MOLECULAR CONFORMATIONS OF MET-ENKEPHALIN: COMPARISON OF THE ZWITTERIONIC AND CATIONIC FORMS

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Summary

Apparent discrepancies in published assignments of the pmr spectrum of Met^5-enkephalin are due to the existence of two forms, cationic and zwitterionic. The pmr parameters of these two forms indicate different secondary conformations. The pmr parameters of the zwitterionic form are indicative of a 2-5 β-I turn but no secondary conformation can be suggested for the cationic form with the present data.

Introduction

Enkephalin, the endogenous brain peptide Tyr-Gly-Gly-Phe-Met (1), has been shown to be an analgesic (2) and to operate at the same receptors (3) as natural opiates and their antagonists. In preliminary pmr studies (4) of enkephalin in [$^2H_6$]-DMSO* a very small temperature dependence was found for the Met^5 amide proton chemical shift, typical of a hydrogen bonded or solvent shielded proton. This suggested a model for Met-enkephalin with the following features:

(a) Met^5 amide proton hydrogen bonded to the Gly^2 carbonyl

(b) the COO^- and NH_3^+ termini in close proximity

(c) a β-I turn involving residues 2-5 for most of the molecular forms of enkephalin

(d) Side chains of residues of 1,4 and 5 exhibited extensive C_α-C_β internal rotation. Thus based upon side chain internal motions alone, many tertiary enkephalin conformations exist simultaneously in solution, most of which possess

*[$^2H_6$]-DMSO is hexadeutero-dimethylsulfoxide

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the β-I turn secondary conformation. The β-turn model is consistent with certain of the exogenous opiate structure-function relationships (5, 6). Particularly important among these is that the β turn keeps the L-Tyr^1 and L-Phe^4 aromatic rings at the distance required for enhanced activity. D-Phe^4 substitution at the β-turn does not and hence is inactive.

Garbay-Jaureguiberry et al. (7) also concluded that a β-I turn was present in [$^2H_6$]-DMSO solutions but Bleich et al. (8, 9) obtained results which differ in three respects. First, assignments in the amide region of the spectrum are different even though the spectra appear similar. In particular, the assignment of the two amide doublets of Phe^4 and Met^5 are reversed. Second, there are some differences in the appearance of the spectrum in the higher field regions. Third, they find no evidence for a hydrogen bonded structure or for an NH_3^+----COO^- interaction in either [$^2H_6$]-DMSO or H_2O solutions.

Here we show that these apparently contradictory results are due to the existence of two different secondary conformations of Met-enkephalin. One set of secondary conformations predominates when the peptide is in the zwitterionic form and different conformations predominate when the peptide is in the cationic form. The zwitterionic or physiological form is the one we studied originally (4) and for which we proposed the β-I turn model. Bleich et al. (8, 9) studied the cationic form.

Materials and Methods

For these experiments and for our previous studies Met-enkephalin was prepared in the zwitterionic form by neutralizing an aqueous solution of the peptide, lyophilizing and redissolving in [$^2H_6$]-DMSO. Lyophilized peptide was also redissolved in H_2O: this gives a solution of pH = 5, confirming that it was near its isoelectric point. The pmr spectrum of the cationic form was obtained both by dissolving 5 mg/ml of the hydrochloride salt in [$^2H_6$]-DMSO and by the addition of trifluoroacetic acid to a [$^2H_6$]-DMSO solution of the zwitterionic form. Spectra were obtained with a Bruker WH-270 equipped with a Nicolet 1180 computer. Temperature was controlled at +1°C. Double resonance and pH titration were used to make assignments.

