CONFORMATIONAL BEHAVIOR OF [Trp^4,Met^5]-ENKEPHALIN: COMPARISON OF FLUORESCENCE PARAMETERS AT DIFFERENT PH

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SUMMARY

The conformational behavior of the biologically active [Trp^4,Met^5]-enkephalin was elucidated by evaluation of intramolecular energy transfer between Tyr^1 and Trp^4. Identical transfer efficiencies and tyrosine fluorescence quantum yields were observed in aqueous solution at pH 1.5 and 5.5 and the use of these parameters in Förster's equation resulted in the same average Tyr-Trp separation (9.3 Å) under these two conditions. The invariability of these sensitive parameters indicates the existence of very similar types of a folded conformation in the cationic and zwitterionic form of the analog at low concentrations.

INTRODUCTION

The ability of the opioid peptide [Met^5]-enkephalin (H·Tyr-Gly-Gly-Phe-Met·OH) to compete with the rigid, morphine-derived ligands for the opiate receptor render it an attractive object for studies of the conformational requirements of a polypeptide-receptor interaction. Both spectroscopic approaches (1,2,3,4,5) and theoretical conformational analyses (6,7,8) have produced a variety of different models for the conformation of the pentapeptide in solution. Some of the conflicting results obtained from NMR experiments have been attributed to dimerization at high concentration (5) and to pH-dependent conformational changes (1).

[Trp^4,Met^5]-enkephalin (H·Tyr-Gly-Gly-Trp-Met·OH) has recently been shown to possess the same affinity for the opiate receptor in a rat brain membrane preparation as [Met^5]-enkephalin (9) and a potency ratio of 0.27 relative to the latter compound in the vas deferens bioassay (4). This active analog permits the measurement of the average intramolecular distance between the phenol

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ring in position 1 (donor) and the indole moiety in position 4 (acceptor) by evaluation of intramolecular energy transfer (10). The efficiency of transfer is dependent on both distance and orientation. Furthermore, the tyrosine fluorescence quantum yield is very sensitive to changes in environment (11). Changes in these two extremely sensitive parameters permit the monitoring of even subtle conformational rearrangements and their determination provides an excellent tool for verification of the conformational transitions which have been proposed to occur upon titration of the terminal carboxyl- and amino groups (1,2). The present study provides a comparison of these parameters obtained at different pH.

MATERIALS AND METHODS

The synthesis and purification of the peptides has been described (4). Aqueous solutions of peptides (3×10^{-5}M) were prepared in 0.1 N KCl and 1 mM Tris and adjusted to the desired pH by addition of 1 N HCl or 1 N KOH. Fluorescence emission spectra were recorded on a Hitachi-Perkin Elmer fluorescence spectrophotometer MPF-2L and, when necessary, corrected by a procedure based on comparison with absolute spectra (12). Fluorescence quantum yields were determined by comparison of the emission spectra with the fluorescence spectra of L-tyrosine and L-tryptophan in H_2O (4), whose quantum yields were taken as 0.14 and 0.13, respectively (13). Tyr-Trp separations, r, in [Trp^4,Met^5]-enkephalin were calculated on the basis of Förster's equations (14) (10) using the relation

$$r = (E^{-1} - 1)^{1/6} R_o \qquad (1),$$

whereby the Förster critical distance, R_o, was computed using a published value of the overlap integral, J_{AD}, (15) and under the assumption of random donor-acceptor orientation ($\chi^2 = 2/3$) (4). Transfer efficiencies, E, were calculated from the measured donor quantum yields in the presence, ϕ_D, and absence, ϕ_D^o, of transfer ($E = 1 - \phi_D/\phi_D^o$). At pH 1.5 and 5.5 ϕ_D was obtained by a published procedure (4) and ϕ_D^o was determined with [Met^5]-enkephalin, whose phenylalanine residue, while similar to tryptophan, does not participate in energy transfer at an excitation wavelength of 270 nm. At pH 11.5 ϕ_D and ϕ_D^o were obtained from the emission spectra of [Trp^4,Met^5]-enkephalin and H·Phe-Gly-Gly-Trp-Met·OH, respectively.

