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MECHANISTIC ALTERATION IN MICELLAR PEPTIDE ESTEROLYSIS REACTIONS

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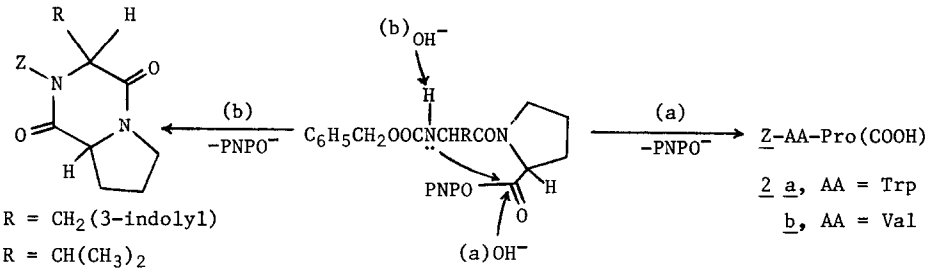
Summary. Buffer or CTACl-catalyzed basic cleavages of Z-D- or L-AA-L-Pro p-nitrophenyl esters proceed mainly via intramolecular cyclization to diketopiperazines. These reactions are more facile with DL than with LL substrates.

Large micellar stereoselectivities were observed in pH 8 esterolyses of the diastereomeric dipeptide esters DL or LL-1.[2] Hydrolysis in buffer or in nonfunctional micellar cetyltrimethylammonium chloride (CTACl) was DL-diastereoselective, but thiolysis by the micellar thiocholine surfactant 16-SH [n-$C_{16}H_{33}\overset{+}{N}(CH_3)_2CH_2CH_2SH$, Cl^-] was LL-diastereoselective.[2] For example, pseudo-first-order micellar rate constant ratios for the liberation of p-nitrophenoxide ion from Z-Trp-Pro-PNP (1a) were $(k_\psi^{LL}/k_\psi^{DL})_{buffer} = 0.29$ and $(k_\psi^{LL}/k_\psi^{DL})_{16\text{-}SH} = 5.0$ at pH 8. With Z-Val-Pro-PNP, the analogous ratios were 0.14 and 3.2. Defining the change in diastereoselectivity as $(k_\psi^{LL}/k_\psi^{DL})_{16\text{-}SH}/(k_\psi^{LL}/k_\psi^{DL})_{buffer}$, net diastereoselectivities of 17 and 23 are obtained for the Trp and Val dipeptide esters. Although nonfunctional micellar CTACl accelerated the cleavages of substrates 1 by factors of 10-20, the catalysis was comparable for diastereomers, so that the net diastereoselectivities were very similar to those of the buffer (non-micellar) reactions.

The LL diastereoselectivity of 16-SH/dipeptide esterolyses can be understood in terms of specific substrate surfactant interactions,[2,3] and this rationale has now been successfully extended to the LL-diastereoselective 16-SH thiolyses of four tripeptide-PNP esters.[4] But what is the origin of the DL-diastereoselectivity observed in the buffer or CTACl-catalyzed cleavages? We now demonstrate that (a) buffer hydrolyses of substrates 1 proceed mainly by intramolecular cleavage to diketopiperazines, (b) that such cyclizations are DL-diastereoselective for steric reasons, and (c) that competition between intramolecular and intermolecular esterolysis qualitatively accounts for the observed kinetic diastereoselectivities.

We can imagine two mechanisms for OH^- cleavage of 1: direct attack at the substrate's scissile carbonyl, leading to Z-AA-Pro(COOH) (2, eq. 1, path a), or attack at H-N of the remote amino acid, activating nitrogen as an internal nucleophile, and leading to intramolecular cyclization with diketopiperazine formation (3, eq. 1, path b). The latter process is precedented: hydrolysis of Z-Gly-Pro-PNP (1, R = H) in pH 8 aqueous dioxane gave 3 (R = H) in 62% yield.[5] The same reaction was encountered by Lucente,[6] who showed that related reactions could



(1)

3 a, R = CH_2(3-indolyl)
b, R = $CH(CH_3)_2$

2 a, AA = Trp
b, AA = Val

1 a, R = CH_2(3-indolyl) = Z-Trp-Pro-PNP
b, R = $CH(CH_3)_2$ = Z-Val-Pro-PNP

occur with tripeptide Pro-PNP esters.[6,7] However, these reactions have not been kinetically studied with amino acids other than Gly adjacent to Pro, so that their intrinsic diastereoselectivities remain uncharacterized.

