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STEREOSELECTIVE HYDROLYSIS OF LEUCINE OLIGOMERS

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Abstract—A number of polymers derived from D-, L- and DL-leucine were subjected to partial hydrolysis under comparable conditions, with the finding that the polymers from DL-leucine were hydrolyzed more extensively than those from D- or L-leucine. Leucine polymers containing an excess of one enantiomer were partially hydrolyzed, resulting in an enhancement of the enantiomeric excess in the residual unhydrolyzed polymer and a depletion of the enantiomeric excess in the recovered monomer. These stereoselective hydrolyses are discussed from the viewpoint of the abiotic enhancement of optical activity on the primitive Earth.

The origin of molecular chirality in the biosphere is one of the fundamental problems associated with the origin of life on Earth. Since, with only occasional exceptions, contemporary biological processes utilize only one enantiomeric configuration of amino acids, it is reasonable to assume that complex biotic processes could not have evolved on Earth without a means for the prior stereoselection of such a specific antipodal configuration. A number of abiotic mechanisms for the origin of optical activity have been proposed,[1] and experimental evidence for some of these have been recently obtained using amino acid substrates.[2-5] The enantiomeric excess (percent major minus percent minor enantiomer) in the products from such experiments was typically in the range of only 1–5%, however, rather smaller than might be thought adequate for the direct genesis of a biosphere. Thus subsequent mechanisms for the abiotic amplification of such small enantiomeric excesses would seem essential in an overall scheme for the origin of life.

In 1957 Wald[6] suggested that amplification might occur *via* formation of the α-helical secondary structure of proteins, which "if enhanced by the employment of a single configuration of the amino acids ... should provide a sufficient basis for the selection of one configuration out of a mixture of enantiomorphs." The credibility of Wald's hypothesis was enhanced by the finding that the α-helical structure of poly-γ-benzyl-L-glutamate is progressively weakened when configurational randomness is introduced by substituting D-glutamate units for L-glutamate in the peptide chain.[7]

The actual amplification of enantiomeric inequalities in amino acid mixtures has more recently been demonstrated by polymerization experiments involving their N-carboxyanhydrides (NCA's). Thus the enantiomeric excesses in D ≠ L mixtures of alanine, leucine or γ-benzylglutamate have been enhanced in the polymer during partial polymerization of their NCA's.[8-11] If an enhancement of optical purity can occur during peptide formation by stereoselective polymerization, it is perhaps reasonable to suspect that peptides might also show stereoselectivity during their degradations, e.g. by hydrolysis, pyrolysis or radiolysis. Several preliminary model experiments investigating this question as regards peptide hydrolysis are reported below. Polyleucine was selected as the model substrate since, when optically pure, it is reputed to possess one of the strongest α-helix structures known among the polyamino acids.[12]

RESULTS AND DISCUSSION

Samples of poly-D-, -L-, and -DL-leucine were partially hydrolyzed by heating at 160° in 3:1 2-propanol-6N HCl for 21 hrs, whereupon the amount of monomeric leucine produced by hydrolysis was determined. The results are shown in Table 1.

In all cases ($DP_n \geq 16,30,40-50$) the DL-polymer was hydrolyzed more extensively than the corresponding stereohomogeneous D- or L-oligomer. No particular correlation was apparent between the polymer size (DP_n) and the percent monomer recovered after hydrolysis. As poly-D- or -L-leucine is known to exist in predominately the α-helical conformation[12,13] and since configurational randomness is known to weaken such structures,[7] it seems possible that the increased yield of monomer obtained from DL- *versus* D- or L-oligomers after hydrolysis may be due to the conformational integrity of the latter. In the α-helix, the amide moiety is tightly H- bonded internally along the peptide chain, and would thus seem relatively protected from hydrolyzing agents. The amide groups in the more random DL-polymer, on the other hand, would presumably be more vulnerable to hydrolysis due to less ordered hydrogen bonding. This suggestion is partially substantiated by the fact that deuterium exchange involving the amide proton is slow when γ-benzyl-L-glutamate is in the helical conformation, yet rapid when in the random coil state.[15]

As we were primarily interested in possible prebiotic mechanisms for the amplification of optical activity, the results in Table 1 suggested a more pertinent experiment. A D ≠ L leucine polymer of intermediate enantiomeric excess was partially hydrolyzed and the hydrolysate, as well as the residual unhydrolyzed polymer, were each analyzed for any change in enantiomeric excess. The results are shown in Table 2.