RESULTS

Fluorescence emission spectra of [Met^5]-enkephalin and [Trp^4,Met^5]-enkephalin are shown in Fig. 1. Titration of the terminal carboxyl group causes no change in the tyrosine quantum yield of [Met^5]-enkephalin (Fig. 1, Table I). The emission spectra of [Trp^4,Met^5]-enkephalin at pH 1.5 and 5.5 both show the

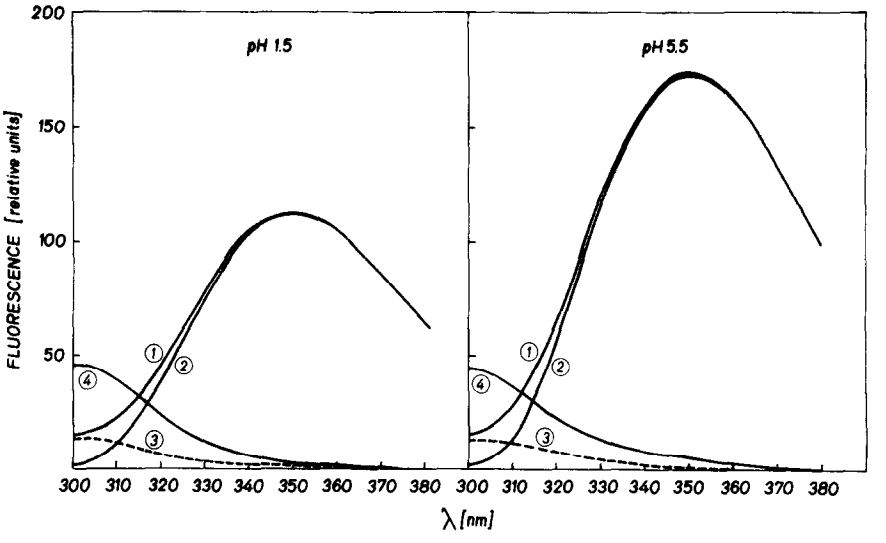

Figure 1. Fluorescence emission spectra at pH 1.5 and 5.5 of [Trp^4,Met^5]-enkephalin with excitation at 270 nm (curve 1) and 293 nm (curve 2) and of [Met^5]-enkephalin with excitation at 270 nm (curve 4). Curve 3 was obtained by subtraction of curve 2 from curve 1 after normalization at 370 nm and represents the contribution of tyrosine in the emission spectrum of [Trp^4,Met^5]-enkephalin. All spectra are corrected for concentration differences.

typical maximum at 350 nm normally observed for a completely aqueous environment. Both protonation of the carboxyl group and quenching by $H^{\oplus}$ (16) are responsible for the decrease in tryptophan fluorescence observed with [Trp^4,Met^5]-enkephalin (ϕ^{Trp} = 0.034) and the reference compound H·Phe-Gly-Gly-Trp-Met·OH (ϕ^{Trp} = 0.036) at pH 1.5. Identical contributions of tyrosine are observed in the emission spectra of [Trp^4,Met^5]-enkephalin at pH 1.5 and 5.5 (Fig. 1, Table I). Consequently the same efficiencies for transfer from tyrosine to tryptophan are obtained (E = 0.70) and, therefore, the use of eq 1 results in identical average separations between Try and Trp in the cationic and zwitterionic form of [Trp^4,Met^5]-enkephalin (Table II). This comparison is rigorous, since the same donor-acceptor pair is present and the same values of ϕ_D^o and J_{AD} are observed at pH 1.5 and 5.5. Therefore, identical values of R_o apply in eq 1 under these two conditions.

Comparison of the tryptophan quantum yields of [Trp^4,Met^5]-enkephalin and

Table I: Quantum yields of tyrosine, ϕ^{Tyr}, and tryptophan, ϕ^{Trp}, at various pH

Compound	ϕ^{Tyr}		ϕ^{Trp}		
	pH 1.5	pH 5.5	pH 1.5	pH 5.5	pH 11.5
Tyr-Gly-Gly-Phe-Met	0.027	0.027			
Tyr-Gly-Gly-Trp-Met	0.008	0.008	0.034	0.057	0.032
Phe-Gly-Gly-Trp-Met			0.036	0.058	0.058

Phe-Gly-Gly-Trp-Met at pH 11.5 reveals extensive quenching in the former compound (Table I) caused by energy transfer from tryptophan to tyrosinate ($Tyr^{\ominus}$). On the basis of the measured parameters and a published value for J_{AD} (15) an average Trp-$Tyr^{\ominus}$ separation of 8.2 Å is calculated. However, this distance cannot be rigorously compared with those obtained at lower pH becasue a different donor-acceptor pair characterized by a very small and less accurate overlap integral is evaluated.

