ENKEPHALIN: STRUCTURE–FUNCTION RELATIONSHIPS

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1. Introduction

Met-enkephalin (Met-Ek) is the 61–65 fragment of β-lipotropin with amino acid sequence Tyr^1–Gly^2–Gly^3–Phe^4–Met^5. It is one of the endogenous ligands for morphine receptors in brain and smooth muscle. Recent semi-empirical conformational analysis of the molecule [1,2] showed its high conformational mobility. (It should be noted, however, that in [1] the end groups of the molecule have been assumed to be uncharged, whilst in [2] a number of potentially stable structures has been omitted in the treatment owing to certain features of the calculation pattern.) On the other hand, biological activity tests of Met-Ek analogs with restricted conformational freedom have shown that [D-Ala^2]–Met-Ek possesses increased affinity for morphine receptors, this property being reduced for [D-Ala^3]–Met-Ek [3]. A comparison of these data with known sets of stable conformations of these molecules ought to make it possible to discern the conformation (or set of conformations) of the Met-Ek molecule which is characteristic for receptor binding. This would mean solving the important problem of structural and functional organization of the Met-Ek molecule. Such a 'biologically active' set of conformations must contain stable structures, common for Met-Ek and [D-Ala^2]–Met-Ek, excluding at the same time [D-Ala^3]–Met-Ek structures (elements of such an approach can be found in [4]).

2. Methods and results

To approach the problem, a total semi-empirical conformational analysis of Met-Ek, [D-Ala^2]–Met-Ek and [D-Ala^3]–Met-Ek zwitterionic forms was done. (Since the conformational mobility of the two latter molecules is restricted, only structures belonging to the set of stable Met-Ek conformations were subjected to examination.) The basic methods and calculation patterns have been described [5,6], including the calculation of conformational energy of model fragment Ala–Gly–Gly–Ala–Ala and tripeptides Tyr–Gly–Gly, Gly–Gly–Phe and Gly–Phe–Met for the initial steps of the calculations. The final stage of the calculations consisted in examining the conformational lability of Tyr^1 side-chain for the most stable conformations of the peptide backbone of the molecule, since in most cases the conformations of the tyrosine side-chain are practically not correlated with the conformations of phenylalanine and methionine side-chains.

Results of our calculations show that the set of stable backbone structures of Met-Ek is considerably restricted by electrostatic interaction of the ionogenic end groups, leading to formation of quasicyclic molecular structures (cf. table 1). In addition, there is also a restriction in mobility of the Phe^4 backbone, limiting to the B and R regions only (in terms of local minima of the dipeptide unit potential maps, B corresponding to values $\varphi\sim-140°$, $\psi\sim140°$, R to $\varphi\sim-60°$, $\psi\sim-60°$, L to $\varphi\sim60°$, $\psi\sim60°$ and H to $\varphi\sim80°$, $\psi\sim-80°$ [19]), as well as enhanced conformational lability of dipeptides Tyr–Gly and Gly–Gly, as compared with dipeptides Gly–Phe and Phe–Met, the mobilities of Tyr^1, Phe^4 and Met^5 side-chains remaining approximately equal (with certain limitations for all rotamers with $\chi_1\sim-60°$).

These features of calculated Met-Ek structures are in agreement with NMR data, thus confirming the validity of our approach. Indeed, close spacing of the

Table 1
Stable conformations of Met-Ek, [D-Ala^2]–Met-Ek and [D-Ala^3]–Met-Ek

Backbone information	Conformational energy $U-U_{min}$ (kcal/mol) of Tyr^1 rotamers with different χ_1 values[a]								
	~ 60°			~ 180°			~ −60°		
	Met-Ek	[D-Ala^2]–Met-Ek	[D-Ala^3]–Met-Ek	Met-Ek	[D-Ala^2]–Met-Ek	[D-Ala^3]–Met-Ek	Met-Ek	[D-Ala^2]–Met-Ek	[D-Ala^3]–Met-Ek
RRRBL	–	–	–	4.7	–	–	0.0	–	–
BBRRB	0.9	–	–	2.9	–	–	–	–	–
BHLBB	4.3	5.0	–	1.1	2.6	3.9	1.5	–	–
RBHRB	–	–	–	3.2	–	1.8	2.7	–	0.7
BLLBL	3.2	4.5	0.3	5.7	2.6	3.9	–	–	–
RHRBL	–	–	–	3.5	1.1	3.6	3.4	–	0.0
RRBBL	–	–	–	–	–	–	3.4	–	–
RLRRB	–	–	–	3.4	1.4	–	–	–	–
BHHRB	–	–	–	3.7	4.4	–	4.5	–	–
RLLRB	–	–	–	–	–	–	3.7	–	4.6
BBLRB	–	–	–	3.8	–	0.4	4.2	–	3.3
RHRRB	–	–	–	3.8	–	5.2	–	–	–
BHRRB	–	–	–	5.1	5.6	3.9	4.0	–	5.5
RHBRB	–	–	–	4.2	0.0	–	–	–	–
BHRBL	–	–	–	5.3	3.5	5.6	4.9	5.0	4.8
BLBRB	5.4	–	–	5.1	–	–	5.7	–	–
BLLBB	6.0	–	–	–	–	–	5.1	–	3.6
BLRRB	–	–	–	–	–	–	5.4	–	–
BBLBL	–	–	–	–	–	–	5.6	–	4.4
BHRBB	–	–	–	5.8	1.5	–	–	–	–
RRHRB	–	–	–	5.9	–	3.5	–	–	–
BLHBL	–	–	–	–	–	–	–	–	–

