[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, WEIZMANN INSTITUTE OF SCIENCE]

# Amine Initiated Copolymerization of N-Carboxy-α-amino Acid Anhydrides[1]

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RECEIVED MAY 14, 1959

Kinetic data have been obtained for the diethylamine initiated homopolymerizations of ε,N-carbobenzoxy-α,N-carboxy-L-lysine anhydride (L) and γ-benzyl-N-carboxy-L-glutamate anhydride (G) in dimethylformamide (DMF). On copolymerizing these monomers it was found that the conversion rate in copolymerization is practically the sum of the conversion rates of the individual monomers when polymerized singly, using the same amounts of solvent and initiator. Compositional analysis shows that the more reactive monomer, G, is incorporated into the growing peptide chains about 2.5 times more rapidly than the other monomer, L. From these results it is concluded that in the bimolecular propagation reaction only the monomer, and not the nature of the active end of the growing chain, determines the copolymerization rate. Other pairs of N-carboxy-α-amino acid anhydrides were found to copolymerize in a mechanism similar to that described for L and G. Based on these findings the chain length distribution and the compositional distribution of the copolymers were calculated.

The synthesis of a considerable number of amino acid copolymers from the corresponding N-carboxy-α-amino acid anhydrides (NCAs) has been described in the literature.[3] No detailed experimental analysis of the kinetics of copolymerization, however, is available. Since the mechanism and kinetics of the primary and secondary amine initiated homopolymerization of these monomers has been satisfactorily clarified,[4] a study of the amine initiated NCAs-copolymerization was undertaken. The copolymerization experiments to be reported were performed in dimethylformamide (DMF) using diethylamine as initiator. DMF was chosen as solvent, since the homopolymerization of NCAs in this medium was found to be first order with respect to monomer throughout the reaction.[5] Furthermore, as DMF is a good solvent for polyamides, the copolymerization could be performed in homogeneous phase.

A detailed analysis of the kinetics of the copolymerization of γ-benzyl-N-carboxy-L-glutamate anhydride (G) and ε,N-carbobenzoxy-α,N-carboxy-L-lysine anhydride (L) was carried out. The copolymers formed at the different stages of the reaction were isolated and analyzed for their amino acid composition. General conclusions as to the mechanism of copolymerization were deduced. Finally it was possible to calculate the expected molecular weight distribution as well as the composition distribution of the copolymers obtained. For comparison the kinetics of copolymerization of other pairs of NCAs was also included.

## Experimental

**N-Carboxy-α-amino Acid Anhydrides.**—The NCAs of ε,N-carbobenzoxy-L-lysine,[6] γ-benzyl-L-glutamate,[6] L-phenylalanine,[7] glycine,[8] DL-alanine[8] and sarcosine[9] were prepared according to the literature. The monomers were recrystallized from ethyl acetate and petroleum ether. A theoretical neutralization equivalent was obtained on titration with sodium methoxide.[10]

Kinetic experiments were performed only with NCAs containing less than 0.1% Cl.

**Kinetic Method.**—The following is a typical kinetic polymerization experiment: The monomer ($5 \times 10^{-4}$ mole) was dissolved in 10 ml. of freshly distilled dimethylformamide. The temperature of the solution was brought to 25° in a thermostat. One ml. of 0.025 *M* diethylamine in DMF was added and the rate of polymerization followed by measuring the amount of $CO_2$ evolved. Carbon dioxide-free nitrogen was bubbled through the reaction mixture to transfer the $CO_2$ formed into a 10% solution of benzylamine in ethanol (20 ml.).[11] The carbamic acid derivative formed was titrated with 0.1 *N* sodium methoxide using thymol blue as indicator.[12]

**Isolation and Analysis of ε,N-Carbobenzoxy-L-lysine–γ-Benzyl-L-glutamate Copolymers.**—The composition of the copolymers of ε,N-carbobenzoxylysine and γ-benzyl-glutamate in a given polymerization mixture, at any instant, was determined as follows: Copolymer precipitation and decomposition of excess monomer was effected by adding aqueous 3 *N* hydrochloric acid (2 ml.) to an aliquot (2 ml.) of the polymerization mixture. The precipitate obtained was filtered and washed with 1 *N* hydrochloric acid (20 ml.). Further purification was obtained by dissolution of the copolymer in dimethylformamide (2 ml.) and reprecipitation with 3 *N* hydrochloric acid (2 ml.). The final precipitate (~50 mg.) was filtered, washed, dried and decarbobenzoxylated and debenzylated, by treatment with 30% HBr in glacial acetic acid (2 ml.), for 6 hours at room temperature.[13] Precipitation of the lysine–glutamic acid copolymer liberated was brought to completion by the addition of ether (10 ml.). The precipitate was filtered, dried and hydrolyzed with 3 *N* hydrochloric acid (2 ml.) in a sealed test-tube at 100° for 24 hours. The hydrolyzate was dried in a desiccator over sulfuric acid and sodium hydroxide and analyzed chromatographically for lysine and glutamic acid.[14]

The estimation of N-terminal amino acids of the copolymers of ε,N-carbobenzoxy-L-lysine and γ-benzyl-L-glutamate was performed as follows: The polymers (10 to 50 mg.) isolated from the reaction mixture as above, were coupled with 2,4-dinitrofluorobenzene ($5 \times 10^{-5}$ mole in 0.25 ml. of ethanol) in 1 ml. of DMF containing 1% (v./v.) triethylamine. The reaction mixture was left at room temperature overnight and the dinitrophenylated polymers were precipitated with 2 ml. of 3 *N* HCl and washed with ethyl ether. They were hydrolyzed in 6 *N* HCl at 105° for 24 hours, and the hydrolyzates analyzed for

(1) For a preliminary note *cf.* Y. Shalitin and E. Katchalski, *Bull. Res. Council Israel*, **7A**, 115 (1958).

