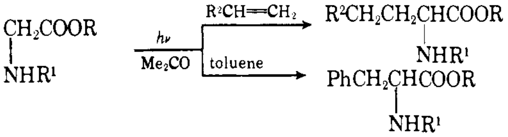
Photochemical Modification of Glycine-Containing Polypeptides

Joseph Sperling[1] **and Dov Elad***

Contribution from the Department of Chemistry,
The Weizmann Institute of Science, Rehovot, Israel. *Received July 2, 1970*

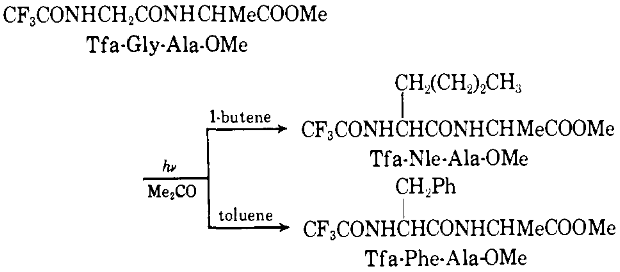
Abstract: Ultraviolet light induced alkylation of random as well as sequential glycine-containing polypeptides is described. The reactions were initiated with acetone and led to the selective conversion of glycine residues in the polypeptides into residues of a variety of branched α-amino acids, the side chain of which could be preselected by the use of the appropriate alkylating agents. The degree of selectivity and asymmetric induction has been found to depend on the location of the glycine residue and the conformation of the peptide.

The study of the photoalkylation reactions in the amino acid series aims at developing these reactions as a tool for the modification of peptides and proteins. This modification consists of the conversion of a glycine derivative into the appropriate derivative of a branched α-amino acid.[2] The reactions involve an alkylation process, the "alkylating" agent being an olefin or toluene. The net chemical change involved is the substitution of an alkyl or an aralkyl group for an α-hydrogen atom in the glycine. These reactions enabled the conversion of glycine into a variety of branched α-amino acids through the preselection of the appropriate side chain of the newly formed amino acid. Thus, the reactions of glycine with isobutylene or 1-butene lead to its conversion into leucine or norleucine, respectively, while the reaction with toluene leads to phenylalanine. Initiation was achieved through excitation of a ketonic compound, usually acetone, which serves as the light-absorbing system. The use of the photoinitiator enables the induction of the reactions with light of longer wavelengths (λ >290 nm), which is not absorbed by the amino acids, thus avoiding the direct excitation of the amino acids which might lead to their destruction.[3] The reactions can be summarized as follows.



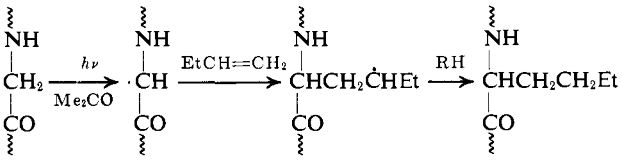
$$\underset{NHR^1}{CH_2COOR} \xrightarrow[Me_2CO]{h\nu} \begin{cases} \xrightarrow{R^2CH{=}CH_2} R^2CH_2CH_2\underset{NHR^1}{CHCOOR} \\ \xrightarrow{\text{toluene}} PhCH_2\underset{NHR^1}{CHCOOR} \end{cases}$$

In previous studies of this series dipeptides incorporating glycine residues have been chosen as models for the study of the various aspects of these photoalkylation reactions and of their scope. It was shown[2] that glycine residues in protected glycylalanine and glycylleucine could be converted into residues of various branched α-amino acids. Furthermore, it was found that the reactions were selective to the glycine residues in the dipeptides, whereas the other residues were attacked to a small extent only.



$$CF_3CONHCH_2CONHCHMeCOOMe \ (\text{Tfa-Gly-Ala-OMe}) \xrightarrow[Me_2CO]{h\nu} \begin{cases} \xrightarrow{\text{1-butene}} CF_3CONH\overset{CH_2(CH_2)_2CH_3}{CH}CONHCHMeCOOMe \ (\text{Tfa-Nle-Ala-OMe}) \\ \xrightarrow{\text{toluene}} CF_3CONH\overset{CH_2Ph}{CH}CONHCHMeCOOMe \ (\text{Tfa-Phe-Ala-OMe}) \end{cases}$$

