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Stereochemical Aspects of Forcing Special Substrate Hydrazides to Behave Either as Electrophiles or as Nucleophiles during Catalysis by Crude Papain[1]

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Restriction of hydrazides of N-blocked amino acids mainly to electrophilic action, in acylating crude papain, has been achieved by means of a large amount of aniline, with formation of insoluble anilides of *N*-acylamino acids. Similarly, nucleophilic behavior, on the part of a hydrazide, has been promoted by introducing a large proportion of an *N*-acylamino acid to produce an insoluble N^1,N^2-diacylhydrazine. Achiral, chiral and racemic hydrazides and their corresponding *N*-acylamino acids were utilized in the study. Among the more informative combinations of reactants were *Z*-DL-alanine hydrazide with aniline and then with *Z*-glycine. A stereospecific response in the former situation produced *Z*-L-alanine anilide. In the latter case, a stereoselective interaction produced *Z*-Gly-NH–NH-LAla-*Z* more rapidly than *Z*-Gly-NH–NH-D-Ala-*Z*. The final incubation period yielded an optically pure *D* product. Differences in stereochemical control have been delineated in terms of different spatial aspects for interactions at the S and S′ subsites of sulfhydryl proteolytic enzymes. A racemic reactant encountered firm stereospecificity as an electrophile at the S subsite but only modest stereoselectivity as a nucleophile at the S′ subsite. The ready availability of crude papain allows an effective procedure for the synthesis of substantial quantities of diacylhydrazines.

INTRODUCTION

Recent research (*1*) has demonstrated that hydrazides of *N*-acylamino acids can serve in a difunctional manner, if subjected as sole substrates to catalysis by crude papain. As an electrophile, the carbonyl portion of a suitable hydrazide causes acylation of the sulfhydryl group of the enzyme, to form the thioester moiety of the enzyme–substrate intermediate. Other substrate molecules subsequently attack the thioester unit with resultant precipitation of an insoluble N^1,N^2-diacylhydrazine:

$$\text{Enz–SH} \xrightarrow[\text{electrophilic action}]{\text{R–CO–NHNH}_2} \underset{+\ \text{NH}_2\text{NH}_2}{\text{Enz–S–CO–R}} \xrightarrow[\text{nucleophilic action}]{\text{NH}_2\text{NH–CO–R}'} \underset{+\ \text{Enz-SH}}{\text{R–CO–NH–CO–R}'} \qquad (1)$$

[1] This preparation of crude papain contains a mixture of sulfhydryl proteolytic enzymes.

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In the current study, simple, restrictive procedures were devised that largely segregate electrophilic from nucleophilic action of the hydrazides. Their capacity to function mainly as electrophiles was effected by using excess aniline as a competitive nucleophile, as in Eq. [2]. Products were the readily identified anilides of *N*-acylamino acids.

$$\text{Enz–SH} \xrightarrow[\text{electrophile}]{\text{R–CO–NHNH}_2} \begin{matrix}\text{R–CO–S–Enz}\\ +\\ \text{NH}_2\text{NH}_2\end{matrix} \xrightarrow[\text{nucleophile}]{\text{NH}_2\text{Ph}} \begin{matrix}\text{R–CO–NHPh}\\ +\\ \text{Enz–SH}\end{matrix} \cdot \qquad [2]$$

In a similar fashion, a large proportion of a competitive electrophile, such as an *N*-acylamino acid, forced the hydrazide to exhibit predominantly nucleophilic behavior, with precipitation of N^1,N^2-diacylhydrazines from the reaction medium:

$$\begin{matrix}\text{R–CO–OH}\\ +\\ \text{Enz–SH}\end{matrix} \rightarrow \begin{matrix}\text{R–CO–S–Enz}\\ +\\ \text{H}_2\text{O}\end{matrix} \xrightarrow[\text{nucleophile}]{\text{NH}_2\text{NH–CO–R}'} \text{Enz–SH} + \begin{matrix}\text{R–CO–NH}\\ |\\ \text{R}'\text{–CO–NH}\end{matrix} \cdot \qquad [3]$$