DL- and LL-Z-Trp-Pro-PNP[2] and DL- and LL-Z-Val-Pro-PNP[2] (1a and 2a) were hydrolyzed in pH 8 0.02M PO_4 buffer, with or without 4 x 10^{-3}M CTACl, at 25°. Varying quantities of dioxane were added to ensure homogeneity, and reactions were carried to ≥ 90% completion except for LL-1a in CTACl (67%). Conditions are specified in Table I. After hydrolysis, reaction solutions were acidified to pH 1 with HCl and lyophilized to dryness. Residues were leached with 2 ml of CH_2Cl_2, filtered (Millipore, FGLP-013), concentrated to ~0.5 ml and analyzed by hplc.[8] Products from 1a were DKP 3a and free acid (FA) 2a; analogous products, 3b and 2b, were obtained from 1b.[9-11] Product mixtures were quantitatively analyzed,[8] and the results are reported in Table I.

The data clearly show that buffer hydrolyses of dipeptide esters 1a and 1b give extensive DKP formation; eq. (1), path b. Corrected for subsequent hydrolysis, DKP is the major product from DL-1a, LL-1a, and DL-1b, and Z-AA-Pro(COOH) is the minor product; eq. (1), path a. Only with LL-1b does the free acid product exceed DKP.

The intramolecular DKP-cyclizations are more facile with the DL substrates, which afford larger 3/2 (DKP/FA) ratios; cf., Table I. Moreover, the buffer hydrolysis DKP/FA product ratios from DL-1a or DL-1b, when divided by the corresponding product ratios from the LL substrates, afford "final" DL/LL product distribution ratios which are very similar to the (kinetic) rate constant ratios for $PNPO^-$ release from the diastereomeric substrates (cf., the last 2 columns of the table). The simplest interpretation of these results proposes similar origins for the product and rate constant ratios: buffer hydrolysis and product formation proceed largely by intramolecular diketopiperazine (3) formation, and the outcome of the kinetic competition between OH^- assisted cyclization to DKP or OH^- cleavage to dipeptide acid is more strongly biased toward cyclization in the case of the DL substrates. Note that this explanation assumes that direct cleavage to 2 is a minor competitive pathway, the rate of which is relatively insensitive to substrate configuration.

Cyclization of DL-1 to DL-3 is kinetically more facile than in the LL cases because here the D-AA side groups (CH_2-indolyl or i-Pr) are anti to the L-Pro $(CH_2)_3$ substituent, relative to the forming DKP ring. Steric interactions during cyclization are thus minimized in the DL

Table I. Products from the pH 8 Hydrolysis of Dipeptide Esters

Substrate	Conditions	% DKP[a]	Corr. Factor[b]	Corr. % DKP[c]	DKP/FA[d]	$\frac{(DKP/FA)^{DL}}{(DKP/FA)^{LL}}$	$\frac{k_{\psi}^{DL}}{k_{\psi}^{LL}}$[e]
DL-1a	buffer[f], 24 h	75	0.83	90	9.0	3.0	3.4
LL-1a	buffer[f], 24 h	59	0.79[g]	75	3.0		
DL-1a	CTACl[h], 0.25 h	91	0.96	94	16.	5.8	3.5
LL-1a	CTACl[h], 0.50 h	69	0.95[g]	73	2.7		
DL-1b	buffer[i], 18 h	23	0.27	85	5.7	7.0	7.0
LL-1b	buffer[i], 36 h	16	g,j	45	0.82		
DL-1b	CTACl[h], 0.25 h	92	0.94	98	49	73.[k]	10.
LL-1b	CTACl[h], 2 h	32	g,j	40	0.67		

[a]DKP = diketopiperazine (3). [b]Correction for the hydrolysis of 3 to 2 under these reaction conditions; equal to the fraction of surviving 3; the balance is 2. These factors were obtained by hplc analyses of appropriate control hydrolyses of purified DKP. [c]Corrected % DKP = % DKP/correction factor; entries are rounded to 2 places after arithmetical operations. [d]FA = yield of free acid (2), corrected for additional free acid produced by DKP hydrolysis. DKP + FA is normalized to 100%. [e]Data from ref. 2. The addition of 20% dioxane to the buffer hydrolytic media has little effect on this rate constant ratio. [f]0.02M PO_4, μ = 0.05 (KCl) + 20% dioxane; [1] = 4 x 10^{-5}M. [g]The control hydrolysis used mostly LD-DKP. [h]4 x 10^{-3}M CTACl in 0.02M PO_4 buffer, μ = 0.05 (KCl) + 1% dioxane; [1] = 2 x 10^{-4}M. [i]Conditions as in f, but with 2% dioxane. [j]Corrected by extrapolation to zero-time of 4-5 DKP/FA ratios observed during the control hydrolysis of LL-1b. [k]See text.

