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Structure–ionization relationships of enkephalin and related fragments in aqueous solution

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Many compounds of biological interest, like catecholamines, amino acids and peptides, possess more than one dissociable proton. A pentapeptide, tyrosyl-glycyl-glycyl-phenylalanyl-methionine (enkephalin), a natural endogeneous ligand for the opiate receptors in brain (Chang et al., 1976) has 3 dissociable protons, an aromatic hydroxyl, an ammonium group at the N-terminal of tyrosine residue, and a carboxylic acid at the C-terminal of the amino acid. A great deal about the characterization of enkephalin and small peptides in aqueous solution has not yet been studied in detail. During investigations on tyrosine and its derivatives in solution (Ishimitsu

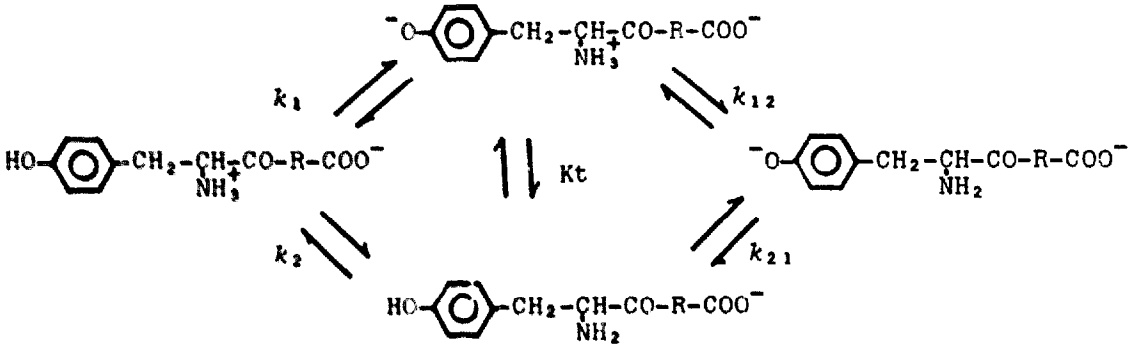

Fig. 1. Scheme of ionization equilibrium of enkephalin and tyrosine-containing peptide.

$$K_1 = k_1 + k_2 \quad (1)$$

$$K_2^{-1} = k_{12}^{-1} + k_{21}^{-1} \quad (2)$$

$$K_1 \cdot K_2 = k_1 k_{12} = k_2 k_{21} \quad (3)$$

$$Kt = \frac{k_2}{k_1} = \frac{k_{12}}{k_{21}} \quad (4)$$

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TABLE I

ACID DISSOCIATION CONSTANTS OF TYROSINE-CONTAINING PEPTIDES [a]

Compound	Titration			CTS method
	pK_{COOH}	pK_1	pK_2	pK_2 [b]
Enkephalin	3.68 ± 0.05	7.77 ± 0.03	9.89 ± 0.03	9.96 ± 0.08
Tyrosyl-glycyl-glycine	3.21 ± 0.03	7.75 ± 0.02	9.78 ± 0.06	9.92 ± 0.09
Glycyl-tyrosyl-glycine	3.10 ± 0.04	8.06 ± 0.03	9.78 ± 0.05	9.97 ± 0.08
Glycyl-glycyl-tyrosine	3.18 ± 0.03	8.17 ± 0.07	9.75 ± 0.09	9.94 ± 0.07
Tyrosyl-glycine	3.51 ± 0.02	7.77 ± 0.04	10.04 ± 0.02	9.97 ± 0.07
Glycyl-tyrosine	3.24 ± 0.06	8.23 ± 0.04	10.55 ± 0.06	10.39 ± 0.04

[a] $\mu = 0.1$ ($NaClO_4$), 25°C.

[b] The pK_2 value, which corresponds to the proton dissociation of aromatic hydroxyl group, determined by CTS method.

et al., 1976, 1977, 1979a and 1979b), we found that a specific sequence of tyrosine residues in peptides plays an important role in the pH-dependent distribution of various ionic forms of a peptide. We report herein the microscopic acid dissociation constants (micro-constants), tautomeric constants (K_t) and the relative concentrations of various ionic forms of enkephalin and related fragment compounds.

The micro-constants of enkephalin and tyrosine peptides, shown in Fig. 1, were determined according to the modified method of complementary tri-stimulus colorimetry (CTS method) (Flaschka, 1960). The acid dissociation and micro-constants thus determined are summarized in Table 1 and Table 2. The relationship between the relative concentrations of 4 chemical species of enkephalin as a function of pH is depicted in Fig. 2.