[a] Side-chain rotamers of other residues: $\chi_1 \sim 180°, \chi_2 \sim 90°$ for Phe, $\chi_1 \sim -80°, \chi_2, \chi_3 \sim 180°$ for Met in all cases with exception of BLLBB, BLHBB, and BHRBB, where $\chi_2 \sim 80°$ for Met; the dihedral angle values are in accordance with [19]. Energies above 6 kcal/mol are not included in the table

N- and C-termini is shown in [7–9]; high mobility of the dipeptide Tyr–Gly features in [10]; the vicinal constant $J_{NHC^{\alpha}H}$ values according to [7,9,11–13] are in good agreement with the majority of structures listed in table 1; finally, in many of the stable structures the amide protons of Gly^3 and Met^5 are situated on the inside part of the β-turn formed by the backbone which, apparently, accounts for the lowering of the $d\delta/dT$ value for these protons, as observed in [7,9,10–12,14].

3. Discussion

The comparatively high rigidity of the Tyr^1 side-chain ($\chi_1 \sim 180°$) is one of the principal structural characteristic features of the [D-Ala$_2$]–Met-Ek. Since the rigid thyramine moiety of morphine is of special functional significance [15], it stands to reason that the very fact of 'fixed' distance between the OH and NH_3^+ groups accounts to some extent for the increased binding capacity of [D-Ala^2]–Met-Ek towards

morphine receptor (the respective distances being: 7.5–8.0 Å in [D-Ala^2]–Met-Ek, 5.6–8.0 Å in Met-Ek and 7.0 Å in morphine, with $NH(CH_3)^+$ in the latter instead of NH_3^+. This circumstance enables us to suggest that the adequate spacing of the Tyr^1 side-chain and of the NH_3^+ group of the Met-Ek molecule is 'selected' by the receptor according to the induced-fit principle. The role of the remaining amino acid residues of the molecule in structure formation is, apparently, confined to maintaining such structures in which the Tyr^1 side-chain is located on the 'bend' of the peptide backbone and points 'outward'. The existence of morphine-mimetic activity of some β-lipotropin fragments starting with Tyr^{61}, as well as decrease in activity of Met-Ek after elimination of the C-terminus [16] also speaks in favour of the above suggestion. Moreover, the loss of activity caused by Phe^4 → Tyr^4 or Met^5 → Gly^5 replacement [16] also points towards participation of the hydrophobic C-terminal moieties of the molecule in receptor binding and, accordingly, towards their location close to the 'main' Tyr^1 residue.

The data listed in table 1 enable us also to select the set of 'biologically active' Met-Ek conformations: it includes RLRRB, BHHRB, RHBRB, BLHBB and BHRBB (BLHBB differing from BHRBB only by a 180° turn of the Gly^2–Gly^3 peptide bond plane). Only one of these structures, however, namely BHRBB, is found to be stable, according to conformational energy calculations for a Met-Ek molecule with uncharged end groups (only optimal rotamers of the Tyr^1 side-chain were considered; calculation results are in good agreement with those from [1]). Comparison with the high activity of the [D-Ala^2]–Met-Ek–NH_2 speaks in favour of selecting one single 'biologically active' Met-Ek structure (selection scheme presented in fig.1). The structure suggests a β-I turn with participation of residues 1–4; more stable Met-Ek structures would contain a β-I turn with residues 2–5.

The concept of 'biologically active' conformation of Met-Ek, namely BHRBB, easily explains the loss of receptor affinity of [L-Ala^2]–Leu-Ek and [L-Ala^2, L-Ala^3]–Leu-Ek [17], since the H-type conformation is sterically inconsistent for L-type amino acids (it explains also loss of affinity of the [D-Phe^4]–Met-Ek [3], the B-type conformation being inconsistent in this case). 'Biologically active' conformation is also

