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CONFORMATIONAL STUDIES OF PEPTIDES IN THE PRESENCE OF LANTHANIDE SHIFT REAGENT, $Eu(DPM)_3$

L. C. MARTINELLI*, I. L. HONIGBERG and L. A. STERNSON
Department of Medicinal Chemistry, School of Pharmacy, University of Georgia; Athens, Georgia 30601, U.S.A.

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Abstract—The conformations of diastereomers of Z-Phe-Ala-OCH_3 were investigated by analyzing NMR and IR spectra in the presence and absence of $Eu(DPM)_3$. NMR ΔEu values and ratios of integral intensities for NH(bonded)/NH(free) confirm the stereochemistry of the time-averaged conformation for the peptide-$Eu(DPM)_3$ complex as that proposed by Bystrov for a dipeptide in solution. Total H-bonding by the peptide was increased by the presence of $Eu(DPM)_3$.

Bystrov[1,2] and Mizushima[3,4] independently reported that diastereomeric dipeptides preferentially exist (in a aprotic solvent) as cyclic intramolecularly H-bonded species which are in equilibrium with less populated linear conformations. Individual diastereomers presumably exist in cyclic conformations which are similar, but differ in their equilibrium constants. One significant result of these studies was the postulation of the stereochemistry of these cyclic conformations. Bystrov's model (Fig 1) orients the $C_{(5)}$-CH_3 group of $N_{(6)}$-benzyloxycarbonylalanylalanine methyl ester in a pseudoaxial position with the $N_{(6)}$—$C_{(5)}H$ dihedral angle, θ, at 0°, while according to the conformation suggested by Mizushima (Fig 2) the $C_{(5)}$—CH_3 moiety is in a pseudoequatorial orientation.

To resolve this difference, and to investigate the applicability of shift reagents to the NMR study of peptides, we analyzed the PMR (60 MHz) and IR spectra of two diastereomeric dipeptides, $N_{(6)}$-benzyloxycarbonyl-L-phenylalanyl-L-alanine methyl ester and $N_{(6)}$-benzyloxycarbonyl-L-phenylalanyl-D-alanine methyl ester in the presence of the lanthanide shift reagent tris(dipivalomethanato)europium (III), $Eu(DPM)_3$. The use of lanthanide shift reagents to resolve the NMR spectra of organic molecules is well-documented in the literature[5–8] and has been generally applied to studying semi-rigid molecules with limited conformations.

From ΔEu values[9] ($\Delta Eu = \delta_{CDCl_3} - \delta^{n=1}_{CDCl_3}$) for corresponding proton absorptions of the L, L and L,D diastereomers (Table 1 and from solution IR studies) we are able to (1) approximate the spatial relationship of the shift reagent to the dipeptide in the complex, and (2) to confirm the stereochemistry of the 7-member H-bonded ring as being that proposed by Bystrov and not that proposed by Mizushima. In addition, complexation of $Eu(DPM)_3$ with the dipeptides shifts the equilibrium (H-bonded ⇌ H-free) toward the H-bonded form.

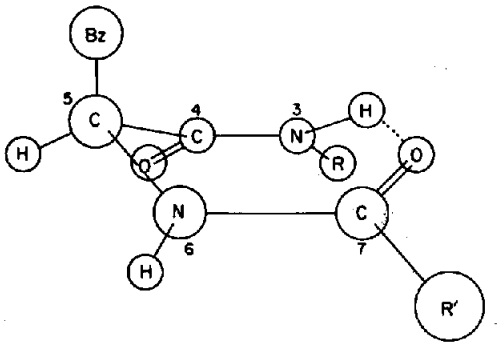

Fig 1. Bystrov's model of the folded dipeptide conformation [R = —$CH(CH_3)CO_2CH_3$: R' = $C_6H_5CH_2O$—].

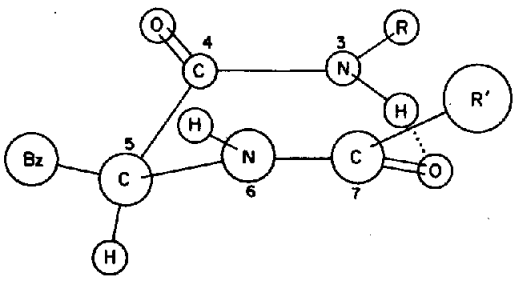

Fig 2. Mizushima's model of the folded form of the dipeptide [R = —$CH(CH_3)CO_2CH_3$: R' = $C_6H_5CH_2O$—].