By utilizing achiral, chiral, and racemic *N*-acylamino acids and their hydrazides, as well as aniline, a thorough exposure of the enzyme's response to diversified reactant combinations was afforded. Although anilides have been prepared from substrate *N*-acylamino acids (*2*) and their amides (*3*), this is the first report of submission of such hydrazides to anilide synthesis, which was brought into clear perspective by enforcing procedures. These forcing methods also revealed an outstanding difference between the stereospecific response of *Z*-DL-Ala-$NHNH_2$ as an electrophile and its stereoselective response as a nucleophile.

RESULTS AND DISCUSSION

Enforced Nucleophilic Action of Hydrazides by Means of Z-Glycine and Z-L-Alanine

An appropriate excess of *Z*-Gly-OH or *Z*-L-Ala-OH with *Z*-Gly-$NHNH_2$, *Z*-D-Ala-$NHNH_2$, *Z*-L-Ala-$NHNH_2$, or *Z*-DL-Ala-$NHNH_2$ smoothly promoted access to desirable N^1,N^2-diacylhydrazines (Eq. [3]). Only recently, N^1,N^2-bis(*Z*-glycyl)hydrazine was prepared (*1*) from *Z*-Gly-$NHNH_2$ as a single substrate for catalysis by crude papain. This same compound was obtained when *Z*-Gly-$NHNH_2$ was used with excess *Z*-Gly-OH. The two chiral hydrazides, in their independent reactions with *Z*-Gly-OH, displayed a considerably more rapid production of N^1-(*Z*-glycyl)-N^2-(*Z*-L-alanyl)hydrazine than N^1-(*Z*-glycyl)-N^2-(*Z*-D-alanyl)hydrazine under equivalent reaction conditions. Important data are compared in Table 1. More significant was the enzyme's moderately stereoselective interaction when racemic *Z*-DL-Ala-$NHNH_2$ and excess *Z*-Gly-OH were the reactants, as shown in Fig. 1. Isolation of mixtures of enantiomeric products after monitored incubation periods permitted specific rotations in DMF to be established. Details are itemized in Table 2. At the start, the principal component of the

TABLE 1

PURE N^1, N^2-DIACYLHYDRAZINES PREPARED FROM EXCESS Z-Gly-OH OR Z-L-Ala-OH WITH THREE ACHIRAL AND CHIRAL HYDRAZIDES[a]

Reactants		Product	Melting point (°C)	$[\alpha]_D^{25}$ in DMF (°)	Percentage yield
Z-Gly-OH	Z-Gly-NH | NH_2	Z-Gly-NH | Z-Gly-NH	216–218	Achiral	69
Z-Gly-OH	Z-D-Ala-NH | NH_2	Z-Gly-NH | Z-D-Ala-NH	228–230	+11.2	53
Z-Gly-OH	Z-L-Ala-NH | NH_2	Z-Gly-NH | Z-L-Ala-NH	228–230	−11.2	71
Z-L-Ala-OH	Z-Gly-NH | NH_2	Z-L-Ala-NH | Z-Gly-NH	228–230	−11.2	86
Z-L-Ala-OH	Z-D-Ala-NH | NH_2	Z-L-Ala-NH | Z-D-Ala-NH	253–255	0.00	54
Z-L-Ala-OH	Z-L-Ala-NH | NH_2	Z-L-Ala-NH | Z-L-Ala-NH	246–247	−21.7	76

[a] See Experimental for conditions of reactions.

product was Z-Gly-NH–NH-L-Ala-Z. As Z-L-Ala-$NHNH_2$ was continuously removed from the medium, Z-D-Ala-$NHNH_2$ became a more important competitor in attacking the enzyme–substrate thioester. Terminally, depletion of Z-L-Ala-$NHNH_2$ was so extensive that optically pure Z-Gly-NH–NH-D-Ala-Z was the sole product. Since the enzyme's stereoselective power was only moderate, it was not possible to secure optically pure Z-Gly-NH–NH-L-Ala-Z from the initial incubation interval.