A mechanism involving free-radical intermediates was proposed for these photoalkylation reactions. This was based on analysis of the peptidic as well as the nonpeptidic products.[2] It has been suggested that the excited photoinitiator abstracts a hydrogen atom from the α-carbon of the glycine residue leading to a free radical located at this site. These free-radical intermediates are scavenged by an olefin resulting in the conversion of the glycine into a branched α-amino acid (*e.g.*, the reaction with 1-butene leading to norleucine). The reactions of glycine and toluene follow a similar path involving glycyl and benzyl free radicals which are generated in the reaction mixture.



$$\begin{matrix} NH \\ | \\ CH_2 \\ | \\ CO \end{matrix} \xrightarrow[Me_2CO]{h\nu} \begin{matrix} NH \\ | \\ \cdot CH \\ | \\ CO \end{matrix} \xrightarrow{EtCH{=}CH_2} \begin{matrix} NH \\ | \\ CHCH_2\dot{C}HEt \\ | \\ CO \end{matrix} \xrightarrow{RH} \begin{matrix} NH \\ | \\ CHCH_2CH_2Et \\ | \\ CO \end{matrix}$$

The conversion of a glycine residue into a residue of a branched α-amino acid involves the generation of a chiral center at the α-carbon atom of that residue. The presence of other chiral centers in the peptide might lead to an asymmetric induction process. We have shown[4] that the conversion of a glycine residue in glycylalanine and glycylleucine into norleucine involves the occurrence of asymmetric induction. This results from the presence of a chiral center in a 1,4 relationship to the α-carbon of the glycine residue

$$-\overset{4}{N}H\overset{3}{C}H_2\overset{2}{C}ONH\overset{1}{C}HRCO-$$

and indicates a 1,4-asymmetric induction effect in free-radical reactions.

The photoalkylation reactions have so far been applied to low molecular weight systems. The application of these reactions to polypeptides serves as an intermediate stage in their application for the modification of proteins. The reactivity of polymers in these reactions, mainly in aqueous or partially aqueous media, seems to be of crucial importance for further studies in this direction. The present article is concerned with photoalkylation reactions of some synthetic glycine-containing polypeptides,[5] which serve as substrates for

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(1) In partial fulfillment of the requirements for a Ph.D. thesis submitted to the Feinberg Graduate School, 1970.

(2) D. Elad and J. Sperling, *J. Chem. Soc. C*, 1579 (1969).

(3) A. D. McLaren and D. Shugar, "Photochemistry of Proteins and Nucleic Acids," Pergamon Press, New York, N. Y., 1964, p 88; R. B. Johns, F. D. Looney, and D. J. Whelan, *Biochim. Biophys. Acta*, **147**, 369 (1967).

(4) D. Elad and J. Sperling, *Chem. Commun.*, 234 (1969).

Table I. Alkylation Products of Glycine-Containing Polypeptides (Initiated Photochemically with Acetone)

Polypeptide	Amino acid^a composition			Mol wt	Alkylating agent	Product: Amino acid^a composition					
	Ala	Gly	Pro			Ala	Gly	Pro	Nle	Leu	Phe
[L-Ala-Gly-L-Ala]$_n$	66.7	33.3		3100	Toluene	68	30				2
[L-Ala-Gly-D-Ala]$_n$	66.7	33.3		1100	1-Butene	70.7	27		2.3		
					Toluene	65.5	31				3.5
(DL-Ala)$_m$(Gly)$_n$	90	10		5100	1-Butene	89.7^b	9.1		1.2		
						91.5^c	8.1		0.4		
					Toluene	90.8^b	6.3				2.9
						91.1^c	7				1.9
(DL-Ala)$_m$(Gly)$_n$	77	23		4800	1-Butene	78^b	21.5		0.5		
						81.9^c	16.5		1.6		
					Toluene	77.6^b	22				0.4
						82.5^c	15.3				2.2
(DL-Ala)$_m$(Gly)$_n$	57	43			1-Butene	56.5	42.7		0.8		
					Toluene	56.5	43.2				0.3
[L-Pro-L-Pro-Gly]$_n$		33.3	66.7	1100	Isobutylene		30	67.6		2.4	
					Toluene		31.2	65.5			3.4
[L-Pro-Gly-L-Pro]$_n$		33.3	66.7	2200	1-Butene		25.8	71	3.2		
					Toluene		28.4	68.4			3.2

