mixture of chloroform and glacial acetic acid (for the swellable resins) or pure glacial acetic acid (for the macroreticular resins) and an excess of 50% potassium iodide solution was added. After stirring under nitrogen for 10–30 min, the iodine liberated was titrated with 0.1 *N* sodium thiosulfate. Blank titrations were carried out on the corresponding Ⓟ-COOH resins and corrections were made when required.

Epoxidation of Cyclohexene. A suspension of 3 g (2.9 mmol) of macroreticular Ⓟ-COOOH in 10 ml of purified dioxane containing 0.2 ml (2 mmol) of cyclohexene was stirred at 40° for 6 hr. Analysis of the product was effected by gas chromatography on a column packed with 5% SE-30 liquid phase on Chromosorb W. The absence of α-glycol by-product was shown by titration with sodium metaperiodate. Results of the epoxidation reactions are shown in Table II.

Acknowledgment. Financial support of this work by the National Research Council of Canada is gratefully acknowledged. The authors also wish to thank Ms. Geneviève Pellé for her assistance.

References and Notes

(1) Presented at the IUPAC International Symposium on Macromolecules, Rio de Janeiro, July 1974, Paper A-1.5.
(2) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963).
(3) R. L. Letsinger and V. Mahadevan, *J. Amer. Chem. Soc.*, **88**, 5319 (1966).
(4) J. M. Frechet and C. Schuerch, *J. Amer. Chem. Soc.*, **93**, 492 (1971).
(5) T. L. Ang and H. J. Harwood, *J. Macromol. Sci., Chem.*, **7**, 1079 (1973); M. B. Shambu and G. A. Digenis, *J. Chem. Soc., Chem. Commun.*, 619 (1974).
(6) F. Helfferich and D. B. Luten, Jr., *J. Appl. Polym. Sci.*, **8**, 2899 (1964).
(7) T. Takagi, *Polym. Lett.*, **5**, 1031 (1967).
(8) W. Heitz and R. Michels, *Angew. Chem.*, **84**, 296 (1972).
(9) N. M. Weinshenker and C. M. Shen, *Tetrahedron Lett.*, 3281 (1972); *ibid.*, 3285 (1972).
(10) C. Yaroslavsky, A. Patchornik, and E. Katchelski, *Tetrahedron Lett.*, 3629 (1970); H. Schuttenberg, G. Klump, U. Kaczmar, S. R. Turner, and R. C. Schulz, *J. Macromol. Sci., Chem.*, **7**, 1085 (1973).
(11) C. C. Leznoff and J. Y. Wong, *Can. J. Chem.*, **50**, 2892 (1972).
(12) J. I. Crowley and H. Rappoport, *J. Amer. Chem. Soc.*, **92**, 6363 (1970).
(13) E. C. Blossey, D. C. Neckers, A. C. Thayer, and A. P. Schaap, *J. Amer. Chem. Soc.*, **95**, 5820 (1973).
(14) R. H. Grubbs and L. C. Kroll, *J. Amer. Chem. Soc.*, **93**, 3062 (1971).
(15) C. G. Overberger and K. N. Sannes, *Int. J. Polym. Mater.*, **2**, 271 (1973). C. C. Leznoff, *Chem. Soc. Rev.*, **3**, 65 (1974).
(16) Although resins containing 2% divinylbenzene were used successfully in this work, all the results reported for the "swellable" resin refer to the 1% cross-linked material.
(17) Usually higher reaction temperature or longer time of reaction.
(18) In some cases air was removed from the pores of the macroreticular resin by applying gentle suction to the suspension of resin in the reaction solvent.
(19) R. L. Letsinger and D. M. Jerina, *J. Polym. Sci., Part A-1*, **5**, 1977 (1967).
(20) J. T. Ayres and C. K. Mann, *Polym. Lett.*, **3**, 505 (1965).
(21) C. R. Dick and R. F. Hanna, *J. Org. Chem.*, **29**, 1218 (1964).
(22) K. W. Pepper, H. M. Paisley, and M. A. Young, *J. Chem. Soc.*, 4097 (1953).

Studies of the Dimensions of Oligopeptides by Singlet–Singlet Energy Transfer and Theoretical Calculations. I. Influence of Glycine on the Dimensions of Tetrapeptides

R. Guillard,[1] M. Leclerc, A. Loffet, J. Leonis, B. Wilmet, and A. Englert*

Laboratoire de Chimie Générale I, Faculté des Sciences, Université Libre de Bruxelles, Brussels, Belgium.
Received August 12, 1974

ABSTRACT: The efficiency of energy transfer between a fluorescent donor, L-tyrosine, and a fluorescent acceptor, L-tryptophan, has been determined in R′-L-Trp-L-Ala-L-Tyr-R″, R′-L-Trp-L-Ala-L-Ala-L-Tyr-R″, and R′-L-Trp-Gly-L-Ala-L-Tyr-R″ in ethanol solution. The protecting groups R′ and R″ were respectively *tert*-butyloxycarbonyl and methyl ester. A conformational theoretical analysis of molecules studied has been performed in parallel on the basis of semiempirical conformational potential energy functions. In the theoretical models all the side chains have been represented by a methyl group. From the distribution of distances between chromophores obtained theoretically, transfer efficiencies have been computed assuming a random orientation of the chromophores ($\kappa^2 = \frac{2}{3}$). The comparison of calculated efficiencies with the values determined experimentally for the same value of κ^2 has been used as a check for the theoretical model. Both experimental and theoretical studies have shown that the glycyl residue produces a reduction of dimensions when it replaces in a tetrapeptide a residue with a β-carbon atom such as the L-alanyl residue. However, only a qualitative agreement between experimental and theoretical values of the efficiencies has been obtained.

It is well established from experimental[2] and theoretical[2,3] investigations that the average dimensions of random coils of poly(L-alanine) or its analogs are markedly reduced when glycine is incorporated into the chain, thus replacing an amino acid residue with a β-carbon atom.

