymerization of γ -benzyl-L-glutamate is less sensitive to the solvent polarity and proposed as an explanation a complex between the benzyl ester group and the terminal amine group.⁷ Because the resulting complex is similar to the expected transition state complex, the effect of changing the dielectric constant of the medium would be small. However, the reaction rate above is ten times faster in benzene than in dioxane and is actually more rapid in this non-polar solvent than in more polar ones. Furthermore, Breitenbach and Allinger⁶ have shown that the reaction rate of DL-phenylalanine anhydride is about the same in nitrobenzene and benzene. Thus, an absence of correlation between solvent polarity and rate is clearly manifested; however, the reaction rate is very dependent on solvent type. Such an effect can be attributed to hydrogen bonding between the terminal amine hydrogens in the growing chain and the carbonyl groups of the anhydride. In this view, benzene would enhance hydrogen bonding, producing thereby a higher rate, whereas oxygenated solvents by competing with the anhydride molecules for the growing chain would suppress the rate. Thus the solvation of the end of the growing chain would be greatly dependent on the solvent type.

The Absence of Two-stage Propagation in Dimethylformamide.—To elucidate further the effect of solvent upon the course of the polymerization, a medium was desired which was strongly solvating and yet inert in its behavior toward the anhydride. Dimethylformamide has been shown to prevent intermolecular association of polypeptides and would appear to satisfy these requirements.

When polymerizations were conducted in this medium it was found that the kinetic behavior contrasted strikingly with dioxane and other solvents. Only linear rate curves for the disappearance of anhydride were obtained at $[A]/[I]$ of 20, 40 and 80 ($k_2 = 0.057 \pm 0.004$). The viscosities of the polymers were much less than those obtained from polymerizations conducted at the same $[A]/[I]$ in dioxane. This indicated a considerably lower DP_w and assuming $[A]/[I] = DP_n$, a narrower distribution.¹⁰ Because of the insensitivity of intrinsic viscosities in this low DP region, the weight average molecular weights were measured directly on several polymers; the results on two are given in Table IV.

TABLE IV
THE POLYDISPERSITY OF TYPICAL POLYMERS PREPARED IN DIMETHYLFORMAMIDE

[A]/[I]	$[\eta]$	DP_w	DP_w/DP_n Poisson expt.	
10	0.081	14	1.1	1.4
40	.13	48	1.03	1.2

Since lyophilization of dimethylformamide is not practical those products had to be isolated through precipitation, and therefore fractionation was a serious problem for the lower $[A]/[I]$ samples. Consequently, the $[A]/[I]$ ratio is not strictly equal to DP_n for $[A]/[I]$ of 10, and by mak-

ing this assumption the distribution appears more broad than it actually is. Therefore, the experimental ratios above represent a maximum for the $[A]/[I]$ 10 case. At an $[A]/[I]$ of 40, however, the isolation of the polymer was nearly quantitative. The molar weight distribution is seen to be quite narrow and closely approximates the Poisson distribution at the higher $[A]/[I]$ where fractionation is negligible. The pronounced difference between these polymers and those obtained in dioxane solution (Table II) confirms the kinetic observations in each case. In the absence of any complicating factors and with only a single propagation rate, the Poisson distribution should be observed. When those requirements are achieved, as in dimethylformamide the molecular weight distribution at least approaches this theoretical limit. Therefore, a means has been found of preparing low molecular weight synthetic polypeptides with a very narrow molecular weight distribution.

Reactions were conducted at several temperatures in dimethylformamide and the rate constants obtained are given in Table V.

TABLE V
TEMPERATURE DEPENDENCE OF PROPAGATION CONSTANTS IN DIMETHYLFORMAMIDE

$T, ^\circ C.$	0	25	40
$k_2 \times 10^3$	19	56	87

At higher temperatures the observed rate decreased with time, evidently because the cyclization reaction was taking place more rapidly. A rather low activation energy of 6.6 kcal. is obtained from the constants in Table V. Apparently a higher activation energy is characteristic of the cyclization reaction. As a consequence, there will be a larger rate of termination at high temperatures ($T > 50^\circ$) and polymers with broader molecular weight distributions will result.