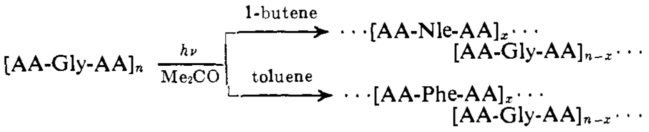
^{a} Residues per 100 total residues. ^{b} Of the water-soluble product. ^{c} Of the water-insoluble product.

the study of the problems involved in the application of these reactions to polymeric systems.

Results and Discussion

Sequential polypeptides containing glycine and alanine as well as those of glycine and proline, and random copolymers incorporating varying contents of glycine, have been employed for the present study. The sequential polypeptides are substrates with definite structure and are models for some proteins, *e.g.*, [L-Pro-Gly-L-Pro]$_n$ as a model for collagen.[6] The random copolymers are useful as substrates containing various contents of glycine. The sequential polypeptides were prepared by polymerization of the appropriate tripeptide *N*-hydroxysuccinimide esters in dimethylformamide,[7] while the random copolymers were prepared by polymerization of the appropriate mixtures of the corresponding *N*-carboxyanhydrides.[8] The polypeptides were fractionated on Sephadex G-25 columns and fractions of molecular weights ranging from 2000 to 5000 were lyophylized and used for the photochemical reactions. The weight-average molecular weights of the peptides were determined by the Yphantis midpoint method.[9] The number-average molecular weight of the insoluble [L-Ala-Gly-L-Ala]$_n$ was determined by the Van Slyke method[10] and was found to be 3100. No protecting groups have been used for the end groups of the polymers.

This work with polypeptides involved a change from organic solvents used in the case of protected dipeptides to aqueous solvent systems. All polypeptides employed were dissolved in the reaction mixture except for [L-Ala-Gly-L-Ala]$_n$ which was handled in suspension. Irradiation at room temperature of these glycine-containing polypeptides with 1-butene or toluene in the presence of acetone led to the conversion of glycine residues in the polypeptides into norleucine or phenylalanine, respectively.



AA = Ala or Pro

Progress of the reactions was followed by periodic hydrolysis of samples and determination of the amino acid composition with an amino acid analyzer.[11] In the reactions studied, 7–25% of the glycine content in the polypeptides was converted to the appropriate branched α-amino acid residue. Precipitation took place in some of the reactions and the precipitates were removed and analyzed separately. These insoluble polypeptides also contained the new amino acid as did the soluble portion of the polypeptidic products. Molecular weight measurements indicated that the reacted polypeptides had nearly the same molecular weight as the corresponding starting polypeptides, indicating that no major fragmentation of the polymers occurred under the reaction conditions. The reactions studied and the newly formed polypeptides are described in Table I.

A study of the selectivity of the process to glycine residues in the glycine–alanine polypeptides indicated that the glycine residues were more reactive than those of alanine. In order to uncover some of the factors which affect the selectivity, a study of the degree of selectivity in a variety of glycine and alanine systems was undertaken. In this study the selectivity observed in the polypeptides has been compared to that in the unit models, *i.e.*, tripeptides for sequential polymers, and appropriate mixtures of glycine and alanine derivatives for the random ones. Differences in the degree of selectivity in the low molecular weight compounds as compared to the polymers have been observed. The ratio of the α-alkylated products of glycine and alanine residues was determined by glpc.[12] The determination of the 2-methyl-2-amino acids by this method was preferred to methods

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(5) Preliminary communication: J. Sperling, *J. Amer. Chem. Soc.*, **91**, 5389 (1969).