Replacement of Z-Gly-OH with a correspondingly large quantity of Z-L-Ala-OH as the competitive electrophile, in combination with the same set of hydrazides, also exposed an appreciably stereoselective bias toward the L-hydrazide. Properties of pure products formed from achiral or chiral hydrazides are listed in Table 1. Racemic Z-DL-Ala-$NHNH_2$ provided further insight into the extent of stereochemical control when enforced nucleophilic behavior was again implemented, portions of which are summarized in Table 3. Relative rates of precipitation of mixtures of diastereoisomers and calculated amounts of each component are presented in Fig. 2. Mixtures contained optically active N^1,N^2-bis(Z-L-alanyl)hydrazine and meso N^1-(Z-D-alanyl)-N^2-(Z-L-alanyl)hydrazine. Reactions are formulated in Eq. [7], through a simple substitution of each Z-Gly with a Z-L-Ala. The same optically active product was prepared much earlier (*4*) from Z-DL-Ala-OH and hydrazine under papain catalysis and also more recently

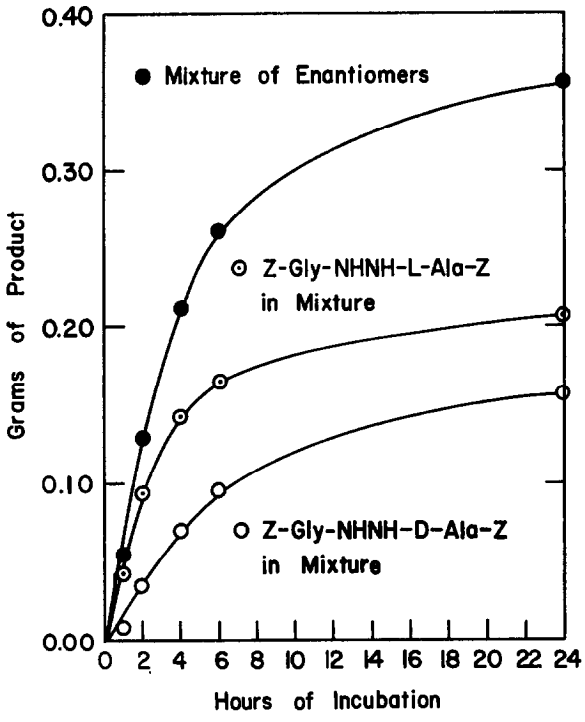


FIG. 1. Rates of precipitation of N^1-(*Z*-glycyl)-N^2-(*Z*-alanyl)hydrazines for reactions between excess *Z*-Gly-OH and *Z*-DL-Ala-$NHNH_2$ under catalysis by crude papain (procedural details under Experimental) at 40°C.

(*1*) from *Z*-L-Ala-$NHNH_2$ as a single substrate. The meso product was previously isolated (*1*) from late incubation periods when *Z*-DL-Ala-$NHNH_2$ served as the sole reactant.

TABLE 2

MIXTURES OF *Z*-Gly-NH–NH-L-Ala-*Z* AND *Z*-Gly-NH–NH-D-Ala-*Z* PRODUCED FROM *Z*-DL-Ala-$NHNH_2$ AND EXCESS *Z*-Gly-OH AT 40°[a]

Incubation period (hr)	Product weight (g)	Melting point (°C)	$[\alpha]_D^{25}$ in DMF (°)	Percentage L enantiomer in mixture
0–1	0.0526	226–228	−7.59	83.9
1–2	0.0772	224–226	−3.33	64.9
2–4	0.0820	220–222	−1.36	56.1
4–6	0.0502	219–221	+0.98	45.6
6–24	0.0960	221–223	+1.85	41.7
24–48	0.0404	224–226	+3.11	36.1
48–168	0.0107	228–230	+11.2	0.00

[a] See Experimental for reaction conditions.

