Solution Conformation of the Thermolysin Inhibitors Carbobenzoxy-L-phenylalanine and β-Phenylpropionyl-L-phenylalanine and Comparison of the Solution Conformation to the Enzyme-Bound Conformation

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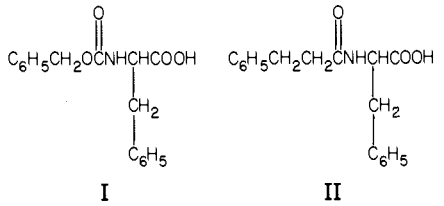
The conformations of enzyme inhibitors in solution and bound to the enzyme thermolysin are investigated as a convenient model for understanding the relationship between the conformation of drugs in solution and at the receptor. The solution conformations of carbobenzoxy-L-phenylalanine (I) and β-phenylpropionyl-L-phenylalanine (II) are determined using nuclear magnetic resonance spectroscopy. These studies show that I and II have essentially the same conformation about both the α–β bond and the C_α–N bond in Me_2SO-d_6, acetone-d_6, methanol-d_4, chloroform-d, and D_2O. In addition, the conformations of I and II are similar to phenylalanine and other phenylalanine derivatives. Comparison of the lowest energy solution conformer to that bound by the enzyme thermolysin shows that the lowest energy conformer (in solution) of carbobenzoxy-L-phenylalanine is bound by thermolysin; on the other hand, it is the highest energy conformer (in solution) of β-phenylpropionyl-L-phenylalanine which is bound to the enzyme. This, to our knowledge, is one of the first experimental confirmations of the hypothesis that an enzyme does not always bind the lowest energy conformer of an inhibitor.

Knowledge of the relationship between the conformation of drugs in the solid state, solution, and at the receptor is an important consideration for drug design and mechanism of action studies. The relationship between the solid state and solution conformation of a number of conformationally mobile drugs has been investigated in our laboratory.[1,2,4] This paper reports studies aimed at understanding the relationship between the solution conformation and the receptor-bound conformation of drugs. Because drug receptors are difficult to purify, we have chosen as a model for drug–receptor interaction the interaction of inhibitors with the enzyme thermolysin. The enzyme-bound conformation of the thermolysin inhibitors carbobenzoxy-L-phenylalanine (I) and β-phenylpropionyl-L-phenylalanine (II) have been determined using crystallographic techniques.[4] Surprisingly, these two phenylalanine derivatives have different enzyme-bound conformations, even though their structures are very similar. Previous studies in our laboratories[5] and other laboratories[6-8] indicate that phenylalanine and its derivatives have similar conformations in a variety of solvents. These two observations can only be explained if it is assumed that the enzyme binds the phenylalanine conformer with the best fit rather than the lowest energy. Otherwise the enzyme-bound conformer would be expected to be the same regardless of the derivative.



I II

This paper reports our investigation of the conformation of the two phenylalanine derivatives I and II using NMR spectroscopy in several solvents. Indeed, both carbobenzoxy-L-phenylalanine and β-phenylpropionyl-L-phenylalanine have essentially the same conformation in all solvents. Thus, this paper shows that an enzyme does not always bind the lowest energy solution conformer of an inhibitor but rather the conformer with the best fit.

Experimental Section

Carbobenzoxy-L-phenylalanine (I) was purchased from Sigma Chemical Co. and used without further purification. β-Phenylpropionyl-L-phenylalanine was prepared following literature procedures.[9]

The NMR spectra were obtained in 5-mm spinning tubes. Tetramethylsilane (Me_4Si) was used as an internal reference. The spectra were measured on a Varian FT-80 spectrometer or a Nicolet 360 MHz superconducting spectrometer. All solvents were purchased from Aldrich Chemical Co. and used without further purification.

Results

The NMR spectra of carbobenzoxy-L-phenylalanine and β-phenylpropionyl-L-phenylalanine were measured in a variety of solvents, and the conformation about the C_α–C_β bond of phenylalanine was analyzed.

NMR Spectra of Carbobenzoxy-L-phenylalanine (I) and β-Phenylpropionyl-L-phenylalanine (II). Tables I and II summarize the results of studies of the effects of solvent on the NMR spectra of I and II.