(6) N. S. Andreeva, N. G. Esipova, M. I. Millionova, V. N. Rogulenkova, and V. A. Shibnev, "Conformation of Biopolymers," Vol. 2, G. N. Ramachandran, Ed., Academic Press, New York, N. Y., 1967, pp 469–481.

(7) D. M. Segal, *J. Mol. Biol.*, **43**, 497 (1969).

(8) E. Katchalski and M. Sela, *Advan. Protein Chem.*, **13**, 243 (1958).

(9) D. A. Yphantis, *Ann. N. Y. Acad. Sci.*, **88**, 586 (1960).

(10) D. D. Van Slyke, *J. Biol. Chem.*, **83**, 425 (1929).

(11) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, 30, 1190 (1958).

(12) C. W. Gehrke and D. L. Stalling, *Separ. Sci.*, 2 (1), 101 (1967).

Table II. Selectivity in the Photoalkylation of Glycine–Alanine Systems[a]

Starting system			
Peptide	Gly:Ala	Phe:MePhe[b]	Nle:MeNle[c]
[L-Ala-Gly-Ala]$_n$	1:2	30:1	
(DL-Ala)$_m$(Gly)$_n$	1:9	30:1	9:1
(DL-Ala)$_m$(Gly)$_n$	1:4	15:1	14:1
(DL-Ala)$_m$(Gly)$_n$	2:3	8:1	13:1
Tfa-Gly-DL-Ala-OMe	1:1	7:1	10:1
Tfa-DL-Ala-Gly-OMe	1:1	20:1	7:1
Tfa-L-Ala-Gly-L-Ala-OMe	1:2	12:1	3:1
Tfa-DL-Ala-OMe + Tfa-Gly-OMe	1:9	2:1	4:1
Tfa-DL-Ala-OMe + Tfa-Gly-OMe	1:4	2:1	5:1
Tfa-DL-Ala-OMe + Tfa-Gly-OMe	1:2	2:1	5:1
Tfa-DL-Ala-OMe + Tfa-Gly-OMe	1:1	2:1	4:1

[a] Obtained by multiplying the absolute ratio of the products by the ratio of Ala:Gly in the starting polypeptide. [b] 2-Methylphenylalanine, resulting from alkylation of alanine residues with toluene; per residue of the respective starting amino acid. [c] 2-Methylnorleucine, resulting from alkylation of alanine residues with 1-butene; per residue of the respective starting amino acid.

which are based on the ninhydrin test, since these acids, which do not possess α-hydrogens, give poor color yields. Determination was achieved by acid hydrolysis, esterification with 1-butanol followed by trifluoroacetylation, and analysis by glpc. Derivatization was practically quantitative (98 ± 2%),[12] and reproducible results of the glpc measurements were obtained. Appropriate authentic mixtures were used as standards for these determinations. The overall experimental error observed in these measurements is in the range of 7%. Results of the reactions of various glycine–alanine systems with toluene or 1-butene are summarized in Table II.