TABLE 3

MIXTURES OF Z-L-Ala-NH–NH-L-Ala-Z AND *meso* Z-D-Ala-NH–NH-L-Ala-Z PRODUCED FROM Z-DL-Ala-$NHNH_2$ AND EXCESS Z-L-Ala-OH AT 40°[a]

Incubation period (hr)	Product weight (g)	Melting point (°C)	$[\alpha]_D^{25}$ in DMF (°)	Percentage *meso* in mixture
0–1	2.7324	231–233	−16.3	25.1
1–2	0.8435	229–230	−14.5	33.2
2–3	0.3303	231–233	−7.80	64.1
3–4	0.2112	236–238	−5.15	76.3
4–5	0.1572	238–240	−3.80	82.5
5–6	0.1279	243–245	−3.50	83.9
6–9	0.2690	245–247	−2.85	86.9
9–12	0.2010	246–248	−2.80	87.1
12–24	0.4010	245–247	−2.70	87.6
24–48	0.2235	244–247	—	
48–90	0.1024	—	—	

[a] Conditions of experiment are given under Experimental.

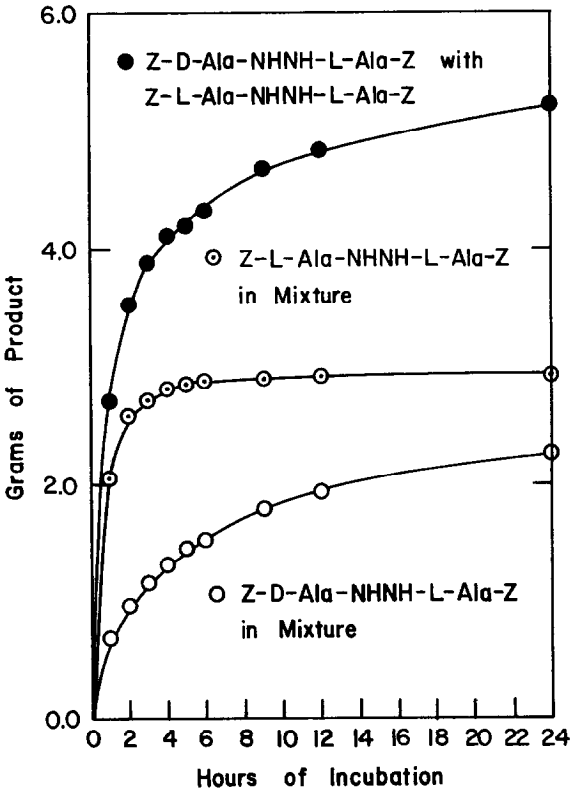


FIG. 2. Rates or precipitation of N^1, N^2-diacylhydrazines for reactions between excess Z-L-Ala-OH and Z-DL-Ala-$NHNH_2$ under catalysis by crude papain (procedural details under Experimental) at 40°C.

Stereospecific Response of Z-DL-Ala-OH When Used to Implement Nucleophilic Behavior from Achiral and Chiral Hydrazides

A large proportion of Z-DL-Ala-OH was utilized in conjunction with achiral Z-Gly-$NHNH_2$ and each of the two chiral hydrazides, Z-D-Ala-$NHNH_2$ and Z-L-Ala-$NHNH_2$, in successful enforcement of nucleophilic behavior on the part of all three hydrazides. Since Z-DL-Ala-OH assumed an electrophilic role, there was an essentially stereospecific interaction with the enzyme. Resultant diacylhydrazines (Eq. [4]) were optically active Z-Gly-NH–NH-L-Ala-Z, *meso*-Z-D-Ala-NH–NH-L-Ala-Z and optically active Z-L-Ala-NH–NH-L-Ala-Z. Properties of the products obtained here and by other means are listed in Table 1.

$$\begin{array}{l} \text{Z-DL-Ala-OH} \\ + \\ \text{Enz–SH} \end{array} \xrightarrow[\text{response}]{\text{stereospecific}} \begin{array}{l} \text{Z-L-Ala} \\ | \\ \text{Enz–S} \end{array} \xrightarrow{\text{NH}_2\text{NH–CO–R}} \begin{array}{l} \text{Z-L-Ala-NH} \\ | \\ \text{R–CO–NH} \end{array} \cdot \qquad [4]$$