The chemical shifts of H_α, $H_{\beta 1}$, and $H_{\beta 2}$ were assigned based on the spectrum of (2*S*,3*R*)-[3-^{2}H]phenylalanine and its Schiff base,[5] (2*S*,3*R*)-[3-^{2}H]tyrosine[9] and (2*S*,3*R*)-2,4-dihydroxy[3-^{2}H]phenylalanine.[9] The spectra of all four of these compounds which contain magnetically different substituents show that $H_{\beta 1}$ appears at a higher chemical shift than $H_{\beta 2}$.

Analysis of the NMR Data for the C_α–C_β Bond of I and II. The coupling data for the H_α, $H_{\beta 1}$, and $H_{\beta 2}$ protons of carbobenzoxy-L-phenylalanine (I) and β-phe-

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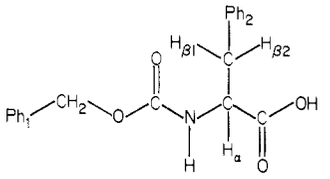
Table I. NMR Spectra (80 and 360 MHz) of Carbobenzoxy-L-phenylalanine (I) in Various Solvents



solvent	chem shift, δ					coupling constant, Hz			
	NH	CH_2	H_α	H_{β_1}	H_{β_2}	$J_{\beta_1-\beta_2}$	$J_{\alpha-\beta_1}$	$J_{\alpha-\beta_2}$	$J_{NH-C_\alpha H}$
Me_2SO-d_6 (5%)	7.67 (8.5 Hz)	4.97 (s)	4.14	2.84	3.05	14.0	10.9	4.9	
acetone-d_6 (5%)	6.45 (d, 8.6 Hz)	5.0 (s)	4.48	3.00	3.22	13.9	8.9	5.0	8.0
Me_2SO-d_6 + D_2O (50% v/v)[a]		4.92 (s)	4.27	2.87	3.11	13.9	10.2	4.8	
CD_3OD (5%)		4.92 (s)	4.38	2.91	3.17	14.0	9.3	4.8	
$CDCl_3$ (5%)[a]	5.30 (d)	5.09 (d)	4.69	3.09	3.19	14.0	6.7	5.6	7.1
D_2O (1%, pH 7.0)[a]			4.10	2.72	3.05	14.0	9.2	4.3	

[a] 360 MHz.

nylpropionyl-L-phenylalanine (II) can be straightforwardly analyzed using equations for ABX systems and the LAOCN3 program.[7,11,12] Table III summarizes the results of this analysis. For this analysis, J_g was assumed to be 2.6 Hz and J_t was assumed to be 13.6 Hz.[7,11] The equations for such a system are

$$N_A = \frac{J_{\alpha-\beta 1} - J_g}{J_t - J_g}$$

$$N_B = \frac{J_{\alpha-\beta 2} - J_g}{J_t - J_g}, \quad N_A + N_B + N_C = 1$$

An examination of Table III shows that for the organic solvents, the $J_{\alpha-\beta 1}$ coupling constant increases with increasing dielectric constant, while the $J_{\alpha-\beta 2}$ decreases. These differences in coupling constants are, of course, reflected in the fraction of rotamers present. As the dielectric constant of the medium increases, the fraction of N_A increases and the fraction of N_C decreases. Table IV reports the relative energies of the rotamers as a function of solvent.

Least-squares analysis of plots of the energy differences $E_B - E_A$ and $E_C - E_A$ vs. dielectric constant gave straight lines with correlation coefficients ranging from 0.93 to 0.99. The best straight-line correlation coefficient, 0.99, was obtained for the plot of $E_B - E_A$ vs. dielectric constant for β-phenylpropionyl-L-phenylalanine.

Analysis of the NMR for the $C_\alpha H$–NH Bond. The $C_\alpha H$–NH proton coupling constants are in the range of 7.1 to 8.0 Hz for carbobenzoxy-L-phenylalanine (I) and 7.2 to 8.5 Hz for β-phenylpropionyl-L-phenylalanine (II). These coupling constants are consistent with H–C_α–N–H dihedral angles in the range of 20–30° or 140–145° for I and 20–35° or 140–150° for II.[11]

Analysis of the NMR Data for the $-CH_2CH_2-$ Bond of β-Phenylpropionyl-L-phenylalanine. The protons of the ethylene group of β-phenylpropionyl-L-phenylalanine give triplets at 360 MHz. At 80 MHz the coupling pattern is of the A_2B_2 type, while at 360 MHz it is of the A_2X_2 type. This result indicates that the gauche and trans rotamers about this bond are nearly equal in energy.