In oligopeptides, the glycyl residue should produce a similar reduction of dimensions but this effect has not been investigated up to now. Such an investigation is reported here, and this is the first part of a more general experimental and theoretical study of the dimensions of oligopeptides undertaken in our laboratory by means of nonradiative energy transfer[4,5] and of theoretical conformational analysis with semiempirical potential functions.[6,7]

The most complete description of the dimensions of flexible molecules is given by the normalized distribution $f(r)$ of the end-to-end distances or of any other representative distances in the molecule. However, $f(r)$ is difficult to obtain experimentally; therefore, only average dimensions of the molecules are studied, principally by measuring the mean dipole moment.[8] The efficiency of singlet–singlet energy transfer between chromophores attached to the ends of the molecule can be used to determine the donor–acceptor distance and can also be correlated to the dimensions of the molecule.[5] In oligopeptides which are flexible, the efficiency of energy transfer is equal to the average value of the ratio $R_0^6/(R_0^6 + r_c^6)$ where r_c is the distance between the chromophores, assumed not to vary during the donor lifetime, and R_0 a characteristic spectroscopic constant,

which depends only on the nature of the solvent and of the chromophores for chromophores oriented at random.[9]

In the present study, the experimental efficiency has been compared with the efficiency calculated theoretically from the distribution of distances $f(r_c)$ obtained by conformational analysis. The comparison is used as a check for the theoretical model.

In the theoretical model, all orientations of chromophores, the aromatic side chains of L-tryptophan and L-tyrosine, have been assumed equally probable. The experimental efficiencies have also been evaluated on the assumption that chromophores are rotating freely.[5] Due to this assumption the number of degrees of freedom in the theoretical model is considerably reduced and consequently a detailed analysis of the distribution functions $f(r)$ and $f(r_c)$ is feasible.

From the distribution functions all the relevant average properties have been calculated. The distribution functions, however, yield much more information on the relation between conformation and the dimensions and on the influence of the side chains than do the average properties.

Unlike the average properties of polypeptides, the distribution of the end-to-end distances cannot be obtained by analytical methods[2] and have to be computed from chains generated numerically. For this reason the distribution functions have not yet been studied very extensively, and the first calculation of $f(r)$ in short unperturbed polypeptide chains composed of from 5 to 20 units, obtained by Monte Carlo sampling technique, has been reported only recently.[10]

In the systems studied here the number of degrees of freedom is small so that all the relevant conformations were generated and conformational energies have been computed from interactions between all the atoms. Thus interactions between distant atoms in the chain have not been neglected, as has been done in previous calculations.[2,10]

I. Method

For a rigid system that contains one fluorescent donor D and one acceptor A, the efficiency of singlet energy transfer predicted by Förster's theory[4,5] is

$$e(R_0) = R_0^6/(R_0^6 + r_c^6) \qquad (1)$$

R_0 is the distance at which the transfer would be half-efficient and depends on the refractive index of the medium between donor and acceptor n, the spectral overlap of donor fluorescence and acceptor absorption J_{AD}, the quantum yield of the donor Q_D and a geometrical factor κ^2. This factor is the angular part of the dipole–dipole interaction.[5] For freely rotating donors and acceptors, $\kappa^2 = 2/3$.

In an oligopeptide the distance r_c between the donor and the acceptor is not rigidly fixed. The energy transfer on the assumption that the distance r_c does not change during the lifetime of excitated state is

$$e(R_0) = \int_0^\infty f(r_c)\,\frac{R_0^6}{R_0^6 + r_c^6}\,dr_c \qquad (2)$$

We have determined the efficiency experimentally for a constant value of R_0, assuming a freely rotating donor and acceptor. From the theoretically determined distributions of the distances between the donor and acceptor and the above value of R_0, the theoretical efficiency is calculated. The distributions of the distances are computed from an appropriate model of the molecules and the semiempirical potential functions.

II. Experimental Section

A. Synthesis. The three following peptides were synthesized: R′-L-Trp-L-Ala-Tyr-R″, R′-L-Trp-L-Ala-L-Ala-L-Tyr-R″, and R′-L-Trp-Gly-L-Ala-R″, where R′ and R″ are protecting groups *tert*-butyloxycarbonyl (*t*-boc) and methyl ester, respectively. The synthesis was performed by a solid-phase method on an automatic apparatus described by Loffet.[11]

The esterification of the first amino acid was carried out as recommended by Loffet,[12] and the *t*-boc protecting group was removed by mercaptoethane sulfonic acid[13] (MESNA). Coupling reactions were carried out with *N*-ethylcarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) as activation reagent to avoid acid acylurea formation that occurs with dicyclohexylcarbiimide.[14] The peptides were removed from the resin by a transesterification reaction.[15]

The resin was a Merck resin for solid-phase peptide synthesis (Cl, 5%). The *t*-boc amino acids were purchased from Fluka A. G., MESNA and solvents from U.C.B., and EEDQ from Pierce Chemical Co.

B. Purification. Molecular filtration on Merckogel 500 in methanol and preparative silica thin layer chromatography (solvent: $CHCl_3$ 95%, methanol 5%) allowed us to obtain pure peptides. The peptides had satisfactory elemental analyses and were chromatographically homogeneous.

Silica thin layer analytical chromatographies at different concentrations (maximum ratio 1:100) did not show any detectable impurities, which means that the rate of impurities does not exceed 1%, and cannot alter significantly the measurements on the peptides.

C. Determination of the Efficiency e(R_0). Absorption spectra were obtained by means of a Cary Model 15 spectrophotometer, and optical densities were obtained by means of a Zeiss Spectrophotometer. Fluorescence spectra were recorded on a Hitachi Perkin-Elmer PF 2 A spectrofluorimeter. All spectra were corrected for the wave-length dependant response of the photomultiplicator. The response was determined by comparison of β-napthol and phenol spectra with absolute ones found in the literature.[16,17] The solutions had low optical densities (lower than 0.1) and the spectra were recorded at a temperature of 25°.