Electrophilicity of Hydrazides Promoted by Aniline as the Competitive Nucleophile

Electrophilic action of four hydrazides, namely, hippuric, Z-glycine, Z-L-alanine, and Z-DL-alanine hydrazides was examined. Aniline, in considerable excess, was the competitive nucleophile. Perusal of the experimental section reveals that limitation of the hydrazides to an electrophilic role was not absolute. Early incubation periods yielded a certain quantity of N^1,N^2-diacylhydrazines, which would necessitate at least some degree of nucleophilic action (Eq. [1]). Subsequent to such introductory periods, successful restriction to electrophilic behavior did produce pure anilides of N-acylamino acids (Eq. [2]), the properties of which are given in Table 4. Crude papain was stereospecific toward Z-DL-Ala-$NHNH_2$ when the racemic hydrazide functioned as an electrophile (Eq. [6]). This was confirmed by the specific rotation of the product, Z-L-alanine anilide, $[\alpha]_D^{25}$ −37.4°, in pyridine, which has been reported for the same compound by another synthesis (5).

TABLE 4

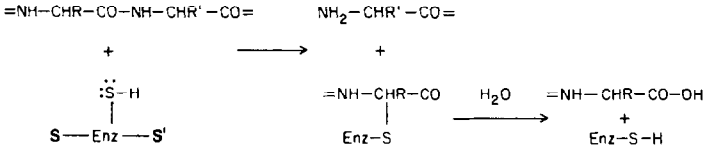
ANILIDES PREPARED FROM HYDRAZIDES OF N-ACYLAMINO ACIDS AND EXCESS ANILINE[a]

Hydrazide reactant	Anilide product	Melting point (°C)	$[\alpha]_D^{25}$ in pyridine (°)	Percentage yield
Hippuric	Hipp-NHPh	209–212	Achiral	44
Z-Glycine	Z-Gly-NHPh	141–143	Achiral	51
A-L-Alanine	Z-L-Ala-NHPh	161–163	−37.4	76
Z-DL-Alanine	Z-L-Ala-NHPh	161–163	−37.4	38

[a] See Experimental for reaction conditions.

Respective Stereospecific and Stereoselective Response at the S *and* S′ *Subsites of Crude Papain*

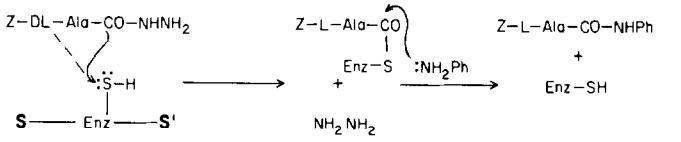
When a polypeptide substrate is cleaved by papain (*6, 7, 8*), a thioester intermediate is produced that includes those substrate residues accommodated at the S-subsite region. Concurrently, a terminal amino group is formed for those residues that reside at the S′-subsite zone. Subsequent attack by water regenerates the sulfhydryl group of the enzyme:



[5]

All of the components that comprise the reactive mixture of sulfhydryl proteolytic enzymes of crude papain (*9, 10*) would also contain S and S′ subsites, with a necessary cysteine residue for catalytic action. By utilizing a p-nitroanilide (*11*) or an appropriate ester (*12*) of an N-blocked amino acid as a substrate under carefully controlled conditions, far more intricate, mechanistic details of catalytic hydrolysis than previously established (*13, 14, 15, 16*) have recently been elucidated.