Results

Spectra of the zwitterionic and cationic forms of Met-enkephalin are shown in Fig. 1a and 1b. While the spectra appear very similar, the amide resonances of Phe^4 and Met^5 are reversed; this demonstrates that the differences between

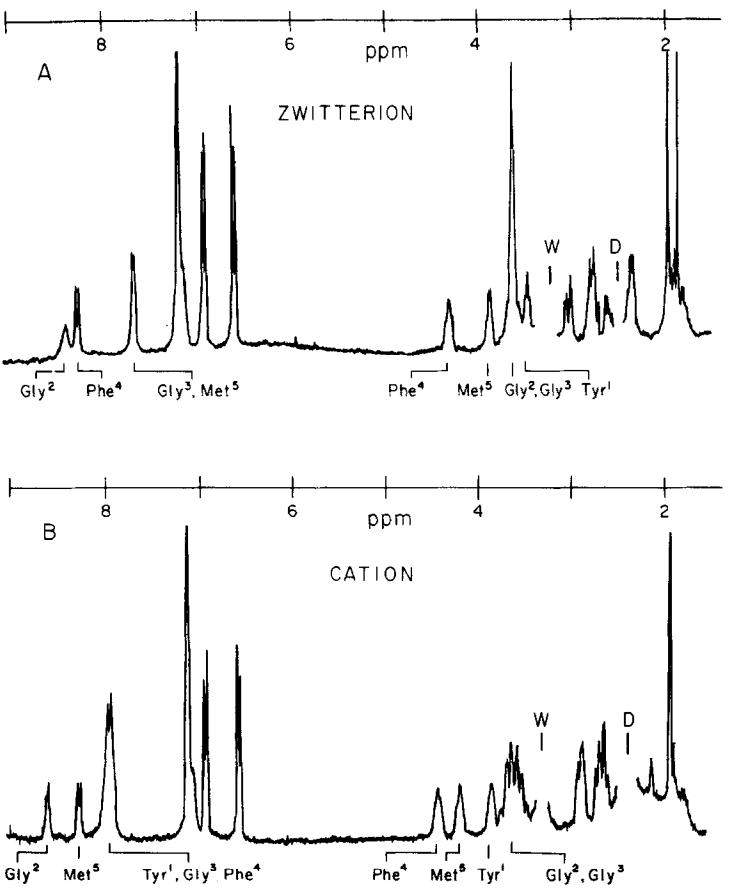

Figure 1. PMR spectra of the zwitterionic and cationic forms of Met-enkephalin in [$^2H_6$]-DMSO. The positions marked W and D are the resonance positions of the residual water and the residual proton in the [$^2H_6$]-DMSO respectively. Comparison of A and B shows a number of differences, most notable of these is the reversal of the Phe^4 and Met^5 amide proton resonance positions. Titration from the zwitterionic to the cationic forms in [$^2H_6$]-DMSO solution produced the same result.

Bleich et al. (8, 9) and ourselves are due to differing ionic forms of enkephalin. These differences are not due to special effects of the chloride counter ion since the same result is obtained in the direct titration with trifluoroacetic acid of the zwitterion to the cation in [$^2H_6$]-DMSO solution.

Differences in chemical shift of a few resonances do not in themselves prove that there are two conformations since chemical shifts are sensitive to charge effects as well as to conformational effects. However, the spectral data summarized in Table I clearly show that the cationic and zwitterionic forms

Table I: PMR Parameters of the Zwitterionic (Z.) and Cationic (C.) Forms of Met^5-enkephalin

	amide proton temperature coefficients*		$<^3J_{NHCH}>$ values†	
	Z.	C.	Z.	C.
Tyr^1	--	--	--	(10.5)
Gly^2	7.8	4.1	--	5.2, 5.9
Gly^3	3.1	4.3	--	5.4, 6.0
Phe^4	7.5	5.7	8.3	8.4
Met^5	0.9	6.3	7.2	7.9

Side-chain analysis

	$<^2J_{CHCH}>$†		$<^3J_{CHCH}>$†		Zwitterion rotamers‡			Cation rotamers+		
	Z.	C.	Z.	C.	tg^+	tg^-	gg	tg^+	tg^-	gg
Tyr^1	-13.7	-14.0	6.2, 7.5	5.9, 8.5	0.44	0.33	0.23	0.50	0.26	0.24
Phe^4	-13.9	-13.9	4.0, 10.1	4.2, 8.4	0.68	0.13	0.19	0.53	0.15	0.32
Met^5	-13.3	--	4.5, 7.3	--	0.43	0.17	0.40	--	--	--