Quantum yields of amino acids and peptides were determined by comparison with that of L-Trp in water (0.14),[18,19] using the formula

$$\frac{Q_x}{Q_{L\text{-trp}}} = \frac{A_x\,OD_{L\text{-trp}}\,n_x^2}{A_{L\text{-trp}}\,OD_x\,n_{H_2O}^2} \qquad (3)$$

where Q is the quantum yield of the sample (x) or of the reference (L-Trp), A is the area of the emission spectrum, and OD is the optical density of the solutions at excitation wavelength. The overlap integral

$$J_{AD} = \int_0^\infty f_D(\nu)\epsilon_A(\nu)\nu^{-4}\,d\nu \qquad (4)$$

where $f_0(\nu)$ is the normalized fluorescence intensity of the donor and $\epsilon_A(\nu)$ is the molar extinction coefficient of the acceptor, was computed on a Hewlett-Packard 9820-A Calculator by numerical integration method.

The Förster critical distance R_0 is given by

$$R_0^6 = 8.8 \times 10^{-25}\kappa^2 Q_D n^{-4} J_{AD} \qquad (5)$$

A random orientation of chromophores was assumed ($\kappa^2 = 2/3$) and n was taken equal to 1.36 for solutions in 95% ethanol.

The efficiency of energy transfer and the distance between chromophores were determined as described by Eisinger.[20]

D. Results. The synthesized protected peptides have been studied in ethanol solution. The critical distance R_0 for the couple *N*-acetyl-L-tyrosine-ethyl ester and *N*-acetyl-L-tryptophan-ethyl ester in ethanol is 15.7 Å. The quantum yields of these amino acid derivatives (0.11 and 0.13, respectively) are equal to the quantum yield of L-tyrosyl-methyl ester and *t*-boc-L-tryptophyl radicals in the peptides assuming an error of 10%. Such a difference is not significant for the determination of r_c because the quantum yield of the donor, Q_D, occurs only as the sixth root.

The overlap integral J_{AD} is equal to 8.82×10^{32} Å mol^{-1}. The fraction of light absorbed by the chromophores (f_{Trp}^{λ} and f_{Tyr}^{λ}) in the peptides is given by

$$f_{Trp}^{\lambda} = \frac{\epsilon_{Trp}^{\lambda}}{\epsilon_{Trp}^{\lambda} + \epsilon_{Tyr}^{\lambda}} \qquad (6)$$

$$f_{Tyr}^{\lambda} = 1 - f_{Trp}^{\lambda}$$

Table I
Efficiencies, $e(R_0)$, Determined Experimentally and the Distances, r_c, as Calculated from Forster's Equation (eq 1)

Molecule	Exptl efficiency	Lower and upper limit of the distance r_c for a rigid molecule	
R'-Trp-Ala-Tyr-R''	0.75	12.1	13.9
R'-Trp-Ala-Ala-Tyr-R''	0.55	14.6	15.7
R'-Trp-Gly-Ala-Tyr-R''	1.00		12.4

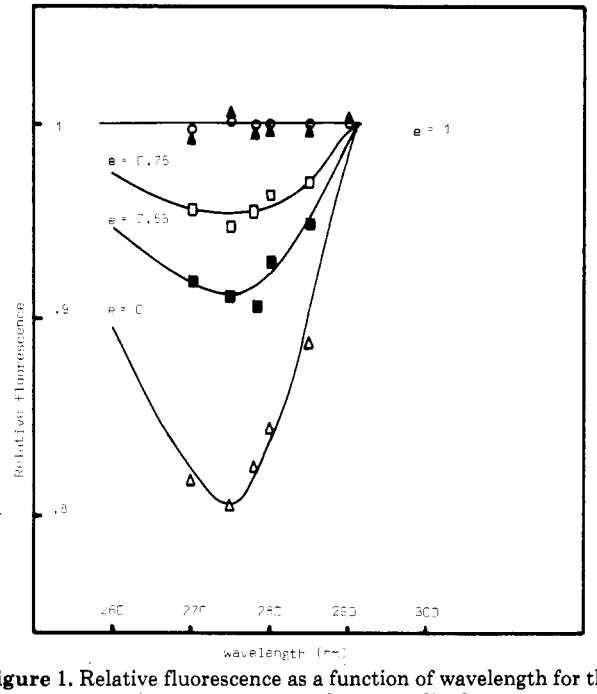

Figure 1. Relative fluorescence as a function of wavelength for the efficiencies equal to 0, 0.55, 0.75, and 1, normalized to 293 nm, and the corresponding experimental points for L-Trp-L-Tyr (○), an equimolar solution of L-Trp and L-Tyr (△), R'-L-Trp-L-Ala-L-Tyr-R'' (□), R'-L-Trp-L-Ala-L-Ala-L-Tyr-R'' (■), and R'-L-Trp-Gly-L-Ala-L-Tyr-R'' (▲).

These ratios were derived from absorption spectra of donor and acceptor and were used to evaluate the efficiency of transfer $e(R_0)$ after the determination of the wavelength dependant relative quantum yield of the peptides ($Q_{Pep}^{\lambda}/Q_{Trp}^{\lambda}$)

$$Q_{Pep}^{\lambda}/Q_{Trp}^{\lambda} = f_{Trp}^{\lambda} + e(R_0)f_{Tyr}^{\lambda} \quad (7)$$

where Q_{Pep} is the quantum yield of the peptide due to an acceptor chromophore at wavelength λ, Q_{Trp} is the quantum yield of an acceptor chromophore in the peptide when no energy transfer occurs (λ_{ex} = 293 nm).

Figure 1 represents the experimental relative fluoresence of the three synthesized peptides of L-Trp-L-Tyr and of an equimolar solution of L-Trp and L-Tyr for different wavelengths. We consider that the various factors discussed above give rise to an error of 10% on the experimental efficiencies. Moreover a good estimate of the distances can only be obtained in the range of efficiencies between 0.2 and 0.8.[9] If the efficiency of energy transfer is greater than 0.8 we only can say that the upper limit of the distance r_c is 12.4 Å (see Table I).

III. Theoretical Model

Theoretical calculations have been performed on the model shown in Figure 2. It is a nonionized tetrapeptide in which the side chains are methyl groups and R represents either a hydrogen or a methyl group. Standard geometry

Table II
Partial Charges on the Terminal Ends of Molecules, Expressed as a Fraction of Electronic Charge

Atom	Charge	Atom	Charge
N_1	−0.202	O'	−0.290
H_1, H_2	0.101	O''	−0.271
C_4'	0.242	H''	0.319

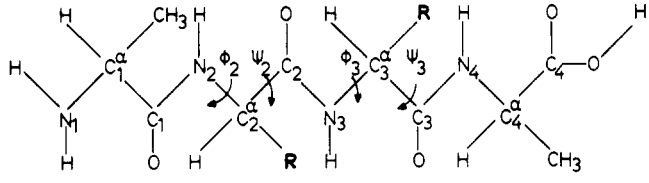

Figure 2. A nonionized tetrapeptide, with either glycyl or L-alanyl residues, at the second and third position.

with a tetrahedral α carbon has been assumed for the backbone and the side chains.