The preferential reactivity of glycine residues in polypeptides, previously observed in dipeptides,[2] is of special interest. This phenomenon, which was studied with peptides containing alanine, indicated the preference of the reaction at a methylene group to that at a methine. The selectivity, which could be observed already in the reactions of the derivatives of the individual amino acids, Tfa-Gly-OMe and Tfa-Ala-OMe, is contrary to the expectation that tertiary free radicals would be formed in preference to secondary ones,[13] *i.e.*, that alanine free-radical centers would be preferred to those of glycine. Although no full explanation for this effect can be presented, few comments based on experimental results can, however, be made. The formation of the product in these photoalkylation reactions results from several steps, the major ones being (i) hydrogen atom abstraction by the excited photoinitiator and generation of a free radical at the α-carbon atom of the amino acid residue,[2] and (ii) interaction of the amino acid free radical with the "alkylating agent," *i.e.*, the olefin or a benzyl radical, to give the branched α-amino acid.[2] It could be assumed that in the first step (i) alanine radical centers are formed faster than those of glycine, and that the former are less reactive in the second step (ii). Consequently, this might lead to smaller quantities of alanine adduct as compared to that of glycine, especially in reactions with olefins where a higher activation energy might be required for the alanine radicals to add to the olefin. However, experiments with derivatives of L-alanine as well as glycyl-L-alanine and toluene ruled out this possibility. These experiments indicated that racemization of alanine occurs to a very small extent during the reaction. Racemization would be expected from alanine free radicals which failed to react with the "alkylating agent" and alternatively abstract a hydrogen atom to give alanine. Comparison experiments, in the absence of toluene, indicated that racemization of L-alanine did not exceed the amount of 2-methylphenylalanine formed in reactions where toluene was present. These experiments indicate that besides the reacted alanine hardly any racemization of alanine takes place.[2] Since glycine is consumed faster than alanine, it is concluded that the observed preferential reactivity of glycine, which is based on product analysis, is already determined at the hydrogen atom abstraction step. Concerning the differences in the degree of selectivity in the various systems examined, it is noteworthy that a different degree of selectivity has been observed in glycylalanine as compared to alanylglycine. This indicates that the location of the glycine residue in the peptide plays a role in determining the degree of selectivity. The selectivity was enhanced in copolymers containing small quantities of glycine, *e.g.*, a random copolymer consisting of 10% glycine and 90% DL-alanine. The increase in selectivity with a decreasing number of glycine residues might arise due to the changes in the conformations of the polymers, which become less reactive with increase of their "polyglycine character."[14] These and other results which are presented in Table II indicate that the location of the glycine residue as well as the conformation of the peptide chain play a role in determining the degree of selectivity of the reactions to the glycine residues. These points are under further investigation.

The effect of the asymmetric induction which was observed in the reactions of glycine-containing dipeptides was further studied with the polypeptides. It has been found in all the reactions studied, either with 1-butene or toluene, that unequal amounts of the two enantiomers of the newly formed norleucine or phenylalanine were obtained. The polypeptides [L-Ala-Gly-L-Ala]$_n$ and [L-Pro-Gly-L-Pro]$_n$ have been employed for this study and comparison was made with the asymmetric induction taking place in the monomer units, *i.e.*, the appropriate protected tripeptides, and also in dipeptides. The results obtained are summarized in Table III.

The asymmetric induction was determined through acid hydrolysis of samples from the reaction mixture

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(13) W. A. Pryor, "Free Radicals," McGraw-Hill, New York, N. Y., 1966, pp 14, 17, 170, 207; A. Heller, *Mol. Photochem.*, 1, 257 (1969).

(14) *Cf.* P. J. Flory, "Statistical Mechanics of Chain Molecules," Wiley, New York, N. Y., 1969, p 282.

Table III. Asymmetric Induction in the Photoalkylation of Di-, Tri-, and Polypeptides

		Formed amino acids			
		Norleucine		Phenylalanine	
Peptide	Alkylating agent	L, %	D, %	L, %	D, %
Tfa-Gly-L-Ala-OMe	1-Butene	54	46		
Tfa-Gly-L-Ala-OMe	Toluene			52.5	47.5
Tfa-Gly-L-Leu-OMe	1-Butene	59	41		
Tfa-L-Ala-Gly-L-Ala-OMe	Toluene			40	60
Tfa-L-Pro-Gly-L-Pro-OMe	1-Butene	48	52		
Tfa-L-Pro-Gly-L-Pro-OMe	Toluene			45	55
[L-Ala-Gly-L-Ala]$_n$	Toluene			70	30
[L-Pro-Gly-L-Pro]$_n$	1-Butene	40	60		
[L-Pro-Gly-L-Pro]$_n$	Toluene			32.5	67.5