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Table 1. Stereoselectivity during hydrolysis of leucine polymers

Polyleucine, from Leucine of Configuration	DP_{av} [a]	Yield of Leucine Monomer, %[b]
D	16.6	45
L	16.2	41
DL	16.0	55
D	29.9	30
L	28.8	31
DL	29.6	39
D	41.4 ± 3.4[c]	30
L	49.2 ± 0.4	35
DL	40.7 ± 2.5	56

a. Determined by end group analysis of the crude polymer.[14]

b. Defined as 100x (amount of leucine recovered)/(theoretical amount of leucine in peptide)

c. Average and standard deviation of three determinations.

In all cases the hydrolyzed leucine monomer is of lower enantiomeric excess than the initial polymer, while the residual unhydrolyzed polymer shows an enhancement of the original enantiomeric excess. Recognizing that the initial polymer, as here prepared, consists of a distribution of oligomers of various sizes and enantiomeric compositions, one can explain the results in Table 2 by assuming that the peptide components of lower enantiomeric excess have been hydrolyzed more rapidly than the peptides of greater enantiomeric purity. The decrease in the enantiomeric excess between the recovered leucine and initial polymer is not exactly mirrored by the increase of the enantiomeric excess in the unhydrolyzed peptide over the original polymer. This is probably due to the presence of smaller water-soluble peptides resulting from the hydrolysis which are not included in the g.c. analysis of the recovered monomer, that is, to hydrolysis products other than monomeric leucine.

In accord with our previous observations regarding the enhancement of optical purity during polymerization,[11] the 45.4% enantiomeric excess L > D polymer in Table 2 was prepared by the partial polymerization of a leucine NCA monomer having a 31.1% L > D enantiomeric excess (Experimental), representing an enantiomeric excess enhancement of

Table 2. Changes in enantiomeric composition on partial hydrolysis of D ≠ L polyleucine

Initial Polymer, % Enantiomeric Excess[a]	Yield of Leucine Monomer, %[b]	Recovered Leucine, % Enantiomeric Excess[a]	Recovered Peptide, % Enantiomeric Excess[a]
45.4[c]	10.4	31.2[c]	49.5[c]
45.4	16.9	30.5	50.1
45.4	27.0	39.2	54.9
45.4	46.4	43.7	54.6
41.4[d]	57.0	39.5[d]	51.5[d]

a) Standard deviations in replicate analyses: ± 0.0-0.2%

b) Defined as in Table I

c) L>D

d) D>L

some 14.3%. Table 2 indicates that the 45.4% enantiomeric excess of this polymer can be increased by as much as 9.5% (to 54.9%) by partial hydrolysis. Thus in one step of a combined polymerization-hydrolysis sequence the initial 31% enantiomeric excess of the monomer was enhanced to *ca* 55%, an increase of some 24%. Thus a combined partial polymerization-hydrolysis process appears potentially quite efficient as a mechanism for the abiotic enhancement of small enantiomeric inequalities. While additional work will be required to see if other polyamino acids behave like polyleucine, it is tempting on the basis of the above model experiments to speculate that repeated polymerization-hydrolysis steps, induced by environmental dry-wet cycles, might have been operative on the primitive Earth to enhance small, abiotically produced enantiomeric excesses in amino acid monomers to a degree of optical purity permitting the eventual evolution of a biosphere.

EXPERIMENTAL

*Poly-*D-, L- *and* DL-*leucines.* The polyleucines utilized in these experiments were prepared by polymerizing D-, L- or DL-leucine-N-carboxyanhydrides (NCA's) with sodium methoxide initiator.[11] The average degree of polymerization (DP_n) was approximately controlled by varying the ratio of NCA substrate to sodium methoxide initiator.[16] and the actual DP_n was subsequently determined for each crude polymer using the end group analysis procedure of Sela and Berger.[14] In a typical polymerization L-leucine NCA (864.5 mg; 5.50 mmoles) was dissolved in anhydrous[17] dioxane (125 ml) and treated with 0.860 ml of 0.124 M $NaOCH_3$ (in 1:3 methanol-dioxane) (0.107 mmole; A/I = 51.4). The mixture was stirred for one week, whereupon the crude polymer (589 mg; 94.3%) was collected by filtration. It proved to have DP_n = 49.2. A portion of this (376 mg) was purified by dissolving in a minimum amount of trifluoroacetic acid, then reprecipitating by the gradual addition of water under rapid stirring. The reprecipitated polymer was collected by filtration, then dried at 25° under vacuum and by subsequent heating at 110° for 24 hr. (Found: C, 63.28; H, 9.69; N, 11.81. Calcd. for $(C_6H_{11}NO)_{49}.H_2O$: C, 63.48; H, 9.80; N, 12.34%). The polymers listed in Table 1 were prepared by the above procedure, varying only the initial ratio of NCA to sodium methoxide initiator.