The conformational energy has been computed assuming that it arises from van der Waals and electrostatic pairwise interactions between all the nonbonded atoms.

The van der Waals interaction energy has been represented by the Buckingham potential function with characteristic parameters given by Liquori.[21] The partial charges on the internal residues which appear in the expression for the electrostatic energy are taken from Brant, Miller, and Flory,[22] those on the terminal groups, shown in Table II, have been chosen by similarity to the peptide bond for the amino terminal end, while for the carboxy terminal end the charge distribution proposed by Poland and Scheraga[23] for the side chain of the aspartic acid in nonionized state has been used.

Calculations have been performed with the dielectric constant equal to 1, 4, and 30. Following Ralston and De Coen,[24] the hydrogen-bond energy has been represented by the sum of electrostatic and van der Waals energies, canceling the van der Waals interaction between oxygen and hydrogen. This purely electrostatic interaction decreases very rapidly when the dielectric constant increases.

IV. Calculation of the Distribution of Distances

In order to generate all the comformations of low energy, a set of values of the angles Φ, Ψ was selected from the dipeptide energy map of glycyl or L-alanyl residues. The dipeptide enrgy maps were computed in intervals of 20°. Only the conformational states with energies of less than 3.25 kcal above the absolute minimum of conformational energy have been considered. Using the above values for each pair of the internal angles of rotation, Φ_2, Ψ_2 and Φ_3 Ψ_3, and a constant set of values for the remaining internal angles of rotation, the molecules have been generated numerically on a CDC 6400 electronic computer.

The values given to the internal angles of rotation Φ_1^1, Ψ_1, Ψ_4^1, θ_4^c, equal to −90, 160, −160, 160, 180°, respectively, correspond to low-energy conformations obtained by varying all the Φ_i, Ψ_i except Φ_1^1 through the values prescribed by the stereochemical code of Liquori[7] and by varying Φ_1^1 and θ_4^c in steps of 90°.[25] The side chains have been held in a staggered conformation. The conformational energy E arising from the interactions between all the atoms, the distance r between C_1^α and C_4^α, and the distance r_c between the chromophores have been computed by the above method as a function of Φ_2, Ψ_2 and Φ_3, Ψ_3. The distance r_c between the chromophores has been assumed to be equal to the average value of the distance between the center of the

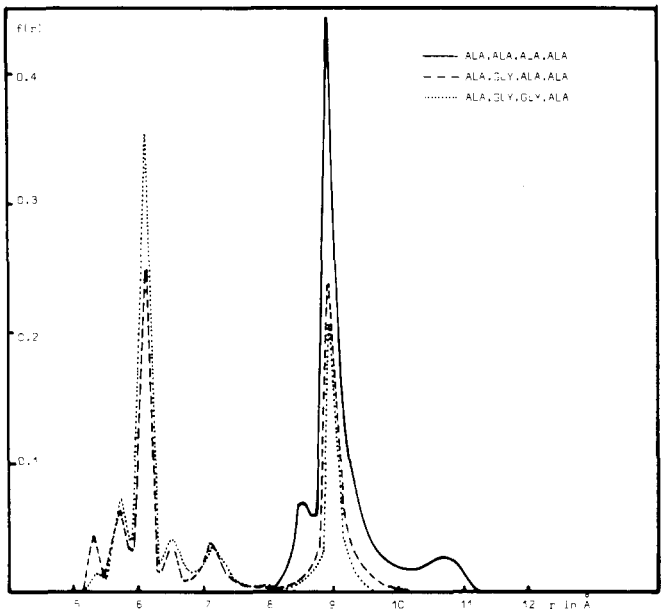

Figure 3. Distributions $f(r)$ of the end-to-end distances $r(C_1^{\alpha}-C_4^{\alpha})$, computed for a dielectric constant $\epsilon = 1$.

benzene ring in L-tyrosine and the center of the pyrrole ring in L-tryptophan. Three orientations corresponding to the low-energy positions of the side chains of the aromatic residues[26] ($\chi^1 = -120, 0, 120°$) have been considered neglecting the energy difference between them. With this method of averaging r_c is determined completely by Φ_2, Ψ_2 and Φ_3, Ψ_3.

The normalized distribution $f(r)$ is computed from the following expression

$$f(r) = z^{-1} \sum_{r=r_k-\Delta r}^{r=r_k+\Delta r} \exp[-E(\Phi_2, \Psi_2, \Phi_3, \Psi_3)/RT] \qquad (8)$$

where the summation is performed on the conformations for which r lies in the prescribed interval $r_k \pm \Delta r$, and z is the partition function.

V. Results

The experimental values of the efficiencies obtained with the two tetrapeptides and a tripeptide R′-L-tryptophanyl-L-alanyl-L-tyrosine-R″ are shown in Table I together with the distances r_c calculated from eq 1 for a rigid system. It is apparent from these results that a glycyl residue produces a marked reduction of dimensions when it replaces an L-alanyl residue. The corresponding reduction of dimensions appears to be more pronounced than that related to the number of units in the molecule.

Table III shows the average square of the end-to-end distance $\langle r^2 \rangle$ and the characteristic ratio $\langle r^2 \rangle / x l_v^2$, where x is the number of peptide units in the molecule and l_v is the length of the virtual bond, calculated from the distributions of distances $f(r)$ with three different values of the dielectric constant. For comparison, the corresponding values for the tetrapeptide L-Ala-Gly-Gly-L-Ala are also included.

The reduction of average dimensions produced by a glycyl residue is also apparent from these theoretical results. However, the computed average dimensions of tetrapeptides containing a glycyl residue are larger than the average dimensions of a trialanine.