followed by digestion of the L-amino acids by L-amino acid oxidase,[15] and quantitative determination of the remaining intact D-amino acids with an amino analyzer. In all measurements it was confirmed that complete digestion of the L enantiomer took place. This was achieved through the use of an internal standard of an appropriate DL-amino acid. The enzymic digestion was regarded as complete when three consecutive measurements of the amino acid composition of the mixture, taken after long intervals (see Experimental Section), showed the disappearance of 50% of the internal standard and identical amino acid composition. Glycine and proline, which are not digested by the enzyme,[15] were used as additional internal standards. The experimental error in the determination of the asymmetric induction by this method was ±2%. Results obtained by the enzymatic method were compared to those obtained by gas chromatographic determinations. At the dipeptide level the diastereoisomers were separated on the appropriate column and determined directly.[4,16] Gas chromatographic determination of asymmetric induction in polypeptides was conducted on a column with an optically active stationary phase (Tfa-L-Val-L-Val-O-cyclohexyl ester).[17] These were achieved through acid hydrolysis of the peptide followed by esterification with 2-propanol and trifluoroacetylation. The resolved mixture of the enantiomer derivatives was determined by comparison with authentic mixtures. The results obtained for the L and D enantiomers of the new branched amino acids were found to be the same (±4%) as those obtained by the L-amino acid oxidase method.

Previous studies indicated that the amino acid adjacent to the glycine residue plays a role in determining the degree of the asymmetric induction. Thus, 8% of induction has been observed in glycylalanine as compared to 20% induction in glycylleucine.[4] Comparison of the degree of asymmetric induction obtained in the monomer units, *i.e.*, the tripeptides, and the corresponding sequential polypeptides indicates that they vary considerably. Thus, while Tfa-L-Ala-Gly-L-Ala-OMe showed 20% induction with preference of the D enantiomer in the reaction with toluene, the corresponding polymer, [L-Ala-Gly-L-Ala]$_n$, gave 40% induction with preference of the L enantiomer. In both monomer and polymer the glycine is located between the same residues, and thus the same degree of asymmetric induction would be expected had the adjacent amino acid been the only factor which determines the asymmetric induction. It must be assumed, therefore, that an additional factor, besides the adjacent residue to the glycine, affects the degree of asymmetric induction. It is conceivable that this factor is the conformation of the peptide which presents an additional asymmetry element to the reactive species in the reactions described. At a certain stable conformation of a peptide the two α-hydrogens of the glycine residue may have different reactivities. This difference arises from the fact that one hydrogen is directed toward the solvent, while the other is masked by the peptidic chain. Thus, the former is more prone to an attack by the excited photoinitiator and consequently is more easily abstracted. Furthermore, at a given conformation the incorporation of one enantiomer of the newly formed amino acid may be preferred to the other. Thus, the incorporation of D-phenylalanine into [L-Pro-Gly-L-Pro]$_n$ is preferred to the L enantiomer. The latter was incorporated, probably, at the end of the chain where the conformation is less rigid. It is assumed that in high molecular weight polypeptides of the type [AA-Gly-AA]$_n$, which have the collagen structure,[18] the asymmetric induction would tend toward a stereospecific conversion of glycine. Studies toward the solution of these problems with a variety of polypeptides are in progress.

Experimental Section

Chromatography was carried out on columns (100 cm long, 2.5 cm in diameter) filled with Sephadex (G-25 medium; Pharmacia, Upsala). The eluent was aqueous acetic acid (0.01 *M*) and the flow rate was 1 ml/min. The void volume of 195 ml was determined with Blue Dextran 2000 (Pharmacia, Upsala). Fractions of 15–20 ml were collected and the optical density at 220 nm was recorded. Ascending tlc was performed with Kieselgel G (Merck) using 1-butanol–acetic acid–water (3:1:1, v/v) mixture as eluent for the amino acids. Spots of amino acids were revealed with ninhydrin (0.5% in acetone). Amino acid compositions were determined by hydrolysis of peptides in 6 *N* hydrochloric acid at 110° for 22 hr and analysis on a Beckman Spinco amino acid analyzer, Model 120 C. Glpc was carried with a Varian Aerograph 1200-2 instrument on 10% silicone oil DC 710 on Celite (5 ft × $^1/_8$ in.). Molecular weights of polymer fractions were determined with a Spinco Model E ultracentrifuge using the midpoint method of Yphantis. All measurements were carried out at 22° in 0.2 *N* NaCl solution and a polymer concentration of 0.8%.