DISCUSSION

A recent study (9) of several [Trp^4,Met^5]-enkephalin analogs with substitutions in position 2 revealed small, but significant differences in both tyrosine quantum yield and average Tyr-Trp separation and thus indicated the sensitivity of these two parameters to subtle conformational changes. The invariability of <u>both</u> ϕ^{Tyr} and the Tyr-Trp distance observed during titration of the carboxyl group suggests the existence of very similar, if not identical conformations or distributions of conformations at pH 1.5 and 5.5. Furthermore, this finding indicates that a possible $NH_3^{\oplus}$ —— $COO^{\ominus}$ interaction is not an important factor in stabilizing the conformation at physiological pH, as it has been suggested (1,2). As a result of the low peptide concentrations (3×10^{-5}M) used, the

Table II: Calculated intramolecular Tyr-Trp separations

pH	donor-acceptor	R_o [Å]	E	r [Å]*
1.5	Tyr-Trp	10.7	0.70 ± 0.01	9.3 ± 0.1
5.5	Tyr-Trp	10.7	0.70 ± 0.015	9.3 ± 0.2
11.5	Trp-$Tyr^{\ominus}$	7.9	0.44 ± 0.02	8.2 ± 0.1

*Experimental errors reflect uncertainties in E.

present study is dealing with the monomeric form of enkephalin, while most NMR studies were carried out at high concentrations where the occurrence of dimerization has been demonstrated (5). The differences between NMR-parameters obtained at pH 1.5 and 5.5 in 10^{-2}M solution (1) might thus be explained by a change in aggregation behavior rather than by a conformational transition in the monomer.

The Tyr-Trp separation of 9.3 Å indicates the existence of a folded conformation both at pH 1.5 and 5.5 and is compatible with the 5 → 2 hydrogen-bonded β-bend model of enkephalin (1,2,5,6,7). However, other types of folded conformations could also accommodate the two aromatic rings at the observed distance. The average Trp-$Tyr^{\ominus}$ distance (8.2 Å) determined at pH 11.5 is less accurate but can be taken as qualitative evidence for the presence of a folded conformation in alkaline solution.

Arguments for the existence of a hydrogen bond between the tyrosine hydroxyl and a carbonyl group of the backbone have been presented (5,7). Intermolecular hydrogen bonding between phenolic hydroxyl and amide carbonyl groups in non-polar solvents quenches phenol fluorescence completely (11). The observed ϕ^{Tyr} of 0.027 in [Met^5]-enkephalin suggests that conformers with this type of

hydrogen bond are not dominant in aqueous solution. The same observation was made in n-butanol (17).

The relationship between solution conformation and receptor-bound conformation remains to be explored. Fluorescence studies with further analogs of [Trp^4,Met^5]-enkephalin are expected to shed some light on this difficult issue.

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REFERENCES

1. Jones, C.R., Garsky, V., and Gibbons, W.A. (1977) Biochem. Biophys. Res. Commun. 76, 619-629.
2. Anteunis, M., Lala, A.K., Garbay-Jaureguiberry, C., and Roques, B.P. (1977) Biochemistry 16, 1462-1466.
3. Bleich, H.E., Day, A.R., Freer, R.J., and Glasel, J.A. (1977) Biochem. Biophys. Res. Commun. 74, 592-598.
4. Schiller, P.W., Yam, C.F., and Lis, M. (1977) Biochemistry, 16, 1831-1838.
5. Khaled, M.A., Long, M.M., Thompson, W.D., Bradley, R.J., Brown, G.B., and Urry, D.W. (1977) Biochem. Biophys. Res. Commun. 76, 224-231.
6. DeCoen, J.L., Humblet, C., and Koch, M.H.J. (1977) FEBS Lett. 73, 38-42.
7. Isogai, Y., Némethy, G., and Scheraga, H.A. (1977) Proc. Natl. Acad. Sci. USA 74, 414-418.
8. Momany, F.A. (1977) Biochem. Biophys. Res. Commun. 75, 1098-1103.
9. Schiller, P.W., and Yam, C.F. (1977) Proc. Fifth American Peptide Symposium, La Jolla, June 20-24, in press.
10. Schiller, P.W. (1975) Biochemical Fluorescence: Concepts, Vol. 1, Chen, R.F., and Edelhoch, H., eds., Dekker, New York, pp. 285-303.
11. Cowgill, R.W. (1976) Biochemical Fluorescence: Concepts, Vol. 2, Chen, R.F., and Edelhoch, H., eds., Dekker, New York, pp. 441-486.
12. Schiller, P.W. (1972) Proc. Natl. Acad. Sci. USA 69, 975-979.
13. Chen, R.F. (1967) Anal. Lett. 1, 35-42.
14. Förster, T. (1948) Ann. Phys. 2, 55-75.
15. Eisinger, J., Feuer, B., and Lamola, A.A. (1969) Biochemistry 8, 3908-3915.
16. Cowgill, R.W. (1970) Biochim. Biophys. Acta 200, 18-25.
17. Schiller, P.W., unpublished results.