*Absolute values of the temperature coefficients in ppb/deg (parts per 10^9/°K)

†Observed coupling constants between the amide and alpha protons ($<^3J_{NHCH}>$), the two beta protons ($<^2J_{CHCH}>$), and the alpha and beta protons ($<^3J_{CHCH}>$) in units of Hertz (Hz).

‡Statistical weights of rotamers using 2.6 and 13.6 Hz as the intrinsic coupling constants. The method does not unequivocally distinguish the tg^+ from the tg^-.

have different conformations. For example, the values of $^3J_{NHCH}$, (for backbone angle ϕ), and the values of $^3J_{CHCH}$ (for angle χ_I) are different. More importantly, the temperature dependence of the Met^5 amide proton chemical shift is quite small (<2 ppb/deg) for the zwitterionic form, indicating a hydrogen bond (10) or solvent shielded environment, but shows a normal, solvent exposed value (>3 ppb/deg) for the cationic form. Consequently, we conclude that the conformation of the cationic form is probably different from the zwitterionic form. Under physiological conditions Met-enkephalin is probably zwitterionic but is almost certainly not cationic.

Table II: Cumulative Statistical Weights

conformer		Zwitterion Tyr^1	Phe^4	Met^5
1	.129	tg^+	tg^+	tg^+
2	.120	tg^+	tg^+	gg
3	.097	tg^-	tg^+	tg^+
4	.090	tg^-	tg^+	gg
5	.067	gg	tg^+	tg^+
6	.063	gg	tg^+	gg
7	.051	tg^+	tg^+	tg^-
8	.038	tg^-	tg^+	tg^-
9	.036	tg^+	gg	tg^+
10	.033	tg^+	gg	tg^-

Discussion

The $^3J_{NHCH}$ values alone are not sufficient to determine the backbone conformation of either the zwitterionic or cationic forms of Met-enkephalin. However, the evidence discussed above for the involvement of the Met^5 amide proton of the zwitterion in a hydrogen bond, combined with $^3J_{NHCH}$ values are indicative of a folded conformation with a 2-5 β-I turn. While both β-I and β-II turns would have such a hydrogen bond, the β-II turn can be excluded (11) since it would give a value of 6.6 for the Phe^3 $^3J_{NHCH}$. Only the Gly^3 amide proton's temperature dependence (3 ppb/deg) is even close to that of the Met^5 amide proton. This value is at most a borderline case for solvent shielding. While both a Met^5 amide and a Gly^3 amide hydrogen bond could be accommodated by a γ-turn, the coupling constants predicted by Nemethy and Printz (11) make it unlikely that this is the major contributing species. These conclusions can

be accepted with reservation until we fully evaluate the effects of conformational averaging on the $^3J_{NHCH}$ values.

The fact that all three residues with side-chains show extensive averaging about the χ_I bond emphasizes the importance of conformational mobility and necessarily implies that there is not a single, unique Met^5-enkephalin conformation. Of the 27 zwitterionic conformations with differing side-chain rotamers, the ten with the highest cumulative statistical weights are listed in Table II. Even the most probable conformer only accounts for 13% of the total and this does not include any additional conformational flexibility such as the special backbone flexibility known for glycine residues (12). For the zwitterion form the deviations of the observed $^3J_{NHCH}$ values from those predicted by idealized models are attributed to the effects of such conformational averaging.

The β-I turn model of the zwitterion form brings the COO^- and NH_2^+ groups close together and this structural feature may make an important contribution to its stability. The necessary lack of such a feature in the cationic form may explain why its conformation is different. With the $^3J_{NHCH}$ values which are available and with no hydrogen bonding or other information no structure can be suggested for the cationic form at this time.

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