Experiments were carried out at room temperature in an immersion apparatus with Hanovia high-pressure mercury-vapor lamps which were cooled internally with running water. Lamps (200 W) with Corex filters and lamps (450 W) with Pyrex filters were used for the reactions with toluene and the butenes, respectively. Typical experiments for homogeneous and heterogeneous mixtures are described; other experiments were conducted under similar conditions unless otherwise stated. The homogeneous solutions were prepared by dissolving the polymer in water followed by addition of the reagents, and homogenization with *tert*-butyl alcohol.

Reaction of (DL-Ala)$_m$(Gly)$_n$ ($m:n = 9:1$) and 1-Butene. A mixture of the copolymer (0.35 g; $\bar{M} = 5100$), water (25 ml), *tert*-butyl alcohol (25 ml), and acetone (15 ml) was irradiated for 72 hr while 1-butene was bubbled through the mixture. The solvents were evaporated and the residue was washed with ethyl acetate or *tert*-butyl alcohol leaving a solid (0.32 g) which was extracted with water. The water-insoluble fraction (0.12 g) was analyzed for the amino acid composition by the usual procedure and was shown to consist of Ala (91.5), Gly (8.1), and Nle (0.4) (residues/100 total residues). The water-soluble fraction was hydrogenated at room temperature and atmospheric pressure over Pd/C (5%) to remove traces of un-

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(15) A. Meister and D. Wellner, *Enzymes*, 7, 609 (1963).

(16) F. Weygand, A. Prox, L. Schmidhammer, and W. Konig, *Angew., Chem., Int. Ed. Engl.*, 2, 183 (1963).

(17) B. Feibush and E. Gil-Av, *Tetrahedron*, 26, 1361 (1970).

(18) *Cf.* A. Yonath and W. Traub, *J. Mol. Biol.*, 43, 461 (1969).

saturation and was filtered through a column of Sephadex. The 220-nm absorption of the various fractions was recorded, and no absorption could be detected in the region of the low molecular weight fractions. The combined fractions were lyophilized leaving a solid (0.2 g) which was shown to contain Ala (89.7), Gly (9.1), and Nle (1.2). The weight-average molecular weight of the latter was determined and was shown to be $\bar{M}$ = 4150.

Reaction of (DL-Ala)$_m$(Gly)$_n$ (m:n = 9:1) and Toluene. A mixture of the polypeptide (0.25 g), water (15 ml), toluene (5 ml), *tert*-butyl alcohol (35 ml), and acetone (10 ml) was irradiated for 170 hr. The usual work-up led to an insoluble fraction (0.14 g), the composition of which has been shown to be: Ala (91.1), Gly (7.0), and Phe (1.9). The water-soluble fraction (0.12 g) consisted of Ala (90.8), Gly (6.3), and Phe (2.9).

Reaction of [L-Pro-Gly-L-Pro]$_n$ and Toluene. A mixture of the peptide (0.05 g; $\bar{M}$ = 2200), water (1 ml), toluene (1 ml), *tert*-butyl alcohol (5 ml), and acetone (2 ml) was irradiated externally for 90 hr. The usual work-up led to the isolation of a water-soluble polypeptide (0.045 g) which contained Pro (68.4), Gly (28.4), and Phe (3.2).

Reaction of [L-Ala-Gly-L-Ala]$_n$ and Toluene. A suspension of the polymer (0.1 g; $\bar{M}$ = 3100) in a mixture of toluene (2 ml), acetone (3 ml), and *tert*-butyl alcohol (15 ml) was irradiated (450-W lamp; Corex filter) for 150 hr. The solid was filtered off and washed successively with acetone and water. The product (0.09 g) was analyzed for the amino acid composition in the usual way and consisted of Ala (68), Gly (30), and Phe (2). The average molecular weight (Van-Slyke) of the product was $\bar{M}$ = 4200.