Racemic Z-DL-Ala-$NHNH_2$, as an electrophile, must approach the sulfhydryl group at the catalytic site from the S-subsite zone, if acylation is to be achieved. Stereospecific interaction then gives rise to Z-L-alanine anilide, when aniline is the nucleophile:

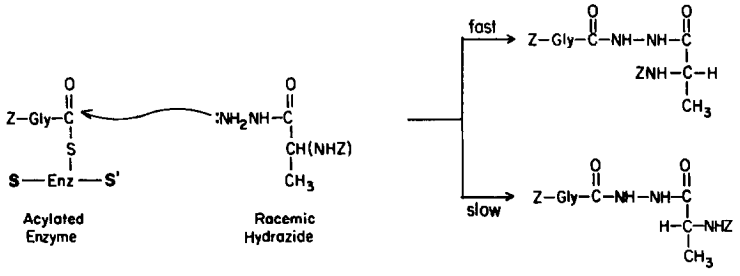


[6]

Racemic *N*-acylamino acids (*2*) and Z-DL-alanine amide (*3*) can likewise encounter the S-subsite as electrophiles with resultant stereospecific interaction prior to yielding anilides. Adequate binding of substrate to the enzyme is required before acylation can occur (*9*). In order for binding to be effective in producing the thioester intermediate, an amino acid residue requires spatial accommodation by the S subsite, so that its carbonyl can be in catalytic contact (*11, 12*) with the sulfhydryl group. A space-filled model of papain (*8, 16*) has shown that factors of such binding are so spatially dependent that a D enantiomer is excluded. When the dominant catalytic components of crude papain exhibit an essentially stereospecific, net interaction toward a racemic substrate, similar spatial considerations at their S subsites would also be involved. Slight spatial tolerance of a D enantiomer might escape polarimetric detection during product analysis. Tolerance would allow both enantiomers to compete for a catalytic site. Extended incubation periods could permit the D enantiomer to become a sufficiently effective competitor so that detection might then be possible. This was not the

case in the current work. Utilization of a large amount of *Z*-DL-Ala-OH as an electrophile source, with achiral and chiral hydrazides as nucleophiles (Eq. [3]), established that *Z*-D-Ala-OH was a spatial misfit at the S subsites in competing with the L enantiomer.

Far less differential spatial restriction was encountered when the D and L enantiomers of *Z*-DL-Ala-$NHNH_2$ entered the S′-subsite zone as nucleophiles. An amino portion of each hydrazide could therefore make proper contact with the carbonyl of a thioester intermediate, with production of an insoluble diacylhydrazine. Although the L-hydrazide provided a better fit, the net result of the concurrent reactions was only modestly stereoselective. This is shown in Eq. [7] for a preformed thioester intermediate from *Z*-Gly-OH as the competitive electrophile.



[7]

The protruding hydrazide group, $-CO-NHNH_2$, might further reduce the extent of stereoselectivity by allowing the chiral centers of *Z*-DL-Ala-$NHNH_2$ to be placed in a less spatially limiting area at the S′ subsite. Additional reduction in the magnitude of stereoselectivity could possibly have arisen due to a reversal in direction of the carbonyl and nitrogen of the amino acid residue, –CO–CHR–NH–, when the hydrazide entered from the S′ subsite, as compared to entrance from the S subsite.

It is apparent that crude papain could be used for the facile synthesis of complex diacylhydrazines, with predetermined configuration, in quantities of several grams.

EXPERIMENTAL

Preparation of Active Crude Papain

The preparation of the active crude papain used in these experiments has been previously described (*1*). Ten grams was ground to a homogeneous powder, carefully sealed in an air-tight bottle and stored under refrigeration for the present research.

Preparation of Substrate Hydrazides

Synthesis of hippuric hydrazide involved conversion of hippuric acid into its methyl ester via a catalytic dehydrator procedure (*3, 17*). Methyl hippurate was

subsequently treated with 98.5% aqueous hydrazine dissolved in absolute methanol to obtain the desired hydrazide (*1*). Esters of the *Z*-amino acids were prepared by treatment of the *Z*-amino acids with dry HCl in an absolute alcohol (*2, 18*). This was followed by a similar exposure to 98.5% aqueous hydrazine in methanol to produce the corresponding hydrazides in excellent yield. All of the hydrazides were known previously through other synthetic methods: hippuric (*19, 20*), *Z*-glycine (*18*), *Z*-D- (*21*), *Z*-L- (*21*), and *Z*-DL- (*22*) alanine hydrazides.