Discussion

Rotamer Populations. The rotamer populations about the α–β bond of carbobenzoxyphenylalanine (I) and β-phenylpropionylphenylalanine (II) were analyzed using NMR spectroscopy. It was assumed that the idealized rotamers A, B, and C were present and a two-parameter equation was used to analyze the data. While reports have indicated that rotamers with nonidealized geometry and six-parameter equations should be used, recent studies indicate that the two-parameter equation used in this paper is nearly as accurate[11] as the more elaborate six-parameter equation. Since the purpose of this paper is to compare the solution and enzyme-bound conformation of two inhibitors, idealized conformations will be assumed to be present in solution and at the enzyme. This assumption adequately represents the accuracy of the X-ray data on the enzyme-bound conformation. The conformations of these inhibitors in solution and at the receptor will only be considered different if an entirely different rotamer is present.

Examination of Table III shows that in all solvents the conformations of the two thermolysin inhibitors I and II are similar, with Me_2SO-d_6 showing the greatest percentage difference, 76% N_A for I vs. 60% N_A for II. The similarity in the proportion of conformers is consistent with the structures of these compounds, which differ only by replacing a CH_2 group with an oxygen atom.

With the exception of D_2O, the variation in the proportion of conformers appears to parallel solvent polarity, with the more polar solvents favoring conformer A. A similar relationship was observed by Cavanaugh.[14] This is probably due to a combination of effects, including steric effects, which would favor A and B over C, and perhaps phenyl–phenyl stacking effects, which would favor A over B. However, the fact that Me_2SO (vs. $CDCl_3$) also increased the proportion of conformer A in both DD- and DL-*N*-acetylalanylphenylalanine from 41 to 64% and from 45 to 69%,[11] respectively, argues against a role for phenyl–phenyl stacking. Obviously, *N*-acetylalanine cannot exhibit phenyl–phenyl stacking. It should be noted that very small energies (see Table IV) are required to shift equilibria by this amount. In water, conformer C is more stable than B, perhaps because phenyl–phenyl stacking overrides the steric effect.

Table V shows the proportion of conformers A, B, and C in a number of phenylalanine derivatives. It is clear that the proportions of conformers of A and B are quite con-