The Role of Optical Isomers in the Polymerization

The Polymerization of Racemic N-Carboxyanhydride in Dioxane.—N-Carboxyanhydrides of γ -benzyl-DL-glutamate were prepared in the same way as the L-isomer. Upon initiation with *n*-hexylamine at $[A]/[I]$ equal to 20 the kinetic data were found to exhibit the same general features as the L-isomer, but quantitatively there were two differences. The propagation constants were substantially lower, $k_{2a} = 0.004$ and $k_{2b} = 0.013$ liter mole⁻¹ sec.⁻¹, and more anhydride was consumed before the transition in rates took place.

In order to ensure that these differences were not due to residual impurities in the racemic anhydride, further experiments were carried out with N-carboxyanhydrides of the pure L- and pure D-isomers and then with the equimolar (racemic) mixture of the two (D + L). The results in dioxane at the usual concentration (4 g. anhydride/100 cc.) and for two $[A]/[I]$ values are summarized in Table VI.

The lower rate of propagation for the racemic mixture is clearly evident: k_{2a} is reduced to about two-third and k_{2b} to about one-half of the values for the pure isomeric forms.

TABLE VI
PROPAGATION CONSTANTS OF ISOMERIC AND RACEMIC N-CARBOXY-ANHYDRIDES OF γ -BENZYL-GLUTAMATE

Isomer or mixture	$[A]/[I] = 4$ $k_{2a} \times 10^3$		$[A]/[I] = 20$ $k_{2a} \times 10^3$	
L	7.4	5.6	32	
D	7.1	7.4	34	
DL	5.1	4.5	13	
D + L	5.5	4.5	15	
		4.2	17	
		4.4	16	

The observation that the onset of the faster propagation constant occurs at a longer chain length in the case of the racemic N-carboxy-anhydrides indicates that a longer chain length is required for the helical configuration to be assumed. This is to be expected, if a copolymer of L- and D-residues has formed, due to the demonstration that helix stability does decrease upon the introduction of increasing amounts of the opposite optical isomer into the polypeptide chain.¹³

Standing in contrast to this correlation, however, is the intriguing possibility of interpreting the lowering of k_{2b} by a factor of two in the following manner. If chain growth in the helical form at least did permit only the addition of pure isomers to themselves so as to produce chains of pure D- and pure L-residues at the usual rate, it would be expected that the observed rate would be only half of the usual one because the concentration of anhydride molecules that could add to any one chain would be only half the total concentration. In order to explore this matter further some investigations were made in dimethylformamide where greater accuracy could be obtained.

The Polymerization of Mixed D- and L-N-Carboxy-anhydrides in Dimethylformamide.—Kinetic measurements were made on polymerizing mixtures of various proportions of D- and L-anhydrides in dimethylformamide. The results were first order in each case; the propagation constants are listed in Table VII.

TABLE VII
DEPENDENCE OF PROPAGATION CONSTANTS ON ANHYDRIDE COMPOSITION IN DIMETHYLFORMAMIDE

% L-isomer	100	92.5	85.5	74.7	50	0
$k_2 \times 10^3$	56	46	39	30	22	58
					22	
					21	

The effect of the opposite optical isomer on the rate of the polymerization is seen to be quite large and about the same as exhibited for k_{2b} in dioxane. Such an effect can be explained only if the propagation step is quite selective with respect to the optical isomers. This can be demonstrated more clearly by analyzing the reaction on the assumption that selectivity is determined only by the terminal residue with which the anhydride reacts. Thus in dimethylformamide solution we have

$$\text{Rate} = -\frac{d[A]}{dt} = k_2[A][I]$$

Since we wish to consider the effect on the rate of varying amounts of L- and D-anhydride ($[A_L]$ and

$[A_D]$) but keeping their sum constant, we shall use mole fractions, denoted by primes. Thus

$$\begin{aligned} [A_L'] &= [I_L'] \\ [A_D'] &= [I_D'] \\ [A_D'] + [A_L'] &= 1 \\ [I_D'] + [I_L'] &= 1 \\ \text{Rate} &= k'([I_D']^2 + [I_L']^2) + 2k''([I_D'][I_L']) \end{aligned}$$

Where

$$\begin{aligned} [I_D'] &= \text{Mole fraction of polymer ending in D-residues} \\ k' &= \text{Propagation constant for reaction between D-anhydride and polymer molecule ending in D-residue} \\ k'' &= \text{Propagation constant for reaction between D-anhydride and polymer ending in L-residue} \end{aligned}$$

The dependence of the rate (and therefore the observed rate constant) on the composition of optical isomer is quadratic. Introduction of a new variable z such that $[I_L'] = z + 0.5$ leads to the simpler form

$$\text{Rate} = z^2(2k' - 2k'') + 0.5(k' + k'')$$

This expression is shown in Fig. 8 as the solid line for $k'' = 0$ (or the case where no cross-reaction exists). The values in Table VII are also plotted. It is rather surprising to find that the experimental points fall consistently below the line, whereas for any positive value of k'' they should fall above this calculated line.