The polyleucine samples listed in Table 2, containing an excess of one enantiomer, were prepared by an alternative partial polymerization technique. The 45.4% enantiomeric excess L > D polymer was prepared by dissolving DL-leucine NCA (182.7 mg; 1.16 mmoles) and L-leucine NCA (82.5 mg; 0.525 mmole) (i.e. a 31.1% enantiomeric excess L > D leucine NCA mixture) in anhydrous dioxane (50 ml). A 1.5-ml aliquot was removed for a control and for initial infrared absorption measurements, and the remainder was treated with a 0.152 M initiator solution of sodium methoxide in 1:3 methanol-dioxane (0.539 ml; 0.082 mmole; A/I ≃ 20). The progress of the polymerization was monitored periodically by measuring the disappearance of the anhydride absorption band at 5.57 μm, using a 0.1 mm path length sodium chloride cell and a Perkin-Elmer infrared spectrometer. When the reaction reached 52% completion the mixture was diluted with ethyl acetate (50 ml) and cooled in ice. The peptide product, recovered by filtration and purified as above, weighed 69.4 mg. Complete hydrolysis of the peptide and gc analysis as described below indicated that, in accord with previously reported[11] enantiomeric excess enhancement during the partial polymerization of D ≠ L leucine NCA's, the initial 31.1% L > D enantiomeric excess had been amplified to 45.4% in the present preparation. By way of its mode of preparation the present product was assumed to approximately average a decapeptide. (Found: C, 63.04; H, 9.52; N, 12.17. Calcd. for $(C_6H_{11}NO)_{10}.H_2O$: C, 62.68; H, 9.82; N, 12.19%).

Partial hydrolyses of leucine polypeptides. The D-, L- and DL-polyleucines in Table 1 (2.0 mg of each) were partially hydrolyzed by sonicating in 4.0 ml of 3:1 2-propanol-6N HCl, then placing the resulting suspensions in culture tubes sealed with teflon-lined caps and heating at 160° for 21 hr, and finally processing as described below. The D ≠ L-polyleucines in Table 2 (1.0 mg of each) were similarly hydrolyzed at 160° for varying reaction times so as to achieve the increasing extents of partial hydrolysis noted. After heating, all samples were evaporated to a volume of *ca* 1 ml and chilled in an ice bath. The residual polymer in each case was removed by filtration and the filtrate was evaporated to dryness. Each residue was dissolved in 2.0 ml of 2N HCl, and 1.0 ml of each solution was removed, evaporated to dryness, and the residue converted to its N-TFA-leucine isopropyl ester derivative for gc analysis.[18] For the hydrolyses in Table 1 the gc analyses of these residues were used to determine the extent of racemization (average *ca* 5%) during the hydrolysis of the poly-D- and poly-L-leucines, so as to permit a correction for racemization in subsequent percent recovery calculations. The remaining portion of each sample was mixed with the appropriate D- or L-leucine marker (0.134 mg of D- or L-leucine in 1.0 ml 1N HCl) for the determination by the enantiomeric mrker technique[19] of the amount of leucine recovered on each partial hydrolysis. These mixtures were similarly evaporated to dryness and converted to N-TFA isopropyl esters for gc analysis.

The recovered peptides from the partial hydrolyses in Table 2 (0.1 mg of each) were suspended in 5.0 ml of 2-propanol-6N HCl reagent, sealed in culture tubes, and hydrolyzed completely by heating at 160° for 48 hr. The reagent was then evaporated and each residue was similarly derivatized for gc analysis, resulting in the final values listed in Table 2 for each recovered peptide.

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