(2) This paper is part of a thesis presented by Y. Shalitin to the Hebrew University, Jerusalem, in partial fulfillment of the requirements of the degree of Ph.D.

(3) C. H. Bamford, A. Elliott and W. E. Hanby, "Synthetic Polypeptides," Academic Press, Inc., New York, N. Y., 1956; E. Katchalski and M. Sela, *Adv. Protein Chem.*, **13**, 243 (1958).

(4) S. G. Waley and J. Watson, *Proc. Roy. Soc.* (*London*), **A199**, 499 (1949); D. G. H. Ballard and C. H. Bamford, *ibid.*, **A223**, 495 (1954); Y. Shalitin, Ph.D. Thesis, Hebrew University, Jerusalem, 1958.

(5) R. D. Lundberg and P. Doty, THIS JOURNAL, **79**, 3961 (1957).

(6) E. Katchalski, "Methods in Enzymology," edited by S. P. Colowick and N. O. Kaplan, Vol. III, Academic Press, Inc., New York, N. Y., 1957, p. 540.

(7) M. Sela and A. Berger, THIS JOURNAL, **77**, 1893 (1955).

(8) A. C. Farthing, *J. Chem. Soc.*, 3213 (1950).

(9) D. Coleman, *ibid.*, 3222 (1950).

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

(11) A. Patchornik and Y. Shalitin, *Bull. Res. Council Israel*, **5A**, 300 (1956).

(12) J. S. Fritz and N. M. Lisicki, *Anal. Chem.*, **23**, 589 (1951).

(13) D. Ben Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(14) R. E. Kay, D. C. Harris and C. Entenman, *Arch. Biochem. and Biophys.*, **63**, 14 (1956).

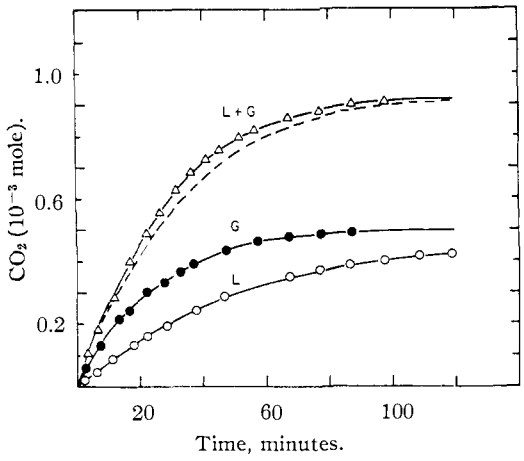


Fig. 1.—Carbon dioxide evolution as a function of time for the diethylamine initiated polymerization of the following NCAs in DMF at 25°: Curve L, ε,N-cbzo-L-lysine-NCA ($5 \times 10^{-4}$ mole); curve G, γ-benzyl-L-glutamate-NCA ($5 \times 10^{-4}$ mole); curve L + G, ε,N-cbzo-L-lysine-NCA ($5 \times 10^{-4}$ mole) and γ-benzyl-L-glutamate-NCA ($5 \times 10^{-4}$ mole). All the polymerization reactions were carried out in 10 ml. of DMF, using $4 \times 10^{-5}$ mole of initiator. The dotted curve is the sum of curves L and G.

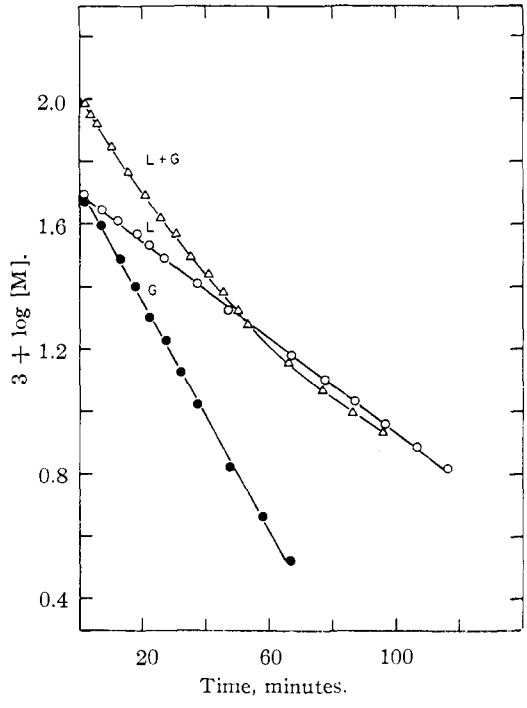


Fig. 2.—Plots of log [M] as a function of time for the cases specified in Fig. 1; [M] is the concentration of intact monomer in moles $l.^{-1}$.

DNP-glutamic acid and α,N-DNP-lysine according to Levy.[15]

**Paper Electrophoresis of Lysine-Glutamic Acid Copolymers.**—Paper electrophoresis experiments with lysine-glutamic acid copolymers, derived from the corresponding ε,N-carbobenzoxylysine-γ-benzylglutamate copolymers, prepared as described above, were carried out on Whatman No. 1 paper, in phosphate buffer, *p*H 7.8, ionic strength 0.28, at a potential gradient of 10 volt/cm. The copolymer spots were detected on the paper by spraying with ninhydrin.

(15) A. L. Levy, *Nature*, **174**, 126 (1954).

## Results

**Polymerization of ε,N-Carbobenzoxy-L-lysine-NCA (L) and of γ-Benzyl-L-glutamate-NCA (G).**—Figure 1, curve L, gives the rate of carbon dioxide evolution in a representative polymerization experiment, with L at 25°. A straight line was obtained when the logarithm of the concentration of the unreacted monomer, log [M], was plotted *versus* time (Fig. 2, curve L). The course of the polymerization may thus be described by a first-order reaction

$$-d[M]/dt = k_{obs}[M] \qquad (1)$$

where [M] denotes the concentration of monomer. Since $k_{obs}$ is constant throughout the polymerization reaction it may be assumed that under the experimental conditions given no termination reaction[7,16] occurs. The absence of such a reaction was proved by the finding that in the final reaction mixtures the amount of terminal amino groups, as determined by titration with perchloric acid in dioxane using thymol blue as indicator,[7] was equivalent to the amount of diethylamine used as initiator.