A better understanding of the influence of the glycyl residue on the dimensions of molecules studied is obtained from the inspection of the distribution functions. The distribution functions $f(r)$ computed with a dielectric con-

Table III
Average Square of the End-to-End Distance $\langle r^2 \rangle$ and the Characteristic Ratio, C_n, Equal to $\langle r^2 \rangle / x l_v^2$ Calculated with the Dielectric Constant ϵ Equal to 1, 4, and 30

	$\epsilon = 1$		$\epsilon = 4$		$\epsilon = 30$	
Molecule	$\langle r^2 \rangle$	C_n	$\langle r^2 \rangle$	C_n	$\langle r^2 \rangle$	C_n
Ala-Ala-Ala	33.64	1.16	41.15	1.42	40.56	1.40
Ala-Ala-Ala-Ala	83.18	1.92	85.34	1.97	80.14	1.85
Ala-Gly-Ala-Ala	58.68	1.35	63.62	1.47	64.82	1.50
Ala-Gly-Gly-Ala	52.56	1.21	56.39	1.30	59.48	1.37

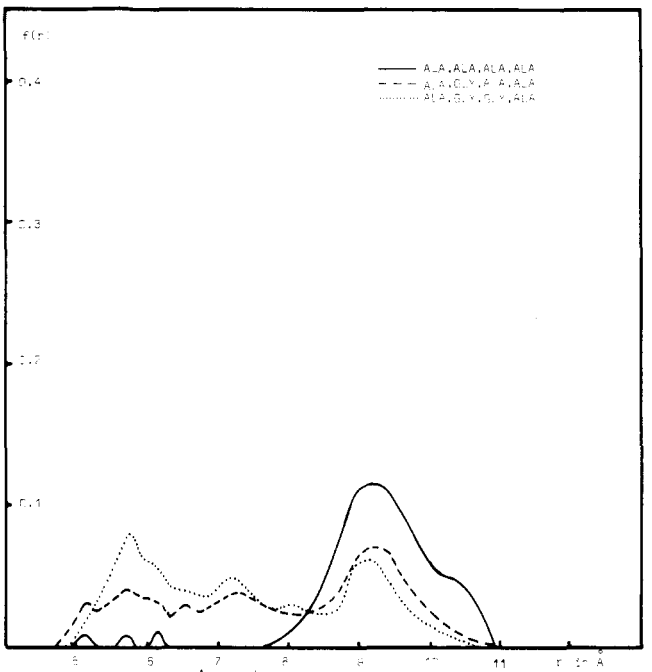

Figure 4. Distributions $f(r)$ of the end-to-end distances $r(C_1^{\alpha}-C_4^{\alpha})$, computed for a dielectric constant $\epsilon = 4$.

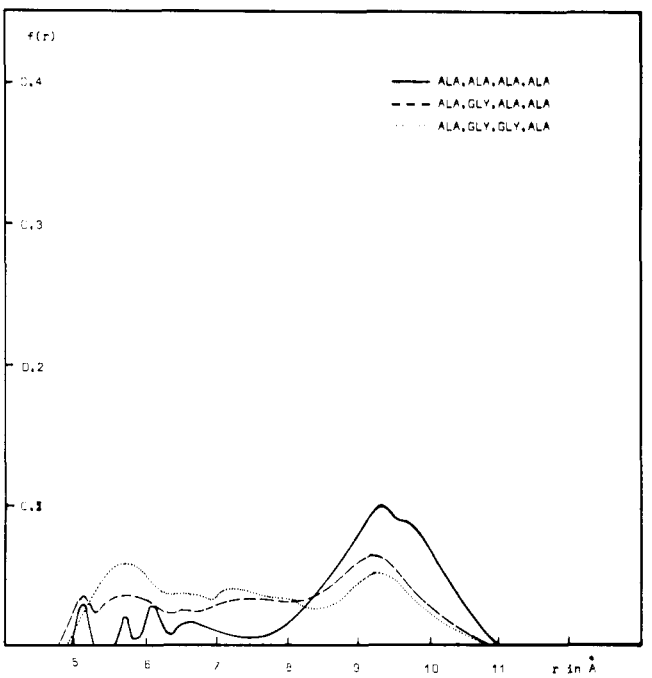

Figure 5. Distributions $f(r)$ of the end-to-end distances $r(C_1^{\alpha}-C_4^{\alpha})$, computed for a dielectric constant $\epsilon = 30$.

Table IV[a]

Molecule	Set	Φ_2	Ψ_2	Φ_3	Ψ_3	Energy, kcal/mol E_{vdw}	E_{elec}	E	$r(C_1^\alpha - C_4^\alpha)$, Å
Ala-Ala-Ala-Ala	1	−80	80	−80	80	0.1	−12.9	−12.8	8.89
	2	−60	90	60	30	2.0	−11.5	− 9.5	4.58
	3	−50	100	50	40	1.4	−12.2	−10.8	4.64
Ala-Gly-Ala-Ala	1	80	−80	−80	80	−0.2	−14.2	−14.4	6.13
	2	−60	90	60	30	1.7	−11.5	− 9.8	4.58
	3	−50	100	50	40	1.2	−12.2	−11.0	4.64
Ala-Gly-Gly-Ala	1	80	−80	−80	80	−0.3	−14.2	−14.5	6.13
	2	−60	90	90	30	1.9	−12.9	−11.0	4.46
	3	−60	90	70	40	0.0	−11.2	−11.2	4.71

[a] Lowest energy conformation for three linked peptide units, the corresponding van der Waals, electrostatic, and total conformational energies, E_{vdw}, E_{elec}, E, and the distances r between C_1^α and C_4^α calculated with $\epsilon = 1$ from the present work (set 1), from Venkatachalam's (set 2), and Chandrasekaran's (set 3) internal angles of rotation.