The K_t-value and Fig. 2 indicate that the concentration of phenolate-ammonium form relatively increases in the N-terminal tyrosine-containing peptides such as enkephalin, tyrosyl-glycyl-glycine and tyrosyl-glycine in comparison to that of 3

TABLE 2

MICROSCOPIC ACID DISSOCIATION CONSTANTS AND TAUTOMERIC CONSTANTS OF TYROSINE PEPTIDES

Compound	pk_1	pk_2	pk_{12}	pk_{21}	$K_t(k_2/k_1)$ [a]
Enkephalin	8.61 ± 0.02	7.99 ± 0.03	9.63 ± 0.04	10.30 ± 0.04	4.2
Tyrosyl-glycyl-glycine	8.18 ± 0.03	7.71 ± 0.02	9.47 ± 0.03	10.00 ± 0.03	3.0
Glycyl-tyrosyl-glycine	9.80 ± 0.03	8.49 ± 0.03	8.85 ± 0.04	9.98 ± 0.04	20.4
Glycyl-glycyl-tyrosine	9.55 ± 0.02	8.36 ± 0.04	8.79 ± 0.04	10.01 ± 0.03	15.5
Tyrosyl-glycine	8.42 ± 0.03	8.04 ± 0.01	9.51 ± 0.03	9.90 ± 0.02	2.4
Glycyl-tyrosine	9.32 ± 0.02	8.41 ± 0.02	9.52 ± 0.04	10.43 ± 0.04	8.1

[a] K_t-value was calculated from the ratio k_2/k_1.