The coefficient, $k_{obs}$, was found to be proportional to the concentration of the initiator, $[I]_0$ (see Fig. 3), in experiments in which the initial monomer concentration was kept constant. In these ex-

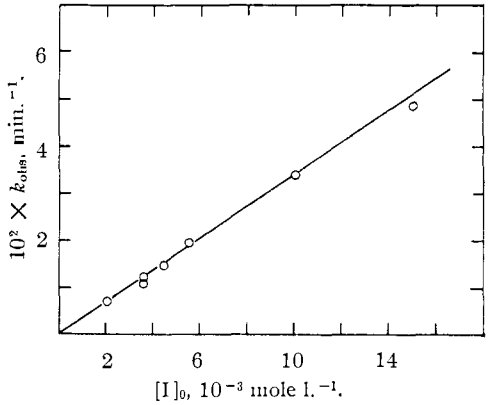


Fig. 3.—The observed first-order coefficient, $k_{obs}$ (min.$^{-1}$), for the homopolymerization of ε,N-cbzo-L-lysine-NCA (0.05 *M*) as a function of the concentration of diethylamine, $[I]_0$, in DMF at 25°.

periments, in which $[M]_0/[I]_0$ varied between 25 to 4, the plot of log [M] *versus* *t* yielded straight lines cutting the ordinate at log $[M]_0$. This shows that the rate of initiation is practically equal to that of propagation, and that both reactions may be represented by the same specific rate constant *k*. The polymerization in the above experiments may therefore be described by (2).

$$-d[M]/dt = k[I]_0[M] \qquad (2)$$

It should be noted, however, that when the initiator concentration, $[I]_0$, was kept constant and the initial monomer concentration, $[M]_0$, was varied, the specific rate constant, *k*, increased slightly with $[M]_0$ (see Fig. 4). A tenfold increase in $[M]_0$ (from 0.0125 to 0.15 *M*) caused only a 50% increase in *k* (from $k = 2.7$ to 4 $l.$ mole$^{-1}$ min.$^{-1}$).

(16) E. Katchalski, Y. Shalitin and M. Gehatia, THIS JOURNAL, **77**, 1925 (1955).

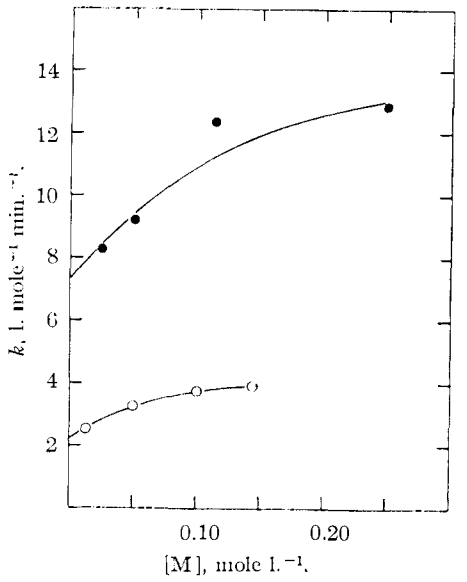


Fig. 4.—The specific rate constant, $k$ (l. mole$^{-1}$ min.$^{-1}$), for the homopolymerizations of ε,N-cbzo-L-lysine NCA (O) and of γ-benzyl-L-glutamate-NCA (●) as a function of monomer concentration; polymerization initiated by 0.004 $M$ diethylamine in DMF at 25°.

Parallel experiments with G revealed a similar behavior to that of L. The course of a representative polymerization experiment performed with this monomer is given in Figs. 1 and 2, curves G. The variation of the specific rate constant with $[M]_0$ is given in Fig. 4.

A comparison of the rates of polymerization of G and L, under similar conditions, shows that the former polymerizes approximately 2.5–3 times faster than the latter.

**Copolymerization of the NCAs of ε,N-Carbobenzoxy-L-lysine (L) and γ-Benzyl-L-glutamate (G).**—The course of copolymerization of a mixture composed initially of $5 \times 10^{-4}$ mole of L, $5 \times 10^{-4}$ mole of G and $4 \times 10^{-5}$ mole of diethylamine per 10 ml. of DMF is given in Fig. 1, curve L + G. For comparison the rate of polymerization of the separate monomers, L and G, at the corresponding concentrations, and the same amount of initiator, is also given. The figure shows that the course of the copolymerization may be represented approximately by the sum of the curves describing the polymerization of the individual component monomers. The copolymerization proceeds at a somewhat faster rate than that derived from the rates of the separate homopolymerization reactions, most likely as a result of the higher concentration of monomers in the copolymerization mixture. It is of interest to note that the rate coefficient of copolymerization, $k_{cop}$, given by the expression

$$-d[L + G]/dt = k_{cop}[I]_0[L + G] \qquad (3)$$

and derived from the slope of the curve L + G in Fig. 2, at the initial stages of the reaction equals $(k_L + k_G)/2$ where $k_L$ and $k_G$ denote the specific rate constants of polymerization of L and G respectively. At the later stages of the copolymerization, $k_{cop}$ decreases and its value approaches that of $k_L$.

