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A MODERATE CHIRAL BIAS OF PAPAIN USED IN PROMOTING UNIQUE CONSECUTIVE SYNTHESES OF ENANTIOMERIC ANILIDES OR PHENYLHYDRAZIDES OF Z-DL-ALANYLGLYCINE

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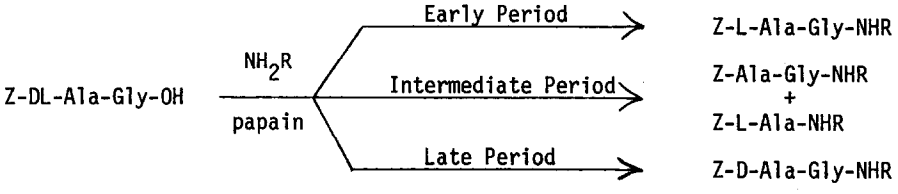
Papain can attack a Z-dipeptide[1], in the presence of a nucleophile such as NH_2Ph or NH_2NHPh, either at the carboxyl terminal to yield a Z-dipeptide anilide or phenylhydrazide, or else at the peptide carbonyl, -CO-NH-, with resultant formation of the anilide or phenylhydrazide of a Z-amino acid. An attack at the carbonyl of an L residue is preferred to an attack at a D residue. For the current research, Z-dipeptides were deliberately prepared[2] with an Ala residue at the N-blocked amino terminal and a Gly residue at the carboxyl terminal, for papain[3] catalysis with these two nucleophiles.

When Z-DL-Ala-Gly-OH was the reactant, Z-L-Ala-Gly-NHR was the early reaction product. On the other hand, only Z-D-Ala-Gly-NHR was formed during the late, prolonged incubation period. For intermediate periods, papain made concurrent attacks at both the peptide carbonyl and the terminal carboxyl, thereby producing a mixture of reaction products.



This stepwise production of enantiomers, at appropriately different incubation periods, was a consequence of the special design of the racemic Z-dipeptide. The D enantiomer was unable to compete successfully for a catalytic site, until the Z-L-Ala-Gly-OH had been sufficiently depleted through attacks on the Gly and the L-Ala residues. Although the D residue of Z-D-Ala-Gly-OH impeded the reaction velocity of this remnant enantiomer, nevertheless, catalysis advantageously proceeded slowly but continuously at the available Gly residue. Interruption of incubation for sampling established that the uncleaved Z-D-Ala-Gly-NHR was always the singular, insoluble product. Subjection of racemic Z-amino acids to papain catalysis[4,5,6,7] has never permitted an optically pure Z-D-a.a.-NHR to be obtained in such a direct manner.

Isolation of separate enantiomeric products in the current research was merely a matter of judgment of spacing the incubation periods at satisfactory time intervals. A better understanding of such spacing was secured by an independent examination of Z-D-Ala-Gly-OH and Z-L-Ala-Gly-OH under similar catalytic conditions. When uninhibited by the presence of the L enantiomer, papain very slowly attacked Z-D-Ala-Gly-OH from the very start of incubation. By comparison, independent Z-L-Ala-Gly-OH reacted far more rapidly, with maintenance of the peptide structure at the onset. Then, both cleaved and intact peptide structures were involved within an intermediate incubation period. However, only cleavage of the peptide structure was encountered during the final phase of catalysis. Early, intermediate and late periods were all confined to a very short duration of time for the L reactant, compared to an extended period when the D enantiomer was utilized. Important details of these experiments are summarized in Table I.

Detection of the cleaved or unsplit nature of the products by means of TLC was reported previously[8]. Identical solvent systems[8] were now employed for thick layer chromatography on ChromAR, after TLC had demonstrated that cleaved and unsplit products actually were both present. Such a mixture was streaked on appropriate sheets of ChromAR, then dried and placed between two glass plates. Following chromatography, the separated bands were located on a dried sheet by means of a short wave length UV lamp. A cleaved product showed a higher R_f value than did a corresponding product that retained the peptide structure. Each separated band was cut into fragments and thoroughly extracted with chloroform. The product was isolated by filtration and removal of the solvent.

When attention was turned to Z-Gly-L-Ala-OH, Z-Gly-DL-Ala-OH and Z-Gly-D-Ala-OH[2], only cleavage of the peptide structure was observed. Z-Gly-NHR[7,9,10] was the sole, insoluble reaction product.

$$\text{Z-Gly-Ala-OH (L, DL, or D)} \xrightarrow[\text{papain}]{NH_2R} \text{Z-Gly-NHR + Ala-OH (L, L > D, or D)}$$

Relative rates of reactions of these substrates were in the order L > DL > D, thus implying that a partial resolution of the racemic residue had taken place. Table II itemizes significant features of this study.