RESULTS AND DISCUSSION

At the concentration of the NMR study (0·10 M) the peptide solutions contain self-associated species (as determined from IR studies) in equilib-

*To whom correspondence should be addressed.

rium with linear and intra-molecular H-bonded species. The complexity of the equilibrium at high concentration is shown by the generalization,

$$X[\text{peptide}] \rightleftharpoons [\text{peptide}]_Y$$

where the term peptide represents the equilibrium

$$\text{linear} \rightleftharpoons \text{pseudo-ring (H-bonded)}.$$

The NMR shift reagent, $Eu(DPM)_3$, can coordinate with the individual components of the equilibrium to form complexes of the general types

$$X[\text{peptide}] \cdot [Eu(DPM)_3]_\alpha \rightleftharpoons [\text{peptide}]_Y \cdot [Eu(DPM)_3]_\beta,$$

which are in equilibrium with uncoordinated peptides and $Eu(DPM)_3$. Plots of Δ ppm ($\delta_{CDCl_3} - \delta_{Eu(DPM)_3}$) *versus* [$Eu(DPM)_3$] for both dipeptides afforded straight lines by least-squares analyses (zero order correlations were $>0{\cdot}99$) to 10^{-1} M $Eu(DPM)_3$, the maximum concentration used. These plots afforded ΔEu values by extrapolation to a mole ratio of $Eu(DPM)_3$ to solute of one ($n = 1$).

In the complex equilibrium mixture, a linear plot of Δ ppm *vs* [$Eu(DPM)_3$] occurs when the ratios X/α, Y/β, and $(X/\alpha)/(Y/\beta)$ remain nearly constant. The individual reactions of the equilibria must be relatively fast on the NMR time-scale so that observed individual proton absorption positions are time-averaged absorptions for all species in solution. The use of a NMR shift reagent such as $Eu(DPM)_3$ thus allows definition of the conformational characteristics from the time-averaged NMR absorptions of a peptide coordinated with $Eu(DPM)_3$. The use of NMR time-averaged peptide spectra at high concentration (0·20 M) to propose definitive peptide conformations was achieved by Bystrov *et al.*, even though these authors failed to clearly indicate the 7-membered pseudo-ring conformation was an average of all conformations present, and best describes this average. The NMR data reported herein (Table 1) and the model we propose for the stereochemistry of the $Eu(DPM)_3$-peptide complex is, therefore, recognized as presenting the average of all $Eu(DPM)_3$-peptide complex species in solution.

The association of the dipeptide with $Eu(DPM)_3$ does not make significant changes in the equilibrium conformation. Bystrov[2] demonstrated by NMR and IR studies that $N_{(3)}CH_3$ derivatized dipeptides do not form intramolecular H-bonds but self-associate by intermolecular H-bonds, while the parent $N_{(3)}H$ compounds form 7-membered pseudo rings in addition to intermolecular bonds. By comparison of NMR chemical shift differences for $C_{(2)}H$ and $C_{(5)}H$ protons in the $N_{(3)}CH_3$ and $N_{(3)}H$ dipeptides one can observe the absence or presence of the intramolecularly H-bonded species. The dipeptide methine protons possess less rotational freedom in the 7-membered intramolecularly-formed pseudo ring than species that exist in linear conformations (intermolecular H-bonds). Disruption of the pseudo ring would result in a significant change in the chemical shift difference between the $C_{(2)}H$ and $C_{(5)}H$ proton absorptions. In Bystrov's $N_{(3)}CH_3$ compounds the chemical shift difference (δ_{int}) between the methine protons are 0·54 ppm and 0·33 ppm for the D,D and D,L isomers respectively; while the $N_{(3)}H$ parents showed these differences to be 0·22 ppm and 0·24 ppm, respectively. Such findings are best explained as a result of going from an acyclic conformation ($\delta_{int} = 0{\cdot}54$ ppm and 0·33 ppm) to a pseudo ring conformation ($\delta_{int} = 0{\cdot}22$ ppm and 0·24 ppm). Chemical shift changes for the remaining protons in these two types of dipeptide derivatives are less than those for the methine protons, thus indicating the methine protons are most sensitive to conformational changes in accord with our expectations for cyclic and acyclic conformations.

In this study, varying concentrations of $Eu(DPM)_3$ resulted in no observed change in chemical shift differences between the $C_{(2)}H$ and $C_{(5)}H$ in either the D,L or L,L dipeptide compared to their values in the *absence* of $Eu(DPM)_3$; these values were approximately 0·2 ppm in each case. It is concluded, therefore, that no significant change in peptide conformation was induced by the addition of shift reagent (to a concentration of 0·1 M).