In experiments similar to those given in Fig. 1, which were performed at different $[L]_0$ and $[G]_0$ (see Table I), it was found that the total monomer

TABLE I

SPECIFIC RATE CONSTANTS OF HOMOPOLYMERIZATION AND COPOLYMERIZATION OF ε,N-CARBOBENZOXY-α,N-CARBOXY-L-LYSINE ANHYDRIDE (L) AND γ-BENZYL-N-CARBOXY-L-GLUTAMATE ANHYDRIDE (G)[a]

| $[L]_0$, mole l.$^{-1}$ | $k_L$, l. mole$^{-1}$ min.$^{-1}$ | $[G]_0$, mole l.$^{-1}$ | $k_G$, l. mole$^{-1}$ min.$^{-1}$ | $k_{cop}$, $t = 0$, l. mole$^{-1}$ min.$^{-1}$ Exptl. | Calcd.[b] |
|---|---|---|---|---|---|
| 0.025 | 3.3 | 0.25 | 13 | 12.5 | 12 |
| .05 | 3.5 | .05 | 10.5 | 7.5 | 7.0 |
| .20 | 3.8 | .02 | 8.5 | 4.4 | 4.2 |

[a] All the polymerizations were initiated with 0.0035 $M$ diethylamine and performed in dimethylformamide at 25°. [b] The calculated copolymerization rate constant was derived from eq. 4.

consumption in the various copolymerization reactions could be described by the sum of the consumptions of [L] and [G] in the corresponding two homopolymerization experiments containing the same initial concentrations of monomer $[L]_0$ or $[G]_0$ and of initiator $[I]_0$. Furthermore, the initial value of $k_{cop}$ in these experiments (see Table I) was given to a good approximation by the equation

$$k_{cop} = \frac{k_G[G]_0 + k_L[L]_0}{[G]_0 + [L]_0} \qquad (4)$$
$$t = 0$$

**Composition of Glutamic Acid–Lysine Copolymers Obtained at Low Conversion.**—In order to determine the effect of the component monomer concentrations ($[L]_0$ and $[G]_0$) on the composition of the copolymers formed at the initial stages of the copolymerization, the following set of experiments was carried out. Mixtures containing a total monomer concentration, $[L]_0+[G]_0$, of $10^{-3}$ mole per 10 ml. of DMF, but different $[G]_0/[L]_0$ ratios, were polymerized in the presence of diethylamine $[I]_0 = 5 \times 10^{-5}$ mole. The copolymers formed at about 20% conversion were precipitated with concentrated hydrochloric acid, decarbobenzoxylated and debenzylated, and hydrolyzed as described in the Experimental part. The relative amounts of lysine and glutamic acid detected in the hydrolyzates are given in Table II. The data re-

TABLE II

COMPOSITION OF GLUTAMIC ACID-LYSINE COPOLYMERS FORMED AT 20% CONVERSION[a]

| Initial monomer mixture | | Copolymer composition | |
|---|---|---|---|
| $X_G$[b] | $[G]_0/[L]_0$[c] | $X'_G$[b] | $[Glu]/[Lys]_{(poly)}$[c] |
| 0.2 | 0.25 | 0.35 | 0.55 |
| .4 | 0.67 | .60 | 1.53 |
| .6 | 1.50 | .76 | 3.10 |
| .8 | 4.0 | .90 | 8.9 |

[a] All the initial copolymerization mixtures contained a total amount of $10^{-3}$ mole monomer per 10 ml. of DMF. The polymerization was initiated with $5 \times 10^{-5}$ mole of diethylamine and performed at 25°. [b] $X_G$ denotes the initial molar fraction of γ-benzyl-N-carboxy-L-glutamate anhydride, G, in the polymerization mixture, while $X'_G$ gives the molar fraction of the glutamic acid residue in the initial copolymer formed. [c] $[G]_0/[L]_0$ gives the initial ratio between the corresponding monomers, while $[Glu]/[Lys]_{(poly)}$ gives the ratio between the corresponding residues in the intial copolymer formed.

ported demonstrate that the ratio between the glutamic acid to lysine residues in all the copolymers investigated is approximately 2 to 2.5 times greater than the molar ratio between $[G]_0$ and $[L]_0$ in the initial copolymerization mixture. These findings demonstrate clearly that at equal molar concentrations the probability of G to react with a growing chain is about 2.5 times greater than that of L. The composition of the copolymers obtained after 20% conversion as a function of initial monomer ratio is represented graphically in Fig. 5.

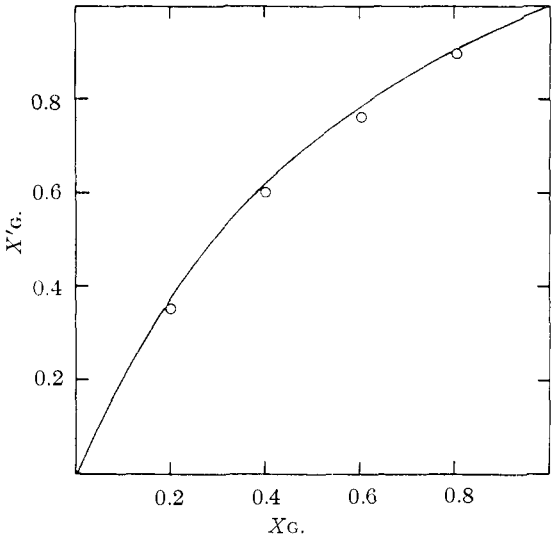


Fig. 5.—Molar fraction of glutamic acid residues in glutamic acid–lysine copolymers, $X'_G$, obtained after 20% conversion, as a function of molar fraction of glutamate component in initial monomer mixture, $X_G$. The conditions of copolymerization are specified in Table II. The full curve gives the values of $X'_G$ calculated according to eq. 8.

**Change in Composition of the Glutamic Acid–Lysine Copolymer during Polymerization.**—As G reacts with the growing peptide chains considerably faster than L, the relative amount of the free lysine monomer will increase during the copolymerization. It could therefore be expected that the relative amount of the lysine residues in the Glu–Lys copolymer formed will also increase with time. Such an increase was demonstrated experimentally in three copolymers derived from different initial $[G]_0/[L]_0$ mixtures (see Table III).