Table I

Products Formed During Reactions between the Z-Alanylglycines and Aniline or Phenylhydrazine[a]

Z-Dipeptide Reactant	Incubation, Hrs at 40°	Product Abbreviation	Weight, g	Melting Point, °C	$[\alpha]_D^{25°}$ in Pyridine	Approximate % of Enantiomer
		1. Reactions with Aniline				
Z-L-Ala-Gly-OH	0-3	Z-L-Ala-Gly-NHPh[10]	0.363	169.5-170.5	+ 13.5°	100, L
	3-6.5	Z-L-Ala-Gly-NHPh	0.253	169.5-170.5	+ 13.5°	100, L
	3-6.5	Z-L-Ala-NHPh[9,10]	0.025	160-162	- 37.0°	100, L
	6.5-48	Z-L-Ala-NHPh[9,10]	0.325	161-163	- 37.5°	100, L
Z-D-Ala-Gly-OH	0-168	Z-D-Ala-Gly-NHPh[10]	0.784	169-170	- 13.6°	100, D
Z-DL-Ala-Gly-OH	0-3	Z-Ala-Gly-NHPh	0.299	163.5-165	+ 9.5°	85, L
	3-6.5	Z-Ala-Gly-NHPh	0.114	163-165	+ 9.5°	85, L
	3-6.5	Z-Ala-NHPh[9,10]	0.145	154-156	- 36.2°	98, L
	6.5-24	Z-Ala-Gly-NHPh	0.156	168.5-169.5	- 12.9°	97, D
	6.5-24	Z-Ala-NHPh[9,10]	0.032	155-158	- 36.0°	98, L
	24-168	Z-Ala-Gly-NHPh	0.203	169.5-170.5	- 13.1°	98, D
		2. Reactions with Phenylhydrazine				
Z-L-Ala-Gly-OH	0-3	Z-L-Ala-Gly-NHNHPh	0.394	186.5-187.5	- 11.7°	100, L
	3-24	Z-L-Ala-Gly-NHNHPh[7,10]	0.507	183-185	- 11.7°	100, L
	3-24	Z-L-Ala-NHNHPh[7,10]	0.186	149-151	- 33.1°	100, L
Z-D-Ala-Gly-OH	0-72	Z-D-Ala-Gly-NHNHPh[10]	0.608	185-187	+ 12.2°	100, D
Z-DL-Ala-Gly-OH	0-3	Z-Ala-Gly-NHNHPh[10]	0.250	182.5-183.5	- 10.9°	95, L
	3-6.5	Z-Ala-Gly-NHNHPh[10]	0.322	179-180	- 10.6°	93, L
	3-6.5	Z-Ala-NHNHPh[7,10]	0.075	148-149	- 26.3°	89, L
	6.5-48	Z-Ala-Gly-NHNHPh	0.248	184-186	+ 11.9°	99, D

[a]Each solution contained 1.000 g Z-dipeptide, 0.52 ml NH_2Ph or 0.60 ml NH_2NHPh, 36 ml 0.50 ml M buffer pH 4.5, 4 ml hexamethylphosphoramide, 0.2000 g L-cysteine·HCl·H_2O and 0.2000 g activated papain[3].

Table II

Products Formed through Reactions between the Z-Glycylalanines and Aniline or Phenylhydrazine[a]

Z-Dipeptide Reactant	Product Formed	Yield in Grams, 0-24 Hrs at 40°	Melting Point, °C
	1. Aniline as the Nucleophile		
Z-Gly-L-Ala-OH	Z-Gly-NHPh[9,10]	0.374	142-144
Z-Gly-D-Ala-OH	Z-Gly-NHPh	0.116	143.5-144
Z-Gly-DL-Ala-OH	Z-Gly-NHPh	0.332	143.5-144
	2. Phenylhydrazine as the Nucleophile		
Z-Gly-L-Ala-OH	Z-Gly-NHNHPh[7,10]	0.436	139.5-144
Z-Gly-D-Ala-OH	Z-Gly-NHNHPh	0.243	139.5-140.5
Z-Gly-DL-Ala-OH	Z-Gly-NHNHPh	0.365	138-140

[a]Each solution contained 0.5000 g Z-dipeptide, 0.26 ml aniline or 0.30 ml phenylhydrazine, 0.1000 g L-Cysteine·HCl·H_2O, 0.1000 g activated papain[3], 18 ml 0.50 M buffer pH 4.5 and 2 ml hexamethylphosphoramide.

References and Notes

1. Z is the abbreviation for N-(benzyloxycarbonyl).

2. All Z-dipeiptides were obtained through Dr. P. Grogg of Biosynthetika, Liestal, Switzerland.

3. E. L. Bennett and C. Niemann, J. Am. Chem. Soc., 72, 1798 (1950).

4. E. L. Bennett and C. Niemann, J. Am. Chem. Soc., 70, 2610 (1948).

5. H. B. Milne and C. M. Stevens, J. Am. Chem. Soc., 72, 1742 (1950).

6. D. G. Doherty and E. A. Popenoe, Jr., J. Biol. Chem., 189, 497 (1951).

7. J. L. Abernethy, E. Albano and J. Comyns, J. Org. Chem., 36, 1580 (1971).

8. J. L. Abernethy and D. Srulevitch, J. Chromatogr., 123, 231 (1976).

9. J. L. Abernethy, F. G. Howell, A. Ledesma, D. Doose and R. Everett, Tetrahedron, 31, 2650 (1975).

10. All nitrogen analyses were run by Mr. C. F. Geiger, Ontario, California. Each analysis deviated less than 0.30 in absolute value from the theoretical value. Mixture melting points were also taken when this was appropriate.