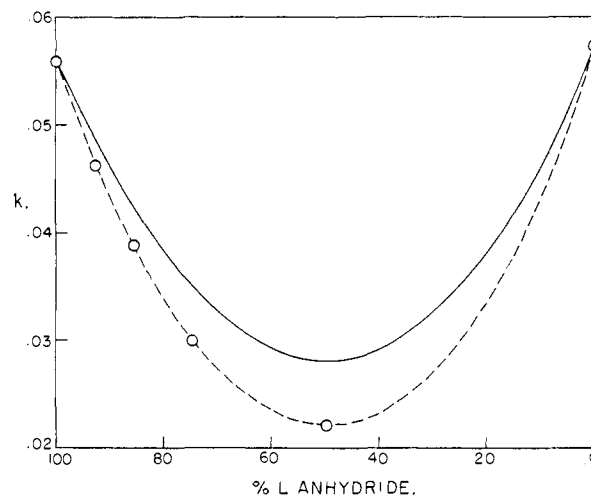


Fig. 8.—Propagation constants obtained in dimethylformamide at $[A]/[I] = 20$ as a function of varying D- and L-anhydride compositions. The solid line is calculated assuming L-anhydride does not react with polymer ending in D-residues (absolute preference).

The data may be treated more quantitatively by taking the log of the above expression together with the experimental points as shown in Fig. 9. The experimental points are seen to fall on a straight line within probable experimental error and this line is below the theoretical one for complete selectivity.

It is apparent therefore, that the rate constants obtained do agree with the interpretation that this polymerization is preferential with respect to the optical isomers. A complication is presented, however, in that the rate constants are lower than the

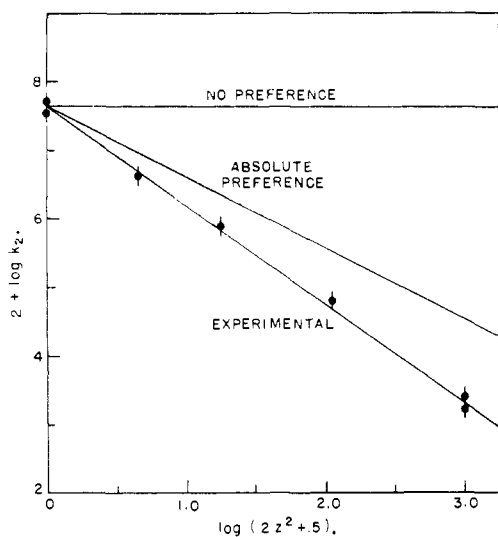


Fig. 9.—Quantitative treatment of data in Table VII according to equations in text.

minimum DPP possible for a preferential reaction of the type assumed. Consequently, the assumption that the selectivity of this reaction is due only to interaction between the anhydride and the last residue in the chain is seen to be an invalid one. The most reasonable alternative seems to be that selectivity depends on one or more residues lying behind the terminal residue of the growing chain. If the growing chain end is in the helical configuration this situation could easily arise and the third residue from the end may play a large role since the residue being added would hydrogen bond with this residue. The strength of the bonding will be influenced by whether these two residues are enantiomeric or not. If the initiation is non-selective, it would be expected that the probability of the second and third residues from the chain end being the same as the terminal residue would remain constant throughout the polymerization and by their multiplicative effects the propagation rate constant could be lowered in the manner shown in Fig. 9. In order to explore the possibility of selective influences arising from non-terminal residues, the following experiments involving initiation with preformed polymer were carried out.