Table V
Experimental and Theoretical Efficiencies $e(R_0)$ for $R_0 = 15.7$ Å

Molecule	Exptl	$e(R_0)$ calculated $\epsilon = 1$	$\epsilon = 4$	$\epsilon = 30$
Ala-Ala-Ala	0.75	0.95	0.98	0.98
Ala-Ala-Ala-Ala	0.55	0.87	0.87	0.93
Ala-Gly-Ala-Ala	1.00	0.92	0.91	0.94
Ala-Gly-Gly-Ala		0.92	0.94	0.93

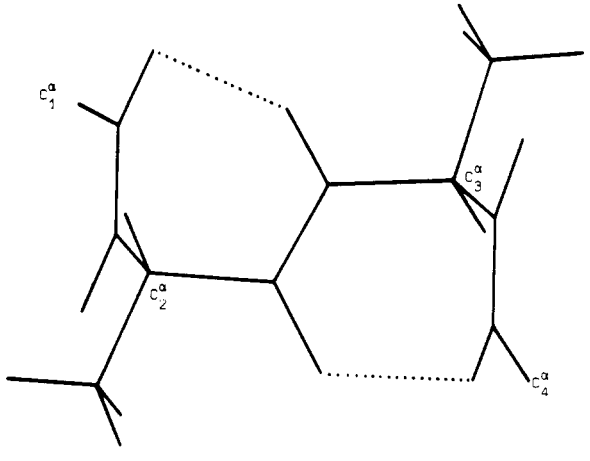

Figure 6. Projection of three peptide units (segment C_1^α to C_4^α) in low-energy conformation *ee*, with an end-to-end distance $r(C_1^\alpha - C_4^\alpha)$ equal to 8.9 Å.

stant ϵ equal to 1, 4, and 30 are shown in Figures 3, 4, and 5, respectively.

We first consider the distribution function $f(r)$ obtained with the dielectric constant equal to 1. In tetralanine there is one single maximum centered at about 9 Å. In L-Ala-Gly-L-Ala-L-Ala and L-Ala-Gly-Gly-L-Ala a new maximum appears at a shorter distance of 6 Å. This maximum is responsible for the reduction of the average dimensions, apparent from the results in Table III.

An increase in the dielectric constant produces a broadening of the distribution functions, but qualitatively the same features are still present.

The maximum of the distribution function $f(r)$ at a larger distance of 9 Å present in all the tetrapeptides investigated theoretically corresponds to a low-energy conformation, defined by the following internal angles of rotation: Φ_2 = −80°, Ψ_2 = 80°, Φ_3 = −80°, Ψ_3 = 80°. This conformation is referred to as *ee* according to the stereochemical code.[7,24]

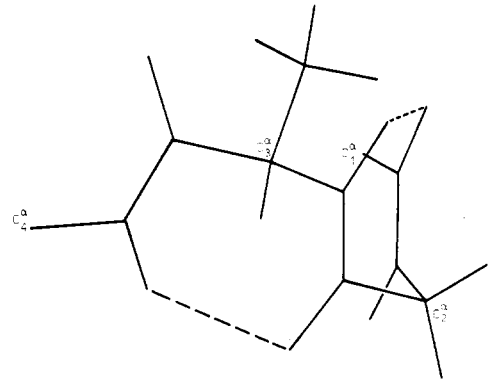

Figure 7. Projection of three peptide units (segment C_1^α to C_4^α) in low-energy conformation *e*e*, with an end-to-end distance $r(C_1^\alpha - C_4^\alpha)$ equal to 6.1 Å.

In the conformation responble for the maximum of $f(r)$ at a smaller distance of 6 Å, the signs of the first two angles of rotation are reversed so that Φ_2 = 80°, Ψ_2 = −80°. The corresponding conformational state *e*e* in the terminology of the stereochemical code is located in the part of conformational energy map accessible only to a glycyl residue or a D amino acid.

The conformations *ee* and *e*e* are shown in Figures 6 and 7. Each is composed of two hydrogen bonded 3 → 1 and 4 → 2 seven-membered rings.

Although isolated seven-membered rings have been observed experimentally,[27,28] a ten-membered hydrogen bonded 4 → 1 ring known as the β bent has been recognized as an essential feature of the tridimensional structure of proteins and peptides.[29]

The ten-membered hydrogen bonded ring has been studied theoretically by Venkatachalam[30] and Chandrasekaran.[31] According to our results, shown in Table IV, the ten-membered ring has a higher energy than the *ee* and *e*e* conformation when three linked peptide units, with L-alanine or glycine on the second and third position, are being considered.

The broadening of the distribution function observed when the dielectric constant increases is due to the absence of conformations stabilized by strong hydrogen bonds.

The experimental efficiencies are compared in Table V with the theoretical values, determined from eq 2 and from the calculated distribution of distances between the chromophores $f(r_c)$. The latter distributions are not markedly

different from the distributions of the distances between C_1^α and C_4^α, $f(r)$.

VI. Discussion

Both experimental and theoretical results show a reduction of dimensions in tetrapeptides containing a glycyl residue, as compared with the dimensions of analogs of the tetraalanine. From the theoretical analysis it is found that conformational states in the lower right quadrant of the conformational energy map, accessible only to a residue without a β-carbon atom or to a D amino acid, give rise to a maximum in the distribution of distances $f(r)$ at a short distance (Figure 3) and are responsible therefore for the observed shortening.

The agreement between experimental and theoretical results is, however, only qualitative. For instance the apparent donor–acceptor distance calculated from experimental efficiencies and from eq 1 is larger for R'-L-Trp-L-Ala-L-Tyr-R'' than for R'-L-Trp-Gly-L-Ala-L-Tyr-R'' (Table I), while the opposite is found for the average end-to-end distances $\langle r^2 \rangle$ computed for the corresponding theoretical models (Table III). Although the same trend is observed for experimental and theoretical efficiencies (Table V), the individual values obtained experimentally and theoretically differ considerably.

Various factors might be responsible for the lack of agreement between experimental and calculated values.