TABLE III

MOLAR RATIO OF GLUTAMIC ACID TO LYSINE RESIDUES IN COPOLYMERS ISOLATED FROM THE POLYMERIZATION MIXTURE AT DIFFERENT TIME INTERVALS

| Initial molar monomer ratio $[G]_0/[L]_0$[a] | Time, min. 15 | 30 | 45 | 75 | 300 |
|---|---|---|---|---|---|
| 0.67 | 1.56 | 1.50 | 1.41 | 1.16 | 0.77 |
| 1.50 | 3.12 | 3.00 | 2.50 | 2.40 | 1.66 |
| 4.0 | 9.00 | 7.70 | 6.66 | 5.00 | 4.30 |

[a] Copolymerizations were performed in 10 ml. of DMF at 25°. The reaction mixtures contained $2 \times 10^{-5}$ mole of diethylamine, and a total amount of $10^{-3}$ mole of monomer.

The significant difference in composition of copolymers derived from L and G copolymerization at the beginning and toward the end of the reaction could also be illustrated by an analysis of the electrophoretic behavior of the peptides obtained after decarbobenzoxylation and debenzylation. Thus a Glu–Lys polypeptide derived from a copolymer obtained, at low conversion (20%), from a polymerization mixture of G ($4 \times 10^{-4}$ mole), L ($6 \times 10^{-4}$ mole) and diethylamine ($4 \times 10^{-5}$ mole), in 10 ml. of $DMF_1$ moved toward the anode at *p*H 7.8 and a potential gradient of 10 volt/cm., while the Glu–Lys polypeptide derived from the same polymerization mixture at the end of the reaction, moved, under similar conditions, toward the cathode (see Fig. 6).

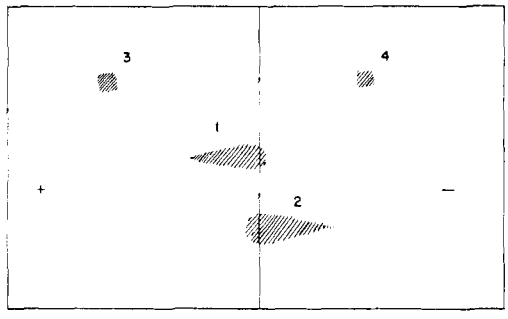


Fig. 6.—Electrophorogram of the initial and final glutamic acid–lysine copolymer: 1, copolymer obtained after 20% conversion; 2, copolymer obtained after complete reaction; 3, glutamic acid; 4, lysine. For details see text.

Additional proof for the change in composition of the polypeptides was given by the estimation of their N-terminal glutamic acid and lysine residues by the dinitrophenylation method described in the Experimental part. Copolymers isolated at 25, 50 and 93% conversion from a polymerization mixture which contained initially $5 \times 10^{-4}$ mole of G, $5 \times 10^{-4}$ mole of L and $2 \times 10^{-5}$ mole of diethylamine in 10 ml. of DMF, possessed N-terminal glutamic acid and lysine residues in a molar ratio of 3.0, 1.3 and 0.22, respectively.

**Copolymerization of Other Pairs of N-Carboxy-α-amino Acid Anhydrides.**—Copolymerization experiments performed with L-phenylalanine-NCA and ε,N-cbzo-L-lysine-NCA, L-phenylalanine-NCA and γ-benzyl-L-glutamate-NCA, glycine-NCA and ε,N-cbzo-L-lysine-NCA, ε,N-cbzo-L-lysine-NCA and DL-alanine-NCA, or γ-benzyl-L-glutamate-NCA and DL-alanine-NCA, in dimethylformamide using diethylamine as initiator revealed that the course of copolymerization of these monomer pairs was similar to the copolymerization of ε,N-cbzo-lysine-NCA and γ-benzyl-L-glutamate-NCA. The rate of copolymerization in all cases equalled the sum of the rates of homopolymerization of the corresponding, individual monomers (see Table IV).

In copolymerization experiments performed in 10 ml. of DMF at 25° with glycine-NCA ($4 \times 10^{-4}$ mole, $k = 40$ l.mole$^{-1}$min.$^{-1}$) and γ-benzyl-L-glutamate-NCA ($6 \times 10^{-4}$ mole, $k = 10$ l.mole$^{-1}$ min.$^{-1}$) or sarcosine-NCA ($5 \times 10^{-4}$ mole, $k = 7$ l.mole$^{-1}$min.$^{-1}$) and γ-benzyl-L-glutamate-NCA ($5 \times 10^{-4}$ mole), using diethylamine ($5 \times 10^{-5}$ mole), as initiator, it was noticed, however, that the copolymerization proceeds in the later stages of the reaction at a considerably lower rate than

TABLE IV

SPECIFIC RATE CONSTANTS OF HOMOPOLYMERIZATION AND COPOLYMERIZATION OF PAIRS OF NCAs (A AND B)[a]

| NCA of: | $[A]_0$, mole l.$^{-1}$ | $k_A$, l. mole$^{-1}$ min.$^{-1}$ | NCA of: | $[B]_0$, mole l.$^{-1}$ | $k_B$, l. mole$^{-1}$ min.$^{-1}$ | $k_{cop}$, $t = 0$, l. mole$^{-1}$ min.$^{-1}$ Exptl. | Calcd.[b] |
|---|---|---|---|---|---|---|---|
| L-Phenylalanine | 0.05 | 11.5 | ε-cbzo-L-lysine | 0.05 | 3.5 | 8 | 7.5 |
| L-Phenylalanine | .05 | 11.5 | γ-benzyl-L-glutamate | .05 | 10 | 10.5 | 11.2 |
| Glycine | .33 | 40 | ε-cbzo-L-lysine | .067 | 4 | 16 | 16 |
| DL-Alanine | .05 | 6 | ε-cbzo-L-lysine | .05 | 4 | 5.5 | 5 |
| DL-Alanine | .05 | 6 | γ-benzyl-L-glutamate | .05 | 11 | 9 | 8.5 |

[a] All the polymerizations were initiated with 0.004 $M$ diethylamine and performed in DMF at 25°. [b] The calculated copolymerization constants were derived from eq. 4.

that expected from the sum of the homopolymerization rates (see Fig. 7).