In the presence of $Eu(DPM)_3$, the *Phe* and *Ala* methine protons, in each dipeptide, shift at identical rates (ΔEu values in Table 1, therefore, represent both methine protons). From the methine ΔEu values, it is clear that complexed $Eu(DPM)_3$ in the complex is equidistant from the *Phe* and *Ala* methine protons in both the L,L and L,D peptides. Since the methine ΔEu values for the two dipeptides are essentially the same and the ΔEu value is the largest for each dipeptide, the 3-dimensional relationship of $Eu(DPM)_3$ to each of the dipeptides is nearly identical and independent of the stereochemistry at the epimeric carbon, $C_{(2)}$.

Mizushima's H-bonded model (Fig 2) orients the benzyl moiety of *Phe* in a pseudoequatorial position. From this model for the *Phe* methine proton to shift at a rate greater than the *Phe* methylene protons, and for the *Phe* and *Ala* methine protons to shift at identical rates, $Eu(DPM)_3$ must coordinate below the plane of the ring and equidistant from the two methine protons. Since ΔEu values for the methine protons are essentially identical in the two dipeptides, the $C_{(2)}$—$N_{(3)}$ bond must rotate to allow the *Ala* methine proton in each dipeptide to assume the same geometry and distance from $Eu(DPM)_3$ as the *Phe* methine; such

an equilibrium conformation is reasoned to occur as a consequence of the steric repulsion between $Eu(DPM)_3$ and the C—CH_3 and $COOCH_3$ groups of alanine. From Mizushima's model, the *Ala* Me would thus be in closest proximity to $Eu(DPM)_3$ in the L,L isomer in contradiction to what is observed on the basis ΔEu values.

Bystrov's model (*vide infra*) (Fig 1) orients the benzyl of *Phe* in a pseudo-axial position. For the same reasons enumerated for Mizushima's model, $Eu(DPM)_3$ must lie below the plane of the ring and equidistant from the *Phe* and *Ala* methine protons in each dipeptide. For the two methine protons to assume the same geometry and distance from $Eu(DPM)_3$ in each dipeptide, rotation about the $C_{(2)}$—$N_{(3)}$ bond axis must also occur. Bystrov's model predicts the *Ala* Me to be in closest proximity to $Eu(DPM)_3$ in the L,D isomer in accord with experimental findings.

Drieding models of Bystrov's folded conformation show that rotation of the $C_{(2)}$—$N_{(3)}$ bond leads to a conformation of the peptide-$Eu(DPM)_3$ complex that adequately explains the ΔEu and Δ(ΔEu) values of Table 1. That conformation is generated when the $C_{(2)}$—H bond axis is slightly below or coplanar with the $N_{(3)}$—C=O group (Fig 3), so that the bulky *Ala* Me and carbomethoxy groups are rotated away from $Eu(DPM)_3$ in the complex, minimizing steric interactions. The differences in ΔEu and Δ(ΔEu) values, between the *Phe* methylene protons of L,D and L,L and the methylene protons of the benzyloxycarbonyl moiety of L,D and L,L are best explained as resulting from small conformational changes induced by steric repulsions with $Eu(DPM)_3$ after complexation.

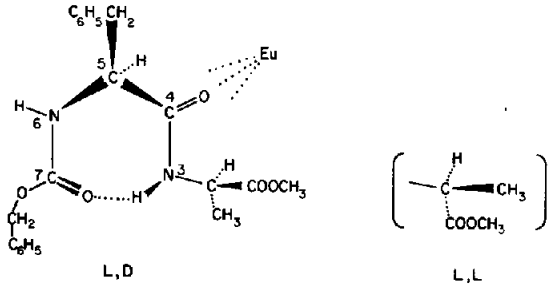

Fig 3. Conformation of dipeptide ring in $Eu(DPM)_3$-dipeptide complexes (Viewed from above).