Let us consider the experimental efficiencies and compare them with the values reported in the literature. According to our theoretical calculations, the dimensions of tetrapeptides containing one or two glycyl residues are almost identical. Our results can thus be compared to those of Edelhoch, *et al.*,[32] for L-Tyr-$(Gly)_2$-L-Tyr. The apparent intertyrosine distance r_c determined by Edelhoch, *et al.*, 13.1 Å, is somewhat larger than the upper limit of r_c determined here for L-Trp-Gly-L-Ala-L-Tyr, 12.4 Å. However, the two experimental systems are not identical. Furthermore, the distances determined by Edelhoch, *et al.*, might be overestimated by 15% due to the value of R_0 adopted in their calculations, which exceeds by the corresponding amount the value of R_0 reported for a similar experimental system by different authors.[33]

Even if our experimental results are of the same order of magnitude as the results obtained by others, it should be pointed out that for tetrapeptides a lower value of R_0 is needed in order to determine the apparent donor–acceptor distance with a better precision. Similarly, the theoretical efficiency computed with a value of R_0 lying above the maximum of the distribution of distances $f(r_c)$ cannot be sensitive to the detailed shape of $f(r_c)$.

The differences between the molecules studied experimentally and their theoretical models represented in Figure 2 can also give rise to a disagreement between theory and experiment. The justification for representing the molecules by the above theoretical models lies in the fact that the dipeptide energy map for the L-alanyl residue is almost identical with that of most amino acids with unbranched side chains of the kind $C^\beta H_2$-R[34a] including L-tyrosine and L-tryptopan residues[35] and that the blocking groups located beyond the three dipeptide units spanned by C_1^α and C_4^α contribute approximately a constant term to the conformational energy, independent of the angles of rotation around C_2^α and C_3^α.[34b]

However, all interactions between aromatic side chains or the blocking groups are necessarily being neglected in the theoretical treatment. Among those the stacking interactions between the aromatic side chains of L-tyrosine and L-tryptophan, estimated to be of the order of several kilocalories, are the most important. The possibility of stacking interactions has been examined with Dreiding stereomodels. It has been determined that the stacking between chromophores is not possible in any of the low-energy conformations represented by the two linked seven-membered rings *ee* and *e*e*, while in a ten-membered ring such an interaction can occur.

While in the absence of the interactions between the side chains the energy of any of the conformations *ee* or *e*e* is much lower than the energy of a ten-membered ring (Table IV), the relative stabilities might be reversed by the stacking interactions between the aromatic side chains and the considerable shortening of the dimansions observed experimentally in tetrapeptides containing one or two glycyl residues could be explained by such a stabilization of the ten-membered ring. Calculations are in progress in order to clarify this point.

Other factors might also be quoted when the reliability of theoretical values is being discussed, such as the uncertainty arising from the use of semiempirical potential functions, among which the potential function representing the hydrogen bond[36] is very critical in determining the relative stabilities of various structures.

However, it appears from Tables II and V that the average properties do not change significantly when the dielectric constant increases, and consequently the hydrogen–oxygen interactions decrease.

Above all, the assumption that the chromophores are orientated at random might be the most critical.

An extensive discussion of the value of the orientation factor κ^2 has recently been given by Eisinger and Dale.[37] As has been pointed out by these authors, the isotropically random orientation of chromophores has only been demonstrated in a few cases[38] although the distances obtained from measurements of transfer efficiencies[5] are based on such an assumption. Further experimental analysis of the dipolar orientation factor κ^2 based on polarized emission spectroscopy is needed in order to demonstrate that chromophores are randomly oriented. In our case specific interactions between chromophores and the backbone, or the interactions between the chromophores, could impose a preferred orientation on the transition dipoles. A theoretical model is under study in which the energetics of the side chains are taken into account and from which a theoretical value for κ^2 will be obtained. The present analysis can give consistent relative values, if the orientations of chromophores are comparable in all the peptides studied.

It is evident that it is not possible to determine explicitly the distribution of distrances from a single experimental value of the efficiency. More information can be obtained if the efficiency $e(R_0)$ is measured as a function of R_0,[9] or from the measurements of the decay of the intensity of fluorescence $I(t)$ with time.[39] The experimental results obtained by either of two methods can be analyzed in terms of a theoretical distribution functions of distances $f(r_c)$, calculated from a specific theoretical model, as discussed elsewhere.[40] The latter method is more suitable for the study of $f(r_c)$ in short peptides.[40]

Due to various assumptions discussed above, the agreement between theory and experiment obtained here is only qualitative and might be improved in further theoretical and experimental investigations.

The present study is, however, the first attempt of correlating the experimental efficiencies of energy transfer with the dimensions of the molecules obtained theoretically from conformational analysis.

Acknowledgment. We wish to thank Professor S. Premilat, Université de Nancy I, for stimulating discussions, Dr. C. Renneboog-Squilbin, Université libre de Bruxelles, for

help in some of the calculations, and Mr. J. Vanophalvens, for technical assistance. The numerical calculations have been performed on the CDC 6400 computer at the Centre de Calcul de l'Université Libre de Bruxelles. This work was supported in part by a grant from the Fonds de la Recherche Fondamentale Collective. One of us (Marc Leclerc) is Aspirant au Fonds National de la Recherche Scientifique.