## Discussion

**Analysis of the Kinetics of Copolymerization.**—As stated above, the data on the homopolymerizations of G and L may be represented by eq. 2 which was derived for a NCA-polymerization devoid of a termination reaction when the specific rate of propagation equals that of initiation.[16]

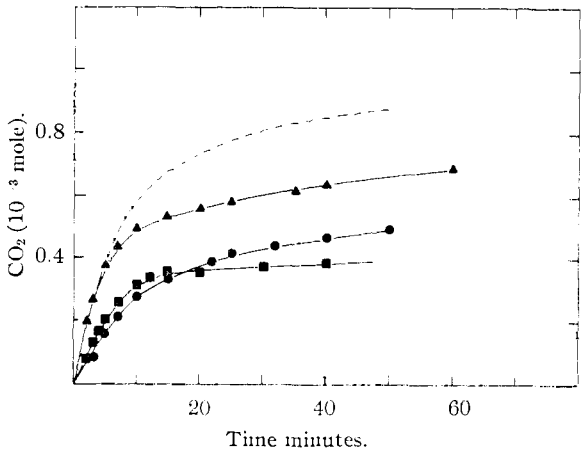


Fig. 7.—Carbon dioxide evolution as a function of time for the diethylamine initiated polymerization of the following NCAs in DMF at 25°: ■—■, glycine-NCA (4 × $10^{-4}$ mole); ●—●, γ-benzyl-L-glutamate-NCA (6 × $10^{-4}$ mole); ▲—▲, glycine-NCA (4 × $10^{-4}$ mole) and γ-benzyl-L-glutamate-NCA (6 × $10^{-4}$ mole). The dotted curve is the sum of curves ■—■ and ●—●. All the polymerization reactions were carried out in 10 ml. of DMF, using 5 × $10^{-5}$ mole of initiator.

The values found for the specific rate constants of homopolymerization of the various NCAs tested are given in Table I. A comparison of the course of copolymerization of G and L with that of the corresponding homopolymerization reactions (see Figs. 1 and 2) shows that the rate of copolymerization, $-d[G+L]/dt$, may be represented to a good approximation, by

$$-d[G + L]/dt = k_G[I]_0[G] + k_L[I]_0[L] \quad (5)$$

or

$$-d[G + L]/dt = k_{cop}[I]_0[G + L]$$

where the coefficient

$$k_{cop} = \frac{k_G[G] + k_L[L]}{[G] + [L]}$$

In eq. 5 it is assumed, in agreement with experiment, that the rate of copolymerization at any instant is given by the sum of the propagation rates of the individual monomers when polymerized at the corresponding monomer and initiator concentrations. The rate of consumption of the individual monomers in the copolymerization reaction may therefore be represented by

$$-d[G]/dt = k_G[I]_0[G]; \quad [G] = [G]_0 \exp(-k_G[I]_0 t) \quad (6)$$
$$-d[L]/dt = k_L[I]_0[L]; \quad [L] = [L]_0 \exp(-k_L[I]_0 t)$$

Formulas 6 are similar to those describing the respective homopolymerization reactions.

Copolymerization reactions are generally governed by the four different propagation constants $k_{11}$, $k_{12}$, $k_{21}$ and $k_{22}$

$$\sim\!A + A \xrightarrow{k_{11}} \sim\!A$$
$$\sim\!A + B \xrightarrow{k_{12}} \sim\!B$$
$$\sim\!B + A \xrightarrow{k_{21}} \sim\!A$$
$$\sim\!B + B \xrightarrow{k_{22}} \sim\!B$$

determining the rates of the different reactions given above, where ~A and ~B denote propagating chains with terminal A or B residues. Two specific rate constants ($k_L$ and $k_G$), however, suffice to describe the copolymerization of L and G. The kinetics of this copolymerization may therefore be described by the assumption that $k_{11} = k_{21} = k_L$ and $k_{22} = k_{12} = k_G$. The rate of propagation in the copolymerization reaction seems therefore to be determined mainly by the chemical nature of the reacting NCA and not by the nature of the terminal amino acid residue, bearing the reacting free α-amino group, of the growing peptide chain. The rate of reaction between NCAs and primary or secondary amines is usually determined by the basicity of the amine and by the chemical structure of the NCA. The basicity of the terminal α-amino groups of the growing peptide chains does not vary significantly with the chain length or with the character of the N-terminal amino acid residue. On the other hand, Ballard and Bamford[17] have shown that substitution at the α-carbon of the NCA may influence the reactivity of the 5-carbonyl of the oxazolidine-2,5-dione.

The over-all specific rate constant of the copolymerization reaction at the early stages of the reaction, is given by eq. 4 as eq. 5 at $t \to 0$ reduces to

$$\left\{-\frac{d[G + L]}{dt}\right\}_{t\to 0} = \frac{k_G[G]_0 + k_L[L]_0}{[G]_0 + [L]_0}[I]_0\{[G]_0 + [L]_0\} \quad (5a)$$

(17) D. G. H. Ballard and C. H. Bamford, *J. Chem. Soc.*, 355 (1958).