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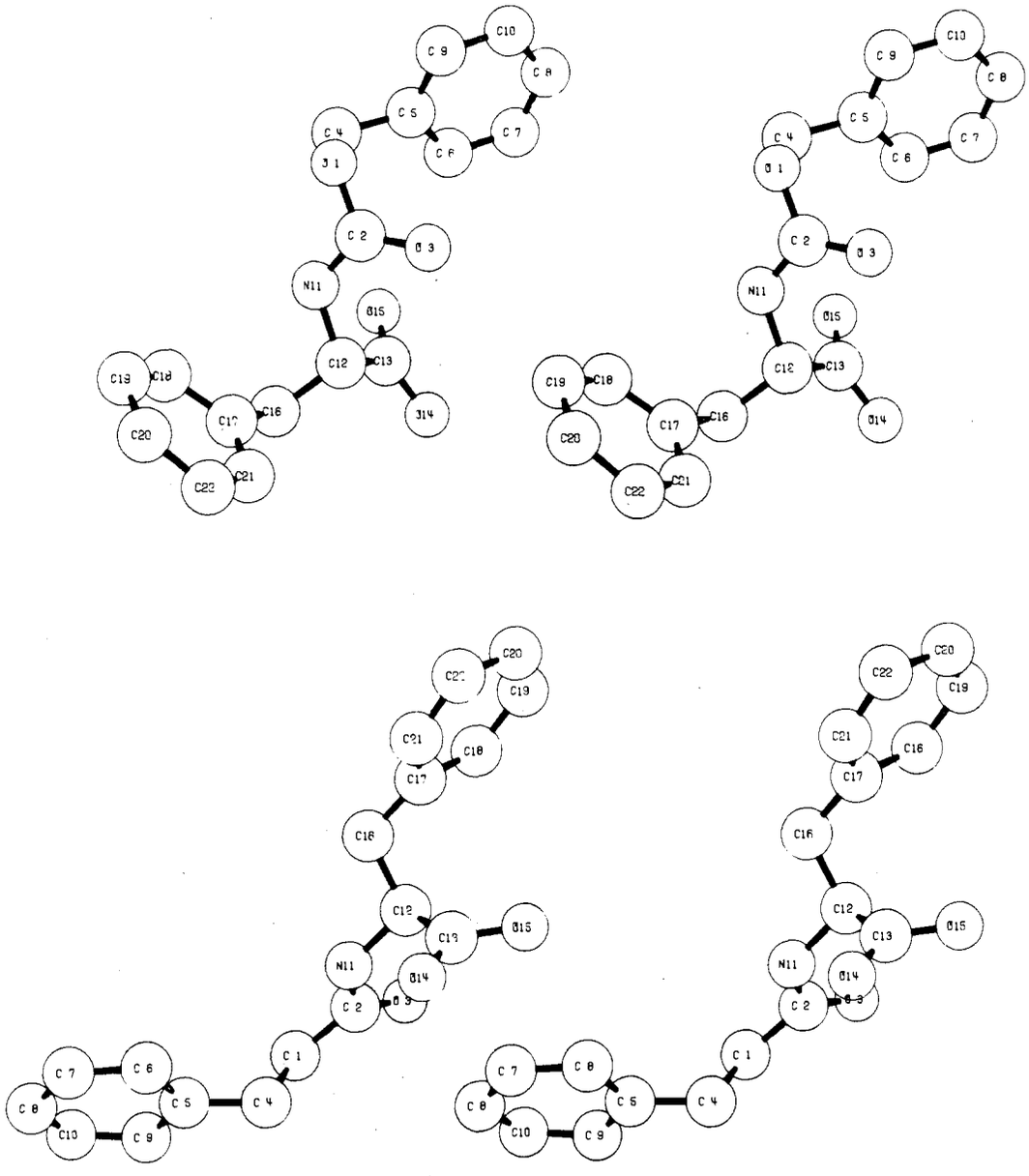


Figure 1. Stereoscopic drawings of carbobenzoxy-L-phenylalanine (top) and β-phenylpropionyl-L-phenylalanine (bottom) in their thermolysin-bound conformation. The coordinates were taken from Kester and Matthews.[6]

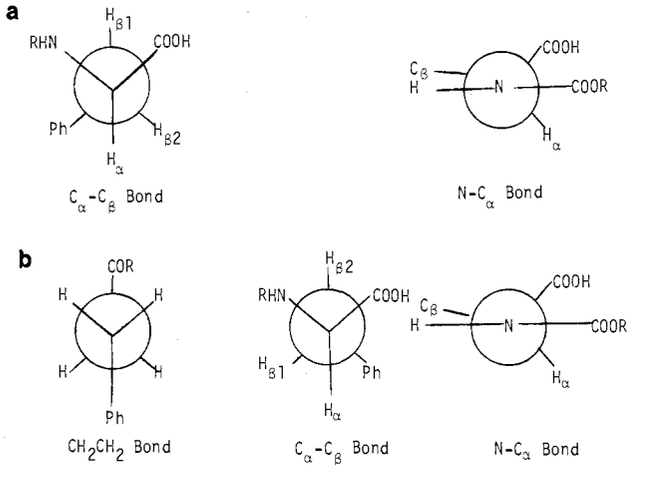


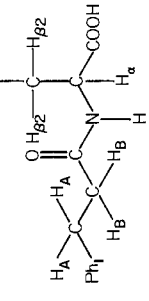
Figure 2. Idealized Newman projections of the enzyme-bound conformation of carbobenzoxy-L-phenylalanine (a) and β-phenylpropionyl-L-phenylalanine (b).

sistent with those of a number of phenylalanine derivatives.