Solution IR data of the diastereomeric dipeptides were obtained from 10^{-2} and 5×10^{-4} M solutions in the presence and absence of molar equivalents of $Eu(DPM)_3$ (Table 2). Integrated intensities for N—H(free) ($\nu_{NH} = 3420\ cm^{-1}$) and N—H(H-bonded) ($\nu_{NH} = 3340\ cm^{-1}$) stretching frequencies show that $Eu(DPM)_3$ promotes H-bonding at both concentrations of the dipeptides. The smaller promotion of H-bonding by $Eu(DPM)_3$ at 5×10^{-4} M peptide concentrations may indicate changes in stabilities of the complexes resulting from changes in solute activities. In similar studies, Bystrov *et al.*[1] attributed H-bonding in dipeptides at similar dilute concentrations to intramolecular associations in the case of $N_{(6)}$—Z-protected dipeptides. We have, therefore, assigned the observed H-bonding at 5×10^{-4} M to intramolecular associations. At 10^{-2} M, however, the observed H-bonding is an unresolved mixture of inter- and

Table 1. Paramagnetic shifts of Z-Phe-Ala-OCH_3 protons at 60 MHZ

Functional group[a]	δ_{CDCl_3} L,L	ΔEu	δ_{CDCl_3} L,D	ΔEu	Δ(ΔEu)[b]
CH_3-C	1·33	1·96	1·25	3·56	−1·60
ϕCH_2-C	3·05	4·31	3·07	4·66	−0·35
O-CH_3	3·68	0·45	3·70	0·50	−0·05
C-H[c]	4·58	7·85	4·53	7·81	+0·04
ϕCH_2-O	5·07	2·58	5·12	2·20	+0·38

[a]Phenyl proton chemical shifts were not measured due to the complexity of their spectra in the presence of $Eu(DPM)_3$. Amido proton chemical shift rates could not be monitored due to the low intensity of their signals in the presence of $Eu(DPM)_3$.

[b]$\Delta(\Delta Eu) = \Delta Eu_{L,L} - \Delta Eu_{L,D}$.

[c]Represents both *Phe* and *Ala* methine protons.

Table 2. Effect of $Eu(DPM)_3$ on H-bonding (IR) in diastereomeric dipeptides[a]

Peptide	Conc. (M)	NH(bonded)/NH(free)[b] No $Eu(DPM)_3$	NH(bonded)/NH(free)[b] $Eu(DPM)_3$[c]	% of H-bonding promoted by $Eu(DPM)_3$[d]
L,L	1×10^{-2}	0·287	0·386	26
	5×10^{-4}	0·031	0·056	45
L,D	1×10^{-2}	0·281	0·470	40
	5×10^{-4}	0·132	0·177	25

[a]All data points at an average of four determinations.

[b]Ratios calculated from relative integral intensities; ratio denoted as I.

[c]Molar ratio of $Eu(DPM)_3$ to solute is one (n = 1).

[d]$(I_{Eu(DPM)_3} - I)/(I_{Eu(DPM)_3}) \times 100$.

intramolecular components. The greater H-bonding of the L,D peptide (at 5×10^{-4} M) compared with the L,L isomer, both in the *absence* of $Eu(DPM)_3$, cannot be adequately explained at this time, however, this is consistent with the discussions of Weinstein[10,11] on increased intramolecular folding for unprotected L,D diastereomeric dipeptides in D_2O as well as protected dipeptides in $CDCl_3$.

Differences in the effect of $Eu(DPM)_3$ on H-bonding in the two dipeptides can be explained as a consequence of the effect the relative steric bulk of the *Ala* $C_{(2)}$—CH_3 and $COOCH_3$ have on the stability of the resultant $Eu(DPM)_3$ complex. In the complex formed with the L,D isomer, the bulkier COOMe group is removed from $Eu(DPM)_3$ while in the L,L isomer it is proximal, introducing steric strain into the complex, reducing its stability. The smaller NH(bonded)/NH(free) ratios for the L,L-$(Eu(DPM)_3$ complex at 10^{-2} and 5×10^{-4} M compared with the same ratios for the L,D-$Eu(DPM)_3$ complex indicates a lesser propensity for the L,L-isomer to participate in intramolecular H-bonding. Such findings are consistent with the stereochemistry of the complex proposed here, in support of the dipeptide model proposed by Bystrov.

EXPERIMENTAL

NMR spectra were determined with a Hitachi Perkin-Elmer R-20A spectrometer with 0·1 M soln in $CDCl_3$. Chemical shifts of all compounds are reported in ppm (δ) and were measured from internal TMS(1%). ΔEu values were obtained from straight lines derived from least-squares analysis and extrapolation to a point where the molar ratio of shift reagent to solute is one ($n = 1$); zero correlations were greater than 0·99 in all cases.

IR spectra were determined with a Perkin-Elmer 237-B spectrometer with 10^{-2} M and 5×10^{-4} M solns in CCl_4: $CHCl_3$ (9 : 1).

Protected dipeptides were prepared by established methods.[11,12] Physical properties were in satisfactory agreement with literature values. All compounds were homogeneous by the criterion of TLC.

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