Since $k_G$ is larger than $k_L$, G is consumed faster than L during the copolymerization. The reaction mixture toward the end of the copolymerization will therefore contain only traces of G and a relatively large quantity of L, if these were initially present in equal amounts. The value of $k_{cop}$ at this stage of the reaction will be given by $k_{cop} = k_L$. $t \longrightarrow \infty$

The assumptions made concerning the mechanism of copolymerization permit the calculation of the composition of copolymers obtained at low conversion. At the initial stages of the copolymerization the ratio between the amounts $\Delta G'$ and $\Delta L'$ incorporated into the polymeric fraction is given by

$$\frac{\Delta G'}{\Delta L'} = \alpha \frac{[G]_0}{[L]_0} \qquad (7)$$

where $\alpha = k_G/k_L$, and $[G]_0$ and $[L]_0$ denote the initial concentrations of the monomers. The factor $\alpha$ thus designates the relative enrichment of the polymeric fraction with respect to the more reactive monomer. Equation 7 may readily be transformed to

$$X_G' = \frac{\alpha X_G}{1 + (\alpha - 1)X_G} \qquad (8)$$

where $X_G$ denotes the molar fraction of the G monomer in the initial polymerization mixture, while $X'_G$ gives the molar fraction of the G residues in the copolymer obtained at the initial stages of the copolymerization. Figure 5 gives the theoretical curve derived from eq. 8 assuming $\alpha = 2.5$. The experimental data (see Table II) concerning the composition of the copolymers formed in the early stages closely fit those expected.

In the type of copolymerization here considered the composition of the copolymers formed varies with time. The ratio $[G]'/[L]'$ between the Glu and the Lys residues of the copolymer as a function of time and initial monomer composition is given in eq. 9 as derived from the equations 6.

$$\frac{[G]'}{[L]'} = \frac{[G]_0}{[L]_0} \times \frac{1 - \exp(-k_G[I]_0 t)}{1 - \exp(k_L[I]_0 t)} \qquad (9)$$

The expected change with time in the composition of a growing copolymer derived from an initial $[G]_0/[L]_0 = 0.67$ monomer mixture, assuming for $k_G$ and $k_L$ the values 10 l. mole$^{-1}$min.$^{-1}$ and 4 l. mole$^{-1}$min.$^{-1}$, respectively, is given in Fig. 8. The experimental data obtained fit closely those calculated.

**Molecular Weight Distribution.**—In a previous paper[16] it was demonstrated that when the polymerization of NCAs is initiated by primary or secondary amines and no termination reaction takes place, polymers with a Poissonian chain length distribution are obtained. Below, it will be shown that a similar chain length distribution is to be expected in amino acid copolymers derived from a reaction mixture in which the rate of propagation is determined by the specific rate constants $k_G$ and $k_L$ defined previously.

Equation 10, describing the rate of initiator consumption

$$-d[I]/dt = [I]\{k_G[G] + k_L[L]\} \qquad (10)$$

may be rewritten in the form

$$-d[I]/d\nu = k[I] \qquad (10a)$$

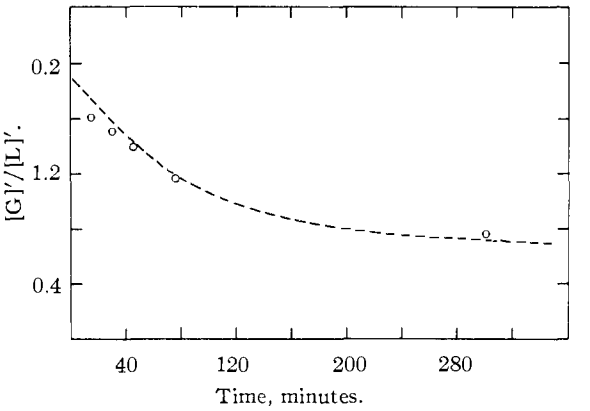


Fig. 8.—The composition $[G]'/[L]'$ of the copolymer formed as a function of copolymerization time. Initial monomer mixture contained ε,N-cbzo-L-lysine-NCA ($6 \times 10^{-4}$ mole) and γ-benzyl-L-glutamate-NCA ($4 \times 10^{-4}$ mole) in 10 ml. of DMF. The polymerization was initiated by diethylamine ($2 \times 10^{-5}$ mole) and performed at 25°. The circles give the experimental findings, while the dashed curve represents the expected values according to eq. 9 assuming $k_G = 10$ l. mole$^{-1}$ min.$^{-1}$ and $k_L = 3.5$ l. mole$^{-1}$ min.$^{-1}$.

where

$$k d\nu = k_G[G]dt + k_L[L]dt \qquad (11)$$

or

$$k\nu = k_G \int_0^t [G]dt + k_L \int_0^t [L]dt \qquad (11a)$$

Integration of (10a) gives

$$[I] = [I]_0 e^{-k\nu} \qquad (12)$$

The concentration $[N_j]$ of growing chains composed of $j$ amino acid residues varies with time according to eq. 13

$$d[N_j]/dt = k_G[N_{j-1}][G] + k_L[N_{j-1}][L] - k_G[N_j][G] - k_L[N_j][L] \qquad (13)$$

which on introduction of eq. 11 gives

$$d[N_j]/d\nu = k[N_{j-1}] - k[N_j] \qquad (14)$$

This procedure is justified in the present case since the specific initiation rate constant is close to that of propagation (see above).

Successive solution of the last set of equations gives

$$[N_j] = [I]_0 \frac{(k\nu)^j}{j!} e^{-k\nu} \qquad (15)$$

The last equation shows that the chain length distribution of the polymer formed is Poissonian, and thus resembles the distribution obtained in a homopolymerization of NCAs, in the absence of a termination reaction.[16] A similar conclusion was derived by Gold.[18]

From eq. 15 it follows that the number average degree of polymerization, $DP_n$, approximately equals the weight average degree of polymerization, $DP_w$, and is given by eq. 16.[16]

$$DP_w = DP_n = k\nu \qquad (16)$$

**Compositional Distribution.**—By a statistical calculation analogous to that of Katchalski, *et al.*[19]

(18) L. Gold, *J. Chem. Phys.*, **21**, 1190 (1953).