The conformations of carbobenzoxy-L-phenylalanine (I) and β-phenylpropionyl-L-phenylalanine (II) about the C_αH–NH bond are also quite similar to that of other phenylalanines. The coupling constants for the C_αH–NH ranged from 7.1 to 8.5 Hz in I and II in various solvents, while the coupling constants for DD- and DL-*N*-acetylalanylphenylalanine ranged from 7.9 to 8.7 Hz,[11] and this coupling constant was 8.0 Hz in Met-Asp-Phe-NHz.[8] These data, along with published conformational maps, indicated that the ϕ value in Met-Asp-Phe-NH_2 was in the range of −90 to −100°.[8] Using similar reasoning, the ϕ value of carbobenzoxy-L-phenylalanine (I) and β-phenylpropionyl-L-phenylalanine (II) is also in the vicinity of −90°.

Comparison of the Rotamer Populations in Solution to the Rotamer Present When I and II Are Bound to Thermolysin. Figure 1 shows stereoscopic drawings of the conformation of carbobenzoxy-L-phenylalanine (I) and β-phenylpropionyl-L-phenylalanine (II) when bound to thermolysin. The coordinates for these drawings were taken from the literature.[4] Figure 2 shows the Newman projections of these enzyme-bound conformations.

Table II. NMR Spectra (80 and 360 MHz) of β-Phenylpropionyl-L-phenylalanine in Various Solvents



solvent	chem shift, δ						coupling constant, Hz				
	NH	CH_A	CH_B	H_α	H_{β_1}	H_{β_2}	J_{AB}	$J_{\beta_1-\beta_2}$	$J_{\alpha-\beta_1}$	$J_{\alpha-\beta_2}$	$J_{NH-C_\alpha H}$
Me_2SO-d_6 (5%)	8.15			4.45	2.70	3.05		14.0	9.2	5.0	8.5
Me_2SO-d_6 + D_2O (50% v/v)[a]		2.75 (t)	2.41 (t)	4.43	2.83	3.03	7.5	13.8	8.9	5.1	
acetone-d_6 (5%)		2.82 (m)	2.45 (m)	4.72	2.96	3.14	8.0	13.6	7.6	5.2	8.7
CD_3OD (5%)		2.75 (m)	2.37 (m)	4.63	2.90	3.14	7.8	14.1	8.8	5.1	
$CDCl_3$ (5%)[a]	5.79	2.93 (m)	2.49 (m)	4.86	3.08	3.16		14.1	6.6	5.9	7.2
D_2O (1%)		2.66 (t)	2.38 (t)	4.29	2.73	2.92	7.3	13.9	8.4	4.8	

[a] 360 MHz

C_α–C_β Bond. Examination of Figures 1 and 2 shows that the enzyme-bound conformation about the C_α–C_β bond is different for carbobenzoxyl-L-phenylalanine (I) and β-phenylpropionyl-L-phenylalanine (II). This is a striking result, since these compounds only differ by the replacement of an oxygen atom with a CH_2 group. Comparison of the Newman projection for I to the rotamers and populations shown in Table III establishes that the most stable and most populated rotamer of carbobenzoxy-L-phenylalanine is the rotamer which is bound by the enzyme. On the other hand, a similar comparison shows that the least stable rotamer of β-phenylpropionyl-L-phenylalanine (II) in D_2O is the rotamer bound by the enzyme. This rotamer is 575 cal/mol less stable than rotamer I in water at pH 7.0.

This is an important result which shows that an enzyme or receptor can bind a conformer other than the lowest energy conformer in solution. It is also important to note that the enzyme does not undergo a major conformational change upon binding these inhibitors. Thus, the energy lost in binding a slightly less stable solution conformer is compensated by inhibitor–enzyme interactions.

The data also rule out an explanation of this result in terms of an unusual solvent effect resulting in the stabilization of a relatively high energy conformer of β-phenylpropionyl-L-phenylalanine (II). The conformer proportions of II are quite similar to those of carbobenzoxy-L-phenylalanine and other phenylalanine derivatives (Table IV). In addition, alanylphenylalanine appears to bind to thermolysin in the same conformation as β-phenylpropionyl-L-phenylalanine, ruling out an anomalous binding mode for only one compound.[6]

C_α–N Bond. The solution NMR studies indicate that the φ angle of both carboxybenzyl-L-phenylalanine (I) and β-phenylpropionyl-L-phenylalanine (II) is in the range of −90 to −100°. Figures 1 and 2 show that the φ angle for both I and II bound to thermolysin is approximately −90°. Thus, the conformation about this bond is similar in solution and at the receptor.