The Polymerization of Racemic and Enantiomeric N-Carboxy-anhydride by Preformed Polypeptide.—The polypeptide used for initiation was that made in dimethylformamide at $[A]/[I]$ equal to 20 from L-anhydride. Consequently, it had a narrow molecular weight distribution and was predominantly in the helical configuration¹⁰ when employed as initiator in dimethylformamide solution. Equal aliquots of this initiating polymer were added to solutions of L-, D- and DL-anhydride in such proportion that an average of 12 residues would be expected to add to each initiating chain. The results are shown in Fig. 10. It is seen that the L-anhydride continues to react at about the same rate as the initiating L-polymer. In the DL-anhydride case the reaction initially has a rate somewhat slower than in the L-anhydride case and then the rate decreases before half of the anhydride has been con-

sumed. This is the behavior which would be anticipated from the earlier observations on the amine initiated case. That is, initially the L-anhydride present would be consumed more rapidly than the D-anhydride and as it is depleted, the rate, calcu-

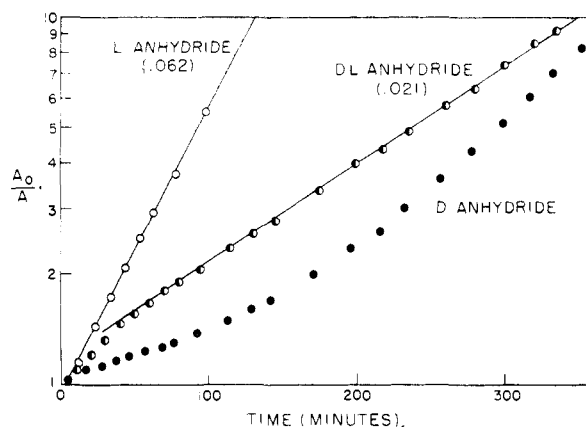


Fig. 10.—Initiation by aliquots of preformed polymer (DP = 20 of polybenzyl-L-glutamate on L (O), DL (◐), and D (●), carboxy-anhydrides of γ -benzyl-glutamate in dimethylformamide; $[A_0] = 0.058$; $[I] = 0.0048$.

lated on the basis of total anhydride concentrations, would be expected to fall. Meanwhile, one would expect the D-anhydride to be gradually added to the growing chains. The result would be an autocatalytic one according to the equation

$$-\frac{d[A_D]}{dt} = k''[A_D][I_L] + k'[A_D][I_D]$$

Since $k'' < k'$, the rate should increase as the more reactive species I_D is produced. The observed behavior is qualitatively similar to this expectation. However, the initial portion of the curve is nearly linear and has a rate constant of *ca.* 0.011 or about $1/8$ of that for the reaction of the pure isomer. The apparent linearity over this region is interpreted as meaning that several D-residues must add to the L-polymer before the period of autocatalysis begins.

A possible explanation for this behavior can be obtained from following the same polymerization by optical rotation. Such a study conducted in dioxane solution is shown in Fig. 11. Here equal amounts of L-, DL- and D-anhydride have been initiated by L-polymer.

The addition of L-anhydride ($[\alpha]^{25}_D - 16^\circ$) to L-polymer ($[\alpha]^{25}_D + 10$) causes the optical rotation to increase continuously throughout the reaction.

The addition of DL-anhydride produces a large increase in the optical rotation because the DL-helix thus formed has a preferred screw sense in the same direction as the initiating polymer and this contributes a large positive rotation.¹³

In the case of the addition of D-anhydride, the first residues to add do so in the same helix sense as that of the initiating L-polymer. Measurement of the number of moles of CO_2 given off at the time of the maximum in the curve indicate that an average of about 4 peptide units have added to each L-polymer chain at this point. The optical rotation of a racemic helix directed in one screw sense at this wavelength has been measured and found to be large and

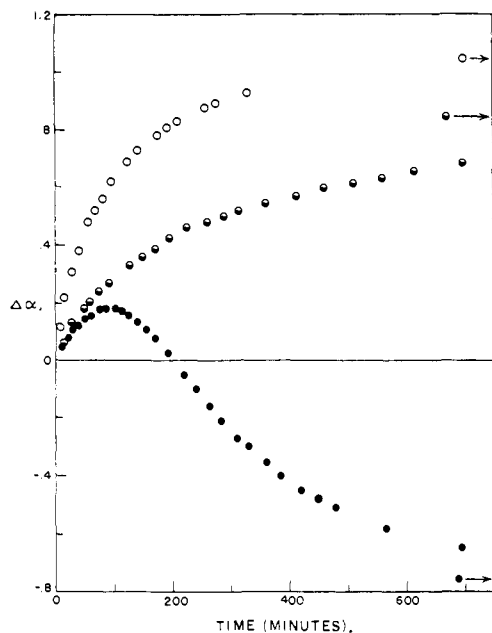


Fig. 11.—The change in optical rotation of L(O), DL (●), and D (●), carboxy-anhydride of γ -benzyl-glutamate during polymerization initiated by aliquots of an L-polymer; $[A_0] = 0.076$; $[I] = 0.0034$; polarimeter tube length 20 cm.