General Procedure for Papain-Catalyzed Reactions of Excess N-Acylamino Acids with Hydrazides of N-Acylamino Acids

A sample of hydrazide (0.5000 g) was added to a 5:1 molar excess of *N*-acylamino acid and ground in a mortar to a fine powder. The solid mixture was immediately dissolved in 225 ml of 0.6 *M* acetate buffer, pH 4.5, and subsequently filtered through a medium porosity, sintered glass funnel. Crude, active papain (0.1000 g) was mixed with an equal quantity of L-cysteine · HCl · H_2O, ground thoroughly and then dissolved in 15 ml of 0.6 *M* acetate buffer, pH 4.5. The solution was then filtered through a medium porosity, sintered glass funnel. The funnel was rinsed under suction into the filtrate with 10 ml buffer. The two filtrates were warmed to 40°C, mixed rapidly with stirring, and then incubated at 40°C. Solid products were collected at monitored intervals on a Büchner funnel, washed with 200 ml of deionized water, and dried under vacuum over P_2O_5. Comparative data are shown in Tables 1, 2, and 3. Periods of incubation, weights of product and other pertinent information are listed below according to reactants.

Z-Glycine and Z-glycine hydrazide. 0–1 hr, 0.2097 g; 1–4 hr, 0.1344 g; 4–24 hr, 0.1760 g; 24–48 hr, 0.0812 g, % N: Calcd 13.42, found 13.67. See Table 1.

Z-Glycine and Z-L-alanine hydrazide. 0–1 hr, 0.1686 g; 1–2 hr, 0.1111 g; 2–4 hr, 0.1323 g; 4–5 hr, 0.0578 g; 5–7 hr, 0.0527 g; 7–24 hr, 0.1964 g; 24–48 hr, 0.0377 g. % N: Calcd 13.08, found 13.05. See Table 1.

Z-Glycine and Z-D-alanine hydrazide. 0–1 hr, 0.0725 g; 1–2 hr, 0.0635 g; 2–4 hr, 0.1047 g; 4–5 hr, 0.0578 g; 5–7 hr, 0.0376 g; 7–24 hr, 0.1331 g; 24–48 hr, 0.293 g; 2–7 days 0.0042 g. % N: Calcd 13.08, found 13.34. See Table 1.

Z-L-Alanine and Z-glycine hydrazide. 0–1 hr, 0.4657 g; 1–2 hr, 0.1098 g; 2–3 hr, 0.0664 g; 3–4 hr, 0.0363 g; 4–24 hr, 0.0577 g; 24–48 hr, 0.0384 g. % N: Calcd 13.08, found 13.05. See Table 1.

Z-L-Alanine and Z-L-alanine hydrazide. 0–1 hr, 0.4722 g; 1–2 hr, 0.0877 g; 2–3 hr, 0.0170 g; 3–4 hr, 0.0058 g; 4–5 hr, 0.0038 g; 5–6 hr, 0.0019 g; 6–24 hr, 0.1200 g. % N: Calcd 12.66, found 12.83. See Table 1.

Z-L-Alanine and Z-D-alanine hydrazide. 0–1 hr, 0.2657 g; 1–2 hr, 0.0790 g; 2–3 hr, 0.0142 g; 3–4 hr, 0.0062 g; 4–5 hr, 0.0080 g; 5–6 hr, 0.0034 g; 6–9 hr, 0.0046 g; 9–24 hr 0.1204 g. % N: Calcd 12.66, found 12.71. See Table 1.

Z-DL-Alanine and Z-glycine hydrazide. 0–1 hr, 0.5064 g; 1–2 hr, 0.0938 g; 2–3 hr, 0.0664 g; 3–4 hr, 0.0374 g; 4–24 hr, 0.0660 g; 24–48 hr, 0.0398 g, % N: Calcd 13.08, found 13.33; 90% yield; mp 228–230°C; $[\alpha]_D^{25}$-11.2° in DMF.