CH_2–CH_2 Bond. In all solvents, the rotamers about this bond are of approximately equal energy. Examination of Figure 1 shows that the enzyme "traps" the trans rotamer out of solution. This is a common result. In addition, this "trapping" by an enzyme is equivalent to the "trapping" of rotamers which occurs during crystallization.

The Influence of Inhibitor Isomerism on the Rates of Reaction of Enzymes. With the knowledge of the relative energies of the conformers of carbobenzoxy-L-phenylalanine (I) and β-phenylpropionyl-L-phenylalanine (II) and data in the literature, it is possible to construct an approximate potential-energy diagram that allows the examination of the influence of conformational isomerism of the inhibitor on the rate of the process. Such an analysis is of interest because of numerous claims of the influence of the conformation of a drug on its biological activity.

In the early 1970's a number of researchers proposed that there was a relationship between the proportion of rapidly equilibrating conformers in solution and the activity of a number of biologically important compounds, including histamine, acetylcholine, hormones, and antihistamines.[15-24] In addition, the most stable conformer

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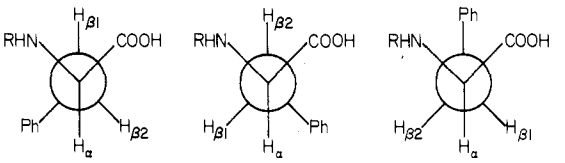
Table III. Approximate Proportion of Conformers of I and II about the C_α-C_β Bond



A B C

	Cbz-Phe (I)					β-PPP (II)				
solvent	$J_{\alpha-\beta_1}$	$J_{\alpha-\beta_2}$	N_A	N_B	N_C	$J_{\alpha-\beta_1}$	$J_{\alpha-\beta_2}$	N_A	N_B	N_C
CD_3OD	9.3	4.8	0.61	0.20	0.19	8.8	5.1	0.57	0.23	0.20
Me_2SO-d_6	10.9	4.9	0.76	0.21	0.03	9.2	5.0	0.60	0.22	0.18
acetone-d_6	8.9	5.0	0.57	0.22	0.21	7.9	5.2	0.49	0.24	0.27
$CDCl_3$	6.7	5.6	0.37	0.27	0.36	6.6	5.9	0.37	0.30	0.33
D_2O	9.16	4.28	0.59	0.15	0.26	9.4	4.8	0.53	0.20	0.27

Table IV. Relative Energies (cal/mol) of Rotamers A, B, and C of Carbobenzoxy-L-phenylalanine (I) and β-Phenylpropionyl-L-phenylalanine

solvent	dielectric constant	β-PPP			Cbz-Phe		
		E_A	E_B	E_C	E_A	E_B	E_C
Me_2SO-d_6	45	0	592	710	0	751	1730
CD_3OD	33.7	0	535	618	0	658	688
acetone-d_6	21.4	0	421	352	0	562	589
$CDCl_3$	4.8	0	124	68	0	186	33

Table V. Proportion of Conformers (%) for Phenylalanine and Related Compounds

compd	N_A	N_B	N_C
Phe[a]	48	24	28
Tyr[a]	46	22	33
Phe-pyridoxal Schiff base[b]	69	15	16
Cbz-Phe (I)	61	20	19
β-PPP (II)	57	23	20
Met-Asp-Phe-NH_2[c]	59	17	24
Gly-Phe[d]	54	22	24
Leu-Phe[d]	60	20	20

[a] Reference 7. [b] Reference 5. [c] Reference 8. [d] Reference 9.

of several drugs in solution has been suggested to be the one bound to the receptor, and numerous attempts to correlate the differences in the proportion of the most stable conformer with activity have been made.[15-24]

Even though the frequency of these suggestions has decreased and these suggestions seem to contradict the postulate of Koshland and Neet,[25] it seems worthwhile to examine whether the proportion of conformers of the inhibitor β-phenylpropionyl-L-phenylalanine (II, β-PPP) would be expected to influence the rate of hydrolysis of 3-(2-furylacryloyl)glycyl-L-leucine (FAGLA) by thermolysin. The reaction scheme for this process is shown in eq 1 and 2.