positive.^{13,19} The intrinsic residue rotation of D-peptide units is also large and positive.¹⁹ The additive nature of these two effects is evidently what makes it possible to observe the increase in rotation at all. The fall in rotation after the maximum has been reached indicates that after sufficient D units have added the helix sense is inverted and the helix characteristic of D-peptides is formed, the mirror image of the L-peptide helix. This same behavior has been observed in dimethylformamide solution.

These results are consistent with the hypothesis that D-anhydride reacts more rapidly with a polymer chain characteristic of D-peptide residues. It is apparent that a number of these must exist in a sequence (at least 4) before this configuration can be realized, and the rate of the pure isomer obtained.

To summarize, the effect of the opposite enantiomorph on the reaction rate is seen to be greater than can be expected if it is assumed that only the last residue of a growing chain exerts selectivity. The most probable explanation is based on the steric influences of residues further removed, and these would be relatively large if the polymer configuration were helical.¹³ The existence of this configuration under the reaction conditions has been amply demonstrated above, and elsewhere.^{10,13}

In dimethylformamide a single propagation rate is observed for the L-isomer of γ -benzyl-glutamate anhydride (Fig. 12). On the basis of the above hypothesis there should be a perceptible difference in rate for the racemic anhydride corresponding to whether the growing chain is helical or not. Because of the additional interference of peptide residues further removed from the last one in a grow-

(19) P. Doty and R. D. Lundberg, *Proc. Natl. Acad. Sci.*, **43**, 213 (1957).

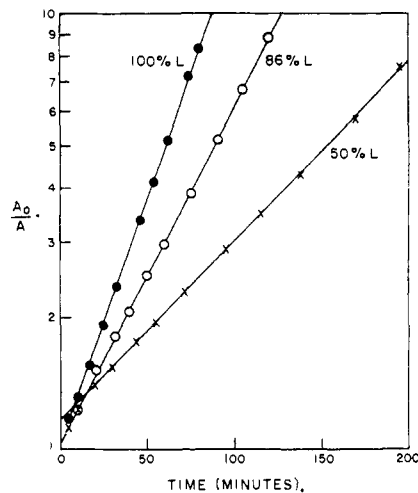


Fig. 12.—Initiation by *n*-hexylamine in dimethylformamide on N-carboxyanhydrides of γ -benzyl-glutamate with various percentages of D- and L-isomers present: 100% L (●), 86% L (O), 50% L (X); $[A_0] = 0.152 M$; $[I] = 0.0076$.

ing chain, it would be predicted that the rate should be less in the region where the helix exists as the stable configuration. Therefore at an $[A]/[I]$ of 20 two rates should be perceptible for the racemic anhydride. Such an effect is detectable in Fig. 12, where a rate corresponding to $\frac{3}{5}$ that of the pure isomer obtains for the first 25–30% of the reaction, and then a lower rate prevails ($\frac{2}{5}$ that of the pure isomer). However, the small magnitude of the change between the rates precludes any definitive test of the hypothesis.

Discussion

Relation to Other Work.—The rather surprising nature of the kinetic results of this investigation invites comparison with other related studies. Actually there appear to be only two other examinations of the kinetics of this polymerization that are relevant: others have dealt with sarcosine N-carboxy-anhydride, the polymer of which cannot form a helical configuration, and other types of initiation which give rise to a quite different mechanism than that explored here.

Ballard and Bamford^{7,14} have reported kinetic studies of the polymerization of the N-carboxy-anhydride of γ -benzyl-L-glutamate in dioxane ($k_2 = 0.022$) and in nitrobenzene ($k_2 = 0.070$) and of DL-leucine in nitrobenzene ($k_2 = 0.042$). In all these cases they have found a behavior that is essentially that of a single propagation constant with occasional mild increases in rate toward the end of the reaction which they attribute to autocatalysis. The rate constants they find are intermediate between the k_{2a} and k_{2b} values we have reported above. These authors place great emphasis on the need of eliminating moisture but it seems unlikely that this can play any role in our results because of the absence of polymerization in blank experiments and the finding of a higher DP than expected, not a lower one that would result from introducing more initiating centers. Thus we find agreement in the magnitude of our rate constants with those of Ballard and Bamford but sharp difference in that

two successive propagation rates were not found by these authors. Our demonstration of the elimination of the second propagation constant with sufficient amine hydrochloride suggests the possibility that this kind of contamination may be responsible for the suppression of the second propagation rate in their work. Breitenbach and Allinger⁵ have studied the polymerization of DL-phenylalanine N-carboxy-anhydride initiated by a diethylamide, and reported only a single propagation rate.