Determination of Selectivity in the Reaction of (DL-Ala)$_m$(Gly)$_n$ (m:n = 9:1) and Toluene. A mixture of the copolymer (0.25 g; $\bar{M}$ = 5100), water (15 ml), toluene (10 ml), acetone (10 ml), and *tert*-butyl alcohol (35 ml) was irradiated (200-W lamp; Corex filter) for 170 hr. The usual work-up as above led to water-insoluble and water-soluble products. An aliquot of 10 mg of each was hydrolyzed in 6 N hydrochloric acid at 110° for 22 hr. The solvent was removed under reduced pressure and the residue treated at room temperature with 1 N HCl in methanol (5 ml) for 30 min, and the solvent was then evaporated. The residue was treated with 1 N HCl in 1-butanol (5 ml) for 3 hr at 90°, followed by evaporation of the solvent. The resulting mixture of ester hydrochlorides was dissolved in methylene chloride (5 ml) and treated with trifluoroacetic anhydride (0.5 ml) for 2 hr at room temperature.[12] The resulting mixture of N-trifluoroacetyl n-butyl esters of Ala, Gly, Phe, and MePhe was dissolved in methylene chloride and injected into the gas chromatography machine. The composition of the amino acids in the mixture was finally determined by comparison with authentic mixtures of the individual amino acid derivatives.

Determinations of selectivity in reactions of other peptides were run in a similar manner. With Tfa-Gly-OMe and Tfa-Ala-OMe, samples from the reaction mixtures were injected and determined directly in glpc, using authentic samples as standards.

Determination of Asymmetric Induction in the Reaction of [L-Ala-Gly-L-Ala]$_n$ and Toluene. (a) With L-Amino Acid Oxidase. The reaction was conducted in suspension as described above. A sample of the product (11 mg) was hydrolyzed with 6 N hydrochloric acid in the usual way. The solvent was evaporated and the residue dissolved in 0.2 M Tris–HCl buffer, pH 8 (9 ml); a 10^{-2} M solution of DL-leucine (1 ml) in the same buffer was added as internal standard. An aliquot of the resulting mixture (1 ml) was diluted with a citrate buffer, pH 2.2 (4 ml), and the amino acid composition was determined on an amino acid analyzer at 0 hr. A solution of L-amino acid oxidase (Worthington Biochemical Corp.) (0.05 ml; 20 mg/ml) was then added to the mixture which was left at room temperature. Aliquots (1 ml) were taken after 6, 24, and 48 hr; these were diluted with citrate buffer, pH 2.2 (4 ml), and samples (1 ml) were analyzed on the amino acid analyzer. A typical analysis is presented in Table IV. The phenylalanine residues in the product polymer were found to consist of 70% L enantiomer and 30% D enantiomer.

Table IV

Time, hr	Amino acid, μmol × 10^2			
	Gly	Ala[a]	Phe	Leu
0	66.7	155.0	4.20	21.2
24	65.5	128.5	1.25	10.6
48	66.7	92.0	1.24	10.5
72	66.0	56.2	1.26	10.3

[a] L-Alanine is being digested slowly by L-amino acid oxidase,[15] as proved by blank experiments.

All other reactions and the determinations of asymmetric induction were conducted similarly. DL-Phenylalanine was used as an internal standard for the butene reactions.

(b) By Glpc. A sample of the product (20 mg) was hydrolyzed with 6 N hydrochloric acid in the usual way. The solvent was removed under reduced pressure and the residue was dissolved in 1 N HCl in 2-propanol (5 ml) and kept for 3 hr at 110° in a sealed tube. The solvent was evaporated and the residue was dissolved in methylene chloride (5 ml) and treated with trifluoroacetic anhydride (0.5 ml) for 2 hr at room temperature. Excess reagent and solvent were removed under reduced pressure; the residue was dissolved in methylene chloride and injected into the gas chromatograph. The mixture of enantiomers of phenylalanine was resolved on a stainless steel capillary column (100 ft × 0.02 in.) coated with Tfa-L-Val-L-Val cyclohexyl ester at 101°, and a carrier gas flow (helium) of 2 cc/min. The mixture was shown to consist of 71.5% L-Phe and 28.5% D-Phe.

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