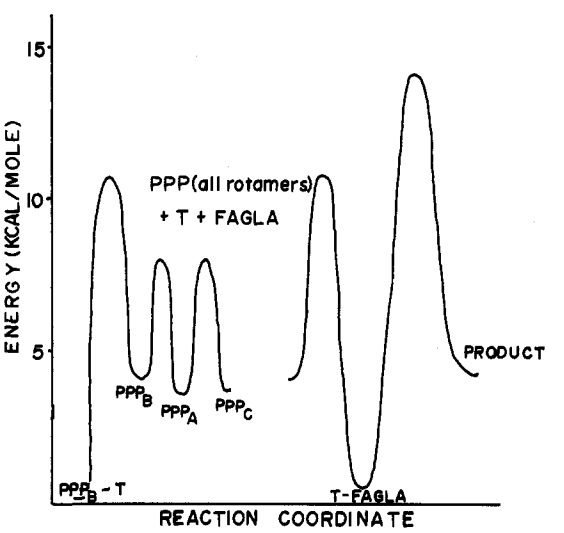


Figure 3. Idealized reaction coordinate diagram for a mixture of thermolysin (T), the reactant FAGLA, and the inhibitor β-phenylpropionyl-L-phenylalanine (PPP). The subscripts refer to the rotamers β-PPP_A, β-PPP_B, and β-PPP_C.

$$\text{FAGLA} + \text{T} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{FAGLA–T} \xrightarrow{k_3} \text{FAGLA} + \text{P} \quad (1)$$

$$\begin{array}{c} \beta\text{PPP}_A \\ \uparrow\downarrow \\ \beta\text{PPP}_B + \text{T} \underset{k_4}{\overset{k_5}{\rightleftharpoons}} \beta\text{PPP}_B\text{-T} \\ \uparrow\downarrow \\ \beta\text{PPP}_C \end{array} \quad (2)$$

The rate of this reaction can be derived in analogy with competitive inhibitors (eq 1).[26] Values for the various rate constants can be estimated or extracted directly from the

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(23) N. Camerman and H. Camerman, *J. Am. Chem. Soc.*, **94**, 8553 (1972).
(24) See, for example, (a) L. B. Kier, "Molecular Orbital Theory in Drug Research", Academic Press, New York 1971; (b) L. B. Kier, *J. Pharm. Pharmacol.*, **21**, 93 (1969).
(25) D. E. Koshland, Jr., and K. E. Neet, *Annu. Rev. Biochem.*, **37**, 359 (1972).
(26) K. M. Plowman, "Enzyme Kinetics", McGraw Hill, New York, 1972, p 22.

$$\text{rate} = k_1k_3[\text{T}][\text{FAGLA}]/\left[(k_2 + k_3)\left(1 + \frac{[\beta\text{PPP}_\text{I}] + [\beta\text{PPP}_\text{II}] + [\beta\text{PPP}_\text{III}]}{K_\text{I}} + k_1[\text{FAGLA}]\right)\right] \quad (1)$$

literature:[27] $k_3 = 7.25 \times 10^2\ s^{-1}$ and $K_m = 2.5 \times 10^{-3}$ M. Since k_1 is probably on the order of $10^8\ M^{-1}\ s^{-1}$,[28] k_2 is approximately $2.5 \times 10^5\ s^{-1}$. Assuming $k_5 = 10^8\ M^{-1}\ s^{-1}$ and using $K_I = 1.6 \times 10^{-3}$ M[29] gives a value for k_4 of 1.6×10^5. These estimated rate constants can be converted into activation energy barriers using the equation $k = Ae^{E_a/RT}$, where A has the assumed value of 10^{13}. Figure 3 schematically relates the energies of the reactants and products based on these energy barriers. For this drawing, FAGLA and β-PPP$_\text{II}$ were assumed to have the same energy. While the rates of interconversion of the conformers of β-phenylpropionyl-L-phenylalanine are not known, they are fast on the NMR time scale, and the barrier for interconversion was assumed to be approximately 4 kcal/mol.