(19) E. Katchalski, M. Gehatia and M. Sela, THIS JOURNAL, **77**, 6175 (1955).

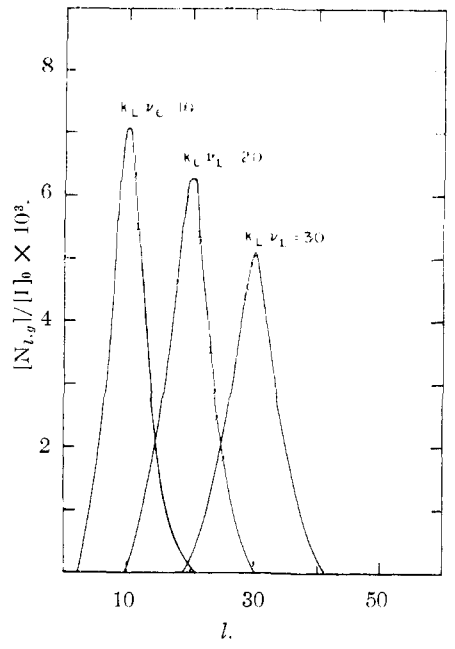


Fig. 9.—The relative number of chains containing $l$ lysine residues and $g$ glutamate residues $[N_{l,g}]/[I]_0$ ($l + g = 60$), in copolymers obtained by the copolymerization of L and G at a monomer to initiator ratio $([L]_0 + [G]_0)/[I]_0 = 60$. The given curves were calculated according to eq. 17 for $k_L\nu_L = 10$, 20 and 30.

(see their eqs. 1 to 9) for the molecular weight distribution of homopolyamino acids, it can readily be shown that the concentration $[N_{l,g}]$ of copolymer molecules composed of $l$ residues of the amino acid L and $g$ residues of the amino acid G is given by

$$[N_{l,g}] = [I]_0 \frac{(k_L\nu_L)^l}{l!} \frac{[k_G\nu_G]^g}{g!} \exp(-k_L\nu_L - k_G\nu_G) \quad (17)$$

where

$$\nu_L = \int_0^t [L]dt \text{ and } \nu_G = \int_0^t [G]dt$$

The expression obtained for $N_{l,g}$ may also be derived by successive solution of the following set of kinetic equations describing the rate of formation of peptide chains composed of $l$ lysine residues and $g$ glutamic acid residues.

$$\frac{d[N_{l,g}]}{dt} = k_L[N_{(l-1),g}][L] + k_G[N_{l,(g-1)}][G] - k_L[N_{l,g}][L] - k_G[N_{l,g}][G] \quad (18)$$

The total concentration $[N_j]$ of copolymer chains composed of $j$ amino acid residues may be derived from eq. (17) on summation over $l$ values from 0 to $j$, for the case $j = l+g$. The expression thus obtained is identical with that given in eq. 15.

The concentration $[N_l]$ of molecules containing $l$ residues of amino acid L, irrespective of the number of residues of amino acid G, is given by

$$[N_l] = \sum_{g=0}^{\infty} [N_{l,g}] = [I]_0 \frac{(k_L\nu_L)^l}{l!} e^{-k_L\nu_L} \quad (19)$$

Equation 19 shows that the Poisson distribution of $N_l$ is the same as that of the corresponding homopolymer.

The distribution of $N_{l,g}$ as a function of $l$ for the case $j = 60$, $g = 60 - l$, and $k_L\nu_L = 10$, 20 or 30 is represented in Fig. 9. The curves given in the figure show that a copolymer fraction with a well defined chain length has a sharp distribution with respect to component amino acid composition. The maximum of $N_{l,g}$ occurs at a value of $l$ at which $l/g = k_L\nu_L/k_G\nu_G$.

The formulas given above for the molecular weight distribution and the compositional distribution derived for the Lys–Glu copolymers obviously apply to the copolymers derived from the other pairs of NCAs which undergo copolymerization at a rate which equals the sum of the rates of the homopolymerization of the corresponding component monomers under similar conditions.

**Acknowledgment.**—This investigation was supported by a research grant (PHS H-2279) from U.S.A. National Institutes of Health, Public Health Service.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, UNIVERSITY OF PITTSBURGH]

# Action of Urea on Tobacco Mosaic Virus[1]

BY ANNE BUZZELL

RECEIVED AUGUST 7, 1959

Six molar urea at 0° degrades tobacco mosaic virus within 20 minutes into apparently intact RNA having sedimentation constant, $S_{20}^w$, 30 and into the smallest subunit of protein with $S_{20}^w$ about 2. Intact monomer virus is less stable than fragments. The monomer is degraded in 2 minutes to rods, measured in electron micrographs, ranging from $^2/_3$ to $^1/_2$ the original length. After 6 minutes these stable intermediates begin to degrade further. Fragments do not degrade appreciably in 6 minutes but are mainly gone in 20.

## Introduction

The mechanism of urea action on tobacco mosaic virus (TMV) has appeared paradoxical in two respects. One paradox is that infectivity of the virus is destroyed by urea[2,3] whereas infectivity of phenol extracted RNA is not.[4] The other paradox is that the virus protein appeared to be

(1) Publication No. 69 of the Department of Biophysics. This research was supported by contract no. AT(30-1)913, Atomic Energy Commission.

(2) W. M. Stanley and M. A. Lauffer, *Nature*, **89**, 345 (1939).

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(4) F. C. Bawden and N. W. Pirie, *J. Gen. Microbiol.*, **17**, 80 (1957).