References and Notes

(1) I.R.S.I.A. Fellow.
(2) W. G. Miller, D. A. Brant, and P. J. Flory, *J. Mol. Biol.*, **23**, 67 (1967).
(3) (a) H. E. Warwari, K. K. Knaell, and R. A. Scott III, *J. Chem. Phys.*, **55**, 2020 (1971); (b) H. E. Warwari and R. A. Scott III, *ibid.*, **57**, 1146 (1972).
(4) Th. Förster, "Modern Quantum Chemistry," O. Sinanoglu, Ed., Istanbul Lectures, Academic Press, New York, N. Y., 1965, part III, pp 93–137.
(5) I. Z. Steinberg, *Annu. Rev. Biochem.*, **40**, 83 (1971).
(6) H. A. Scheraga, *Advan. Phys. Org. Chem.*, **6**, 103 (1968).
(7) A. M. Liquori, *Quart. Rev. Biophys.*, **2**, 65 (1969).
(8) P. J. Flory and P. R. Schimmel, *J. Amer. Chem. Soc.*, **89**, 6807 (1967).
(9) C. R. Cantor and P. Pechukas, *Proc. Nat. Acad. Sci. U.S.*, **68**, 2099 (1971).
(10) S. Premilat and J. Hermans, *J. Chem. Phys.*, **59**, 2602 (1973).
(11) A. Loffet and J. Close, "Peptides 1968," North-Holland Publishing Co., Amsterdam, 1968, p 189.
(12) A. Loffet, *Int. J. Protein Res.*, **3**, 111 (1971).
(13) A. Loffet and C. Dremier, *Experiantia*, **27**, 1003 (1971).
(14) B. Bellau and G. Malek, *J. Amer. Chem. Soc.*, **90**, 1651 (1968).
(15) J. Stewart and J. D. Young, "Solid Phase Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969.
(16) E. Lippert, W. Nägele, I. Seibold-Blankestein, U. Staiger, and W. Voss, *Z. Anal. Chem.*, **170**, 1 (1959).
(17) C. A. Parker, *Anal. Chem.*, **34**, 502 (1962).
(18) J. Eisinger, *Photochem. Photobiol.*, **9**, 247 (1969).
(19) R. Chen, *Anal. Lett.*, **1**, 35 (1967).
(20) J. Eisinger, *Biochemistry*, **8**, 3902 (1969).
(21) A. M. Liquori, A. Damiani, and G. Elefante, *J. Mol. Biol.*, **33**, 439 (1968).
(22) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).
(23) D. Poland and H. A. Scheraga, *Biochemistry*, **6**, 3791 (1967).
(24) E. Ralston and J. L De Coen, *J. Mol. Biol.*, **83**, 393 (1974).
(25) The internal angles of rotation Φ^1, $\Psi_4{}^1$, and $\theta_4{}^c$ define the conformation of the terminal groups accordingly to the convention of the IUPAC-IUB Commission on Biochemical Nomenclature, *J Mol. Biol.*, **52**, 1 (1970).
(26) P. K. Ponnuswamy and V. Sasisekharan, *Int. J. Protein Res.*, **3**, 9 (1971).
(27) (a) M. Avignon, P. V. Huong, J. Lascombe, M. Marraud, and J. Neel *Biopolymers*, **8**, 69 (1969); (b) M. Avignon and J. Lascombe, "The Jerusalem Symposium on Quantum Chemistry and Biochemistry," Vol. 5, E. D. Bergamnn and B. Pullman, Ed., Jerusalem Academic Press, 1973, p 7.
(28) M. Marraud, private communication.
(29) C. M. Deber, *Macromolecules*, **7**, 47 (1974).
(30) C. M. Venkatachalam, *Biopolymers*, **6**, 1425 (1968).
(31) R. Chandrasekaran, A. V. Lakshiminarayanan, U. V. Pandya, and G. N. Ramachandran, *BBA (Biochim. Biophys. Acta) Libr.*, **303**, 14 (1973).
(32) H. E. Edelhoch, R. L. Perlman, and M. Wilchek, *Biochemistry*, **7**, 3893 (1968).
(33) J. Eisinger, B. Jener, and A. A. Lamola, *Biochemistry*, **8**, 3908 (1969).
(34) P. J. Flory "Statistical Mechanics of Chain Molecules," Interscience, New York, N. Y., 1969; (a) p 164, (b) p 252.
(35) S. J. Leach, G. Nemethy, and H. A. Scheraga, *Biopolymers*, **4**, 369 (1969).
(36) A. Hagler and S. Lifson, Proceedings of the Symposium on "Polyamino Acids, Polypeptides and Proteins and Their Biological Implications," Rehovot, 1974, to be published.
(37) J. Eisinger and R. E. Dale, *J. Mol. Biol.*, **84**, 643 (1974).
(38) P. W. Schuller and R. Schwyzer, "Peptides," H. Nesvadba, Ed., North-Holland Publishing Co., Amsterdam, 1974.
(39) A. Grinvald, E. Hass, and I. Z. Steinberg, *Proc. Nat. Acad. Sci. U.S.*, **69**, 2273 (1972).
(40) R. Guillard and A. Englert, to be published.

Conformational Properties of Poly(L-azetidine-2-carboxylic acid) in Solution as Studied by Carbon-13 and Proton Nuclear Magnetic Resonance Spectroscopy

R. Boni, R. Di Blasi,* and A. S. Verdini

Laboratori Ricerche di Base, Snamprogetti S.p.A., Monterotondo, Roma, Italy.
Received July 30, 1974

ABSTRACT: The proton and ^{13}C magnetic resonance spectra of poly(L-azetidine-2-carboxylic acid) were determined in water and in formic acid. The 100-MHz ^1H nmr spectra do not give evidence of cis–trans isomerism in the two solvents. On the other hand, the 220-MHz ^1H nmr spectrum in water shows two peaks for the $^{\alpha}CH$ proton. ^{13}C nmr spectra show two separate peaks for each carbon atom, which are assigned to cis and trans isomers of the amide bond. Some model compounds have been examined to aid this assignment. The percentage of the cis isomer decreases from water to formic acid and increases upon addition of $CaCl_2$ to the water solution. High yields of high molecular weight poly(L-azetidine-2-carboxylic acid) (mol wt = 23,000) have been obtained by the polymeric self-condensation of the $(Aze)_3$ pentachlorophenyl ester trifluoroacetate.

The study of the structure of poly(L-azetidine-2-carboxylic acid) (PLAze) in solution is part of a research program intended to elucidate the conformational effects of the replacement of L-proline with L-azetidine-2-carboxylic acid (L-Aze-COOH) in polypeptide chains.

The optical properties of PLAze in solution have been discussed in a recent paper.[1]

The CD spectra in water and fluorinated alcohols have been interpreted as indicating the occurrence of disordered chain structures. This disordering could originate from cis–trans isomerization and/or increased range of accessible $^{\alpha}CC{=}O$ rotation angles.[2]

Here we report investigations of PLAze in solution by means of ^{13}C and ^1H nuclear magnetic resonance (nmr) spectroscopy.

Materials and Methods

L-Azetidine-2-carboxylic acid (Serva) was used as received; found: $[\alpha]^{22}_{589}$ −123.3° (*c* 3.6% in H_2O). *N-tert*-Butyloxycarbonyl-L-azetidine-2-carboxylic acid, *N-tert*-butyloxycarbonyl-L-azetidine-2-carboxylic acid *N*-hydroxysuccinimide ester, and L-azetidine-2-carboxylic acid pentachlorophenyl ester hydrochloride were prepared as previously described.[3] *N*-Carbobenzoxyglycine-*N*-hydroxy-