Both the numerical values of the rate constants and this diagram clearly show that if the rate constant of equilibration of $\beta\text{PPP}_\text{A} \rightleftarrows \beta\text{PPP}_\text{B} \rightleftarrows \beta\text{PPP}_\text{C}$ is greater than 100 times the rate of conversion of T–FAGLA to product, then relative proportions of the conformers of the inhibitor will not influence its K_I. The K_I clearly depends only on the relative energies of the inhibitor–thermolysin and substrate–thermolysin complex. Only if the barrier to interconversion of the conformers of the inhibitor approaches that for the conversion of the enzyme–substrate–complex to product would the proportions of conformer be expected to influence the K_I. For many drugs this unusual condition implies restricted rotation and would clearly not apply.

Conclusion

These results show that the lowest energy solution conformer as determined by NMR spectroscopy is not always the conformer bound by an enzyme as determined by X-ray crystallography. In particular, thermolysin binds the lowest energy solution conformer of the inhibitor carbobenzoxy-L-phenylalanine but the highest energy solution conformer of β-phenylpropionyl-L-phenylalanine.

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Design of Potent and Selective Antagonists of the Vasopressor Responses to Arginine-vasopressin

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We have synthesized eight new 1-β,β-dialkyl-substituted analogues of [1-deamino]arginine-vasopressin (dAVP) to determine some of the structural features that account for antivasopressor potency and that improve selectivity by reducing antidiuretic agonistic activity. These analogues are as follows: **1**, [1-(β-mercapto-β,β-diethylpropionic acid)]arginine-vasopressin (dEt$_2$AVP); **2**, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine,8-D-arginine]vasopressin (dEt$_2$VDAVP); **3**, [1-deaminopenicillamine,4-valine]arginine-vasopressin (dPVAVP); **4**, [1-(β-mercapto-β,β-diethylpropionic acid),4-valine]vasopressin (dEt$_2$VAVP); **5**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),4-valine]arginine-vasopressin [d(CH$_2$)$_5$VAVP]; **6**, [1-deaminopenicillamine,8-D-arginine]vasopressin (dPDAVP); **7**, [1-(β-mercapto-β,β-diethylpropionic acid),8-D-arginine]vasopressin (dEt$_2$DAVP); **8**, [1-(β-mercapto-β,β-cyclopentamethylenepropionic acid),8-D-arginine]vasopressin [d(CH$_2$)$_5$DAVP]. The protected precursors required for these analgoues were synthesized by a combination of solid phase and 8 + 1 couplings in solutions. These analogues were tested for agonistic and antagonistic activities in rat vasopressor, rat antidiuretic, and rat uterus assay systems. They exhibit no evident pressor activities. They are all highly effective antagonists of the vasopressor responses to AVP. They exhibit the following antivasopressor pA_2 values: **1**, 8.36 ± 0.07; **2**, 8.18 ± 0.06; **3**, 7.92 ± 0.07; **4**, 8.29 ± 0.08; **5**, 7.97 ± 0.06; **6**, 7.45 ± 0.08; **7**, 7.96 ± 0.08; **8**, 8.52 ± 0.03. These findings clearly indicate that in the four series of β,β-dialkyl-substituted analogues now completed, either the β,β-diethyl or the β,β-cyclopentamethylene substitution is far more effective than the β,β-dimethyl grouping in leading to enhanced antivasopressor potency. The antidiuretic activities of these analogues are also dramatically less than in analogues containing a β,β-dimethyl substituent. With antidiuretic activities of only 0.2, 0.06, and 0.3 unit/mg and relatively weak antioxytocic activities, analogues **5**, **7**, and **8** are among the most potent and selective vasopressor antagonists reported to date. These new analogues hold promise as additional tools for studies on the physiological roles of AVP.

We previously reported the synthesis and some pharmacological properties of a number of potent antagonists of vasopressor responses to arginine-vasopressin (AVP).[1-5] Their properties are summarized in Sawyer et al.[6] These antagonists are proving to be valuable tools in a variety of studies on the putative physiological roles of AVP. The

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