Z-DL-Alanine and Z-L-alanine hydrazide. 0–1 hr, 0.1625 g; 1–2 hr, 0.1930 g; 2–3

hr, 0.0808 g; 3–6 hr, 0.0483 g; 6–24 hr, 0.0328 g. % N: Calcd 12.66, found 12.88; 52% yield; mp 241–243°; $[\alpha]_D^{25}$-21.7° in DMF.

*Z-*DL*-alanine and Z-*D*-alanine hydrazide.* 0–1 hr, 0.1374 g; 1–2 hr, 0.0790 g; 2–3 hr, 0.0403 g; 3–6 hr, 0.0698 g; 6–24 hr, 0.0680 g; % N: Calcd 12.66, found 12.73; 43% yield; mp 253–255°; $[\alpha]_D^{25}$-0.00° in DMF.

*Z-Glycine and Z-*DL*-alanine hydrazide.* % N: Calcd 13.08, found 13.20; 45% yield. See Table 2 and Fig. 1.

*Z-*L*-Alanine and Z-*DL*-alanine hydrazide.* Quantities of reactants were eight times those described in the general experimental procedure. % N: Calcd 12.66, found 12.57; 78% yield. See Table 3 and Fig. 2.

General Procedure for Papain-Catalyzed Reactions of Achiral and Chiral Hydrazides of N-Acylamino Acids with Excess Aniline

A mixture of 4.3 ml of redistilled aniline and 100 ml of 0.5 *M* acetate buffer, pH 4.5, was used in small portions to triturate 1.000 g of hydrazide. For hippuric and *Z*-glycine hydrazides, 8 ml of hexamethylphosphoramide[2] was added. When dissolution was complete, the mixture was filtered through a Büncher funnel and then through a medium porosity, sintered glass funnel. Samples of active, crude papain and L-cysteine · HCl · H_2O, each weighing 0.2000 g, were ground thoroughly in a mortar and dissolved with vigorous stirring in the solution containing aniline and hydrazide. The resultant solution was filtered rapidly through a medium porosity, sintered glass funnel and incubated at 40°C. Products were collected at successive time intervals, washed with 200 ml of deionized water and dried over P_2O_5. Periods of incubation, weights of product and other significant details are listed below with respect to anilide product.

Hippuric anilide. 0–4 hr, product was N^1,N^2-dihippurylhydrazine (*1*), 0.0258 g, mp 252-254°C, % N: Calcd 15.81, found 15.87; 4–24 hr, 0.1862 g; 1–2 days, 0.2111 g; 2–4 days, 0.1783 g; 4–7 days, 0.0048 g; % N: calcd 11.02, found 11.23. See Table 4.

Z-Glycine anilide. 0–4 hr, product was *Z*-Gly-NH–NH-Gly-*Z* (*1*), 0.1038 g, mp 217–219°C, % N: Calcd 13.52, found 13.30, 4–24 hr, 0.2508 g; 1–2 days, 0.2007 g; 2–4 days, 0.1629 g; 4–7 days, 0.0302 g; % N: Calcd 9.85, found 9.80. See Table 4.

*Z-*L*-Alanine anilide from Z-*DL*-alanine hydrazide.* All quantities described in general experimental procedure were tripled for this reaction. 0–4 hr, product was a mixture of *Z*-L-Ala-NH–NH-L-Ala-*Z* and *Z*-D-Ala-NH–NH-L-Ala-*Z* (*1*), 0.0881 g, mp 245–255°C, % N: Calcd 12.66, found 12.87, 4–24 hr, 1.1024 g; 1–2 days, 0.3416 g; %N: Calcd 9.39, found 9.26. See Table 4.

Polarimetric Measurements

High optical rotations were measured in either a 1 or 2-dm, water-jacketed polarimeter tube by means of a Rudolph Model 80 high precision polarimeter at 25°C, D-line sodium, *c* 0.01 g/ml. Lower observed values were similarly measured

[2] Hexamethylphosphoramide has been shown to cause nasal cancer in mice and should be handled with extreme care.

by means of a Perkin–Elmer Model 243 polarimeter. Spectrograde solvents were either DMF or pyridine.

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