series. Cyclization of LL-1 to LL-3, however, is comparatively slow due to steric hindrance between the now syn L-AA and L-Pro substituents.[13] DL (or LD) trans DKP isomers [cyclo(AA-Pro); AA = Ala, Phe, Leu, or Val] are known to be thermodynamically more stable than their cis (LL or DD) diastereomers, the extent of DL preference increasing with growing bulk of the AA side group.[14] Although the precise steric interactions which determine the equilibrium diastereomer compositions are complicated,[14-16] and not necessarily identical to those transition state interactions which determine the relative kinetics of DL-1/LL-1 cyclization, we suspect that the thermodynamic preferences are closely related to the observed kinetic selectivities (table and ref. 2). Note that the magnitude of the kinetic DL/LL selectivity rationally depends on the size of the AA side group.[2]

As a further consequence of the steric instability of the LL-cyclo(AA-Pro) isomers, we isolated LD-cyclo(Z-Trp-Pro or Z-Val-Pro) from hydrolyses of LL-1a or LL-1b. These compounds presumably form by epimerization of the initial LL-3a or LL-3b products, and are enantiomers of the DL DKP's formed from DL-1. Rapid epimerization of (e.g.) LL-cyclo(Phe-Pro) to LD-cyclo(Phe-Pro) in dilute base has been reported.[17]

Table I shows that preferential DKP formation from DL-1 also occurs in CTACl micelle-catalyzed[2] hydrolyses; OH^- bound to cationic CTACl micelles presumably accelerates both the intramolecular cyclization and intermolecular cleavage reactions of eq. (1). The final DL/LL DKP/FA product distribution ratio from CTACl hydrolyses of DL and LL-1a (5.8) is somewhat higher

than that observed in the buffer hydrolyses of these substrates (3.0), although the actual product distributions are very similar. However, this ratio appears to be considerably higher for CTACl hydrolyses of DL-1b (73 vs. 7.0). We are uncertain why this is so, although here there is difficulty in obtaining accurate results because the corrected yield of DL-2b is very low (≤ 2%) and its quantitation is imprecise.[8] Note, however, the similarity of the much more precise $(k_{\psi}^{DL})/k_{\psi}^{LL})$ ratios for buffer and CTACl hydrolyses of both 1a and 1b.

Finally, it is now clear that the exceptional net stereoselectivities observed in micellar hydrolyses of diastereomeric dipeptide esters 1 by 16-SH relative to CTACl (see above and ref. 2) originate in the different mechanisms which obtain in the two systems. LL-diastereoselectivity in the 16-SH reactions stems from specific substrate-surfactant interactions operative during intermolecular thiolate nucleophilic cleavage, whereas reversed, DL-diastereoselectivity in CTACl (or buffer) reactions is a consequence of intramolecular DKP-forming cyclization. The contrasting stereoselectivities associated with these two mechanisms add to yield dramatic net stereochemical changes.[2]

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(8) Hplc employed a Waters Radial-Pak A, reversed-phase C_{18} column. The product mixture from 1a was eluted with 25% H_2O/75% CH_3OH (1.3 ml/min); the 1b product mixture required 30% H_2O/70% CH_3OH (2 ml/min). Quantitation employed authentic standards and a (calibrated) uv detector (254 nm) coupled to a Waters Data Module. The analytical precisions (% a.d.) are <4% for 3a/2a in buffer and <6% in CTACl runs. Corresponding precisions are 4% and <9% in 3b/2b analyses. For both reaction series, the elution order was 2, p-nitrophenol, 3 and unreacted 1. Our conditions did not separate the diastereomers of 2 or 3.
(9) Product identities follow from hplc comparisons with authentic standards,[10,11] and from the fact that preparative scale hydrolyses of 1a and 1b gave the same products.
(10) Diketopiperazines were prepared by preparative scale hydrolysis[6] of DL 1a or 1b, purified by chromatography or recrystallization, and characterized by ir, nmr, microanalysis (C, H, N), and optical rotation. Details will appear in the Ph.D. Thesis of Y-S. Lee, Rutgers University, 1981
(11) Authentic Z-dipeptides (2) were prepared by coupling (N-methylmorpholine, hydroxybenztriazole, dicyclohexylcarbodiimide, THF) Z-D- or L-Trp (or Val) to L-Pro-OMe, followed by methyl ester hydrolysis (NaOH, CH_3OH). The crystalline acids were fully characterized, and could be converted (p-nitrophenyl trifluoroacetate, pyridine)[12] to DL or LL 1a or 1b, identical to samples prepared previously by coupling Z-D- or L-Trp (or Val) to L-Pro-PNP.[2]
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