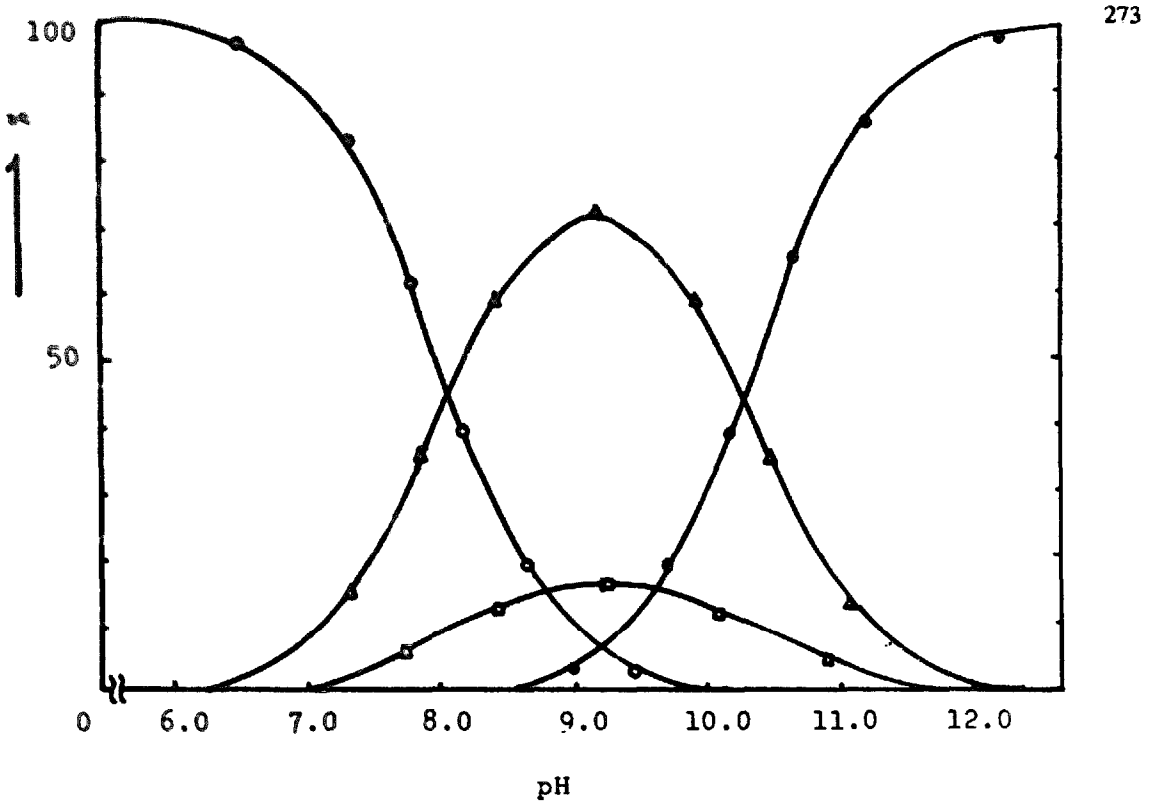
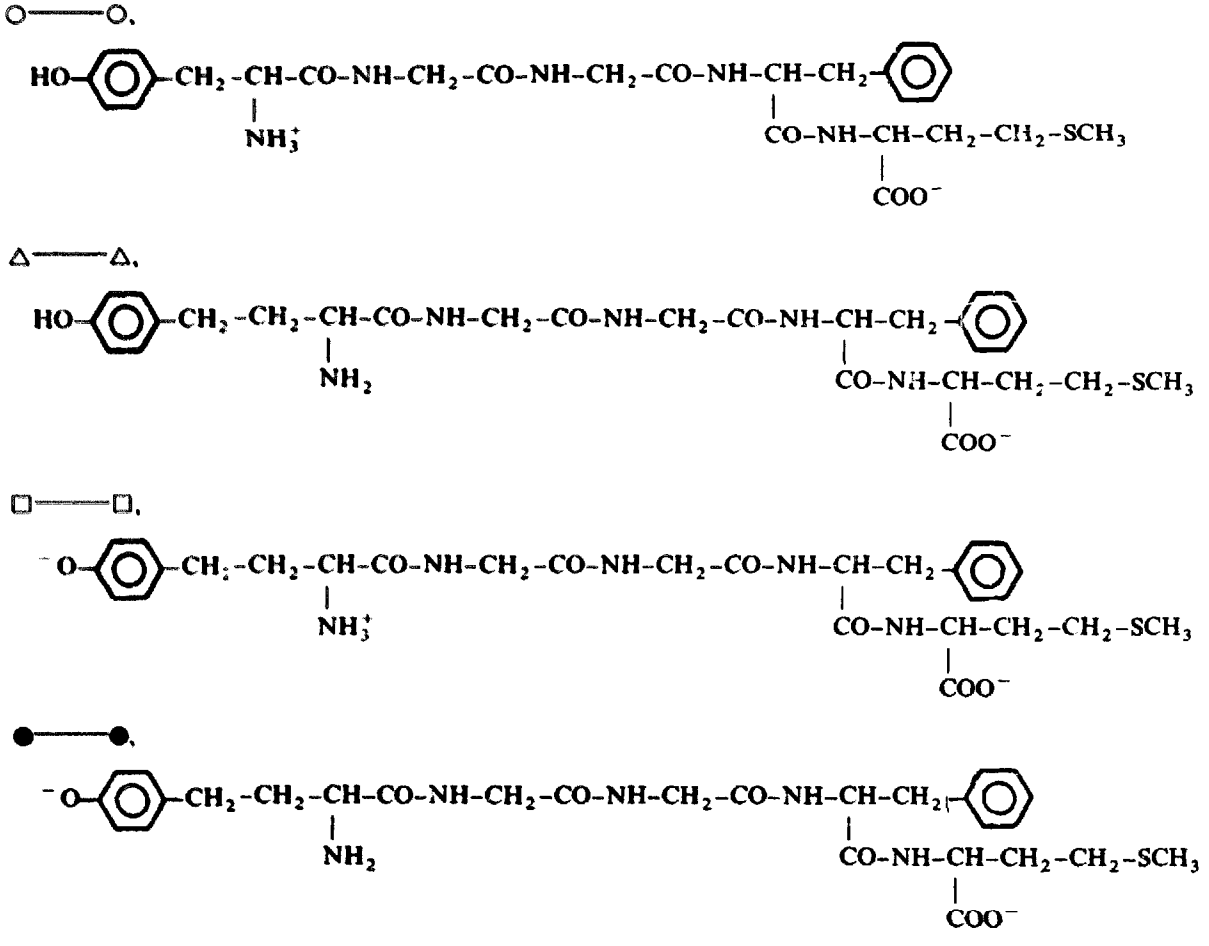


Fig. 2. Relative concentration of various ionic forms of enkephalin.



other peptides (glycyl-tyrosyl-glycine, glycyl-glycyl-tyrosine and glycyl-tyrosine). Therefore, altering partial drug structure, especially the sequence of tyrosine residue, changes the balance between the phenolate-ammonium and phenol-amino form. The significance of this effect to the biological action of enkephalins may be worth exploring.

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