The kinetically simpler behavior of both of these investigations corresponds to the reaction scheme described in the introduction, and its adequacy has been generally accepted. However, the ultimate test of any mechanism of polymerization lies in its ability to predict the molecular weight distribution obtained. This test has not been employed in earlier investigations but clear evidence of the failure of the single propagation rate mechanism is contained in the work of Blout and Karlson⁹ who found that the DP_w did not vary in the predicted manner with the anhydride-initiator ratio. The demonstration of the existence of a broad molecular weight distribution¹⁰ then became the basis of the starting point of this investigation.

Whether the contrast between the experimental findings of this work and that of other investigators is a function of the particular amino acid used, its non-racemic nature, the purity of the anhydride, or other undetermined factors, is not clear. *However, the consistent reproduction of the results reported herein, the abnormally broad molecular weight distribution, the suppression of this anomaly by careful selection of solvent, or by deliberate addition of an impurity, and the infrared spectral studies on both the rate and products¹⁵ provide clear and consistent support for the contention that two successive propagation rates do exist for this polymerization.*

The dependence of these propagation rates on the anhydride-initiator ratio leads to the presumption that the two propagation rates are a development of the configurational change occurring in solution. Independent infrared spectral evidence has been presented¹⁸ to support this postulate.

Role of Optical Isomers in the Polymerization.—The effect of the enantiomorph on the rate of the polymerization has been investigated very little. The evidence presented above demonstrates the presence of a rather large selectivity in the reaction. Therefore the product of the polymerization of racemic N-carboxy-anhydrides is not a polymer composed of randomly mixed D- and L-peptide residues, but approaches very closely that of a block

copolymer. The length of the blocks depends on the particular selectivity of the monomer investigated and on the type of initiation.²⁰ For γ -benzyl-DL-glutamate anhydride a polymer in the helical configuration composed of a single optical isomer reacts about 5 times faster with its own optical isomer than with the opposite enantiomorph.

The rise in optical rotation when a racemic anhydride is initiated by L-polymer offers convincing evidence for the existence of a single screw sense for the helical chain composed of a single optical isomer. By subtracting the optical rotation of the initiating polymer the optical rotary contribution of a racemic helix having the screw sense of the helix composed of L-residues has been obtained and described.¹⁹

The change of optical rotation when D-anhydride is initiated by L-polymer affords an interesting insight into the stability of the α -helix. The initial rise in optical rotation indicates that a number of D-residues are able to assume the configurational requirements of a helix characteristic of L-residues. However, the succeeding decrease in rotation demonstrates that this is a relatively unstable configuration. If the eventual breakup of the helical configuration is assumed to be due to side chain steric repulsion, it can be shown that maximum repulsion is attained when the L-peptide residues are in a right-handed helical sense. In this structure the side chains are directed along the chain such that L- and D-groups would interfere when a block polymer of L and D-residues is prepared. The existence of a left-handed helix for L-residues would result in the side chains being directed in opposite directions for the two residues with a consequent loss of interference. Therefore the helical breakup affords additional indirect evidence that L-peptide residues exist in a helix of right-handed screw sense as first suggested by Perutz.²¹

Acknowledgment.—We are pleased to acknowledge as a gift the N-carboxy-anhydrides used herein from Dr. E. R. Blout and Mr. Roy Karlson. We also would like to acknowledge the help and advice of Dr. J. T. Yang and Mr. J. C. Mitchell, one of whom (J.C.M.) was responsible for many of the molecular weight measurements reported. Financial support was provided by the Office on Naval Research (N5ori-07654).

(20) A more extreme selectivity has been noted in polymerizations initiated by methoxide ion by E. R. Blout and M. Idelson, *This Journal*, **78**, 3857 (1956).

(21) M. F. Perutz, *Ann. Repts. on Prog. Chem. (Chem. Soc. London)*, **48**, 361 (1951).