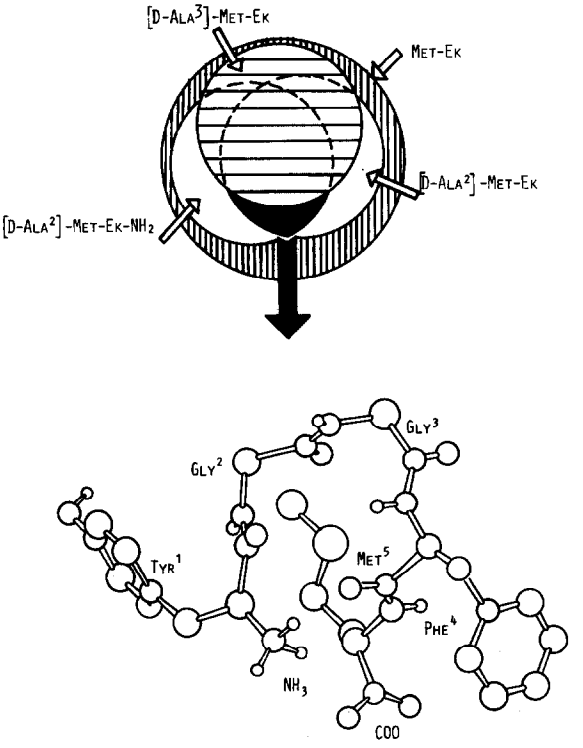

Fig.1. Selection scheme and view of 'biologically active' Met-Ek conformation.

feasible in the case of the highly active [D-Met^2, Pro^5]-Ek analog [18], since, as can be seen from fig.1, the D-Met^2 side-chain is capable of occupying the same place on the receptor surface, as the Met^2 side-chain. On the other hand, loss of affinity of [D-Leu^2]–Met-Ek, [D-Phe^2]–Met-Ek [3] and [L-Ala^3]–Leu-Ek [17] cannot be explained in terms of structural considerations only. It appears that an increase in hydrophobicity of the solvent-facing part of the molecule (cf. fig.1) slackens its binding with the receptor.

References

[1] Isogai, Y., Némethy, G. and Scheraga, H. A. (1977) Proc. Natl. Acad. Sci. USA 74, 414–418.
[2] De Coen, J.-L., Humblett, C. and Koch, M. H. J. (1977) FEBS Lett. 73, 38–42.

[3] Coy, D. H., Kastlin, A. J., Schally, A. V., Morin, O., Caron, N. C., Labrie, F., Walker, J. M., Fertel, P., Bernston, G. G. and Sandman, C. A. (1976) Biochem. Biophys. Res. Commun. 73, 632–637.
[4] Momany, F. A. (1977) Biochem. Biophys. Res. Commun. 75, 1098–1103.
[5] Galaktionov, S. G., Nikiforovich, G. V., Shenderovich, M. D., Chipens, G. I. and Vegner, R. E. (1976) in: Peptides–1976 (Loffet, A. ed) pp. 617–624, Bruxelles.
[6] Nikiforovich, G. V., Shenderovich, M. D. and Galaktionov, S. G. (1976) Bioorg. Khim. 2, 1268–1270.
[7] Jones, C. R., Gibbons, W. A. and Garsky, V. (1976) Nature 262, 779–782.
[8] Combrisson, S., Roques, B. P. and Oberlin, R. (1976) Tetrahedron Lett. 38, 3455–3458.
[9] Garbay-Jaureguiberry, C., Roques, B. P., Oberlin, R., Anteunis, M. and Lala, A. K. (1976) Biochem. Biophys. Res. Commun. 71 (2), 558–565.
[10] Roques, B. P., Garbay-Jaureguiberry, C., Oberlin, R., Anteunis, M. and Lala, A. K. (1976) Nature 262, 778–779.
[11] Bleich, H. E., Cutnell, J. D., Day, A. R., Freer, R. G., Glasel, J. A. and McKelvy, J. F. (1976) Proc. Natl. Acad. Sci. USA 73 (8), 2589–2593.
[12] Jones, C. R., Garsky, V. and Gibbons, W. A. (1977) Biochem. Biophys. Res. Commun. 76, 619–625.
[13] Anteunis, M., Lala, A. K., Garbay-Jaureguiberry, C. and Roques, B. P. (1977) Biochemistry 16, 1462–1466.
[14] Khaled, M. A., Long, M. M., Thompson, W. D., Braedley, R. J., Brown, G. B. and Urry, D. W. (1977) Biochem. Biophys. Res. Commun. 76, 224–231.
[15] Horn, A. S. and Rodgers, J. R. (1977) J. Pharm. Pharmacol. 29, 257–265.
[16] Morgan, B. A., Smith, C. F. C., Waterfield, A. A., Hughes, J. and Kosterlitz, H. W. (1976) J. Pharm. Pharmacol. 28, 660–661.
[17] Terenius, L., Wahlstrom, A., Lindeberg, G., Karlsson, S. and Ragnarsson, U. (1976) Biochem. Biophys. Res. Commun. 71, 175–179.
[18] Bajusz, S., Rónai, A. Z., Székely, J. I., Gráf, L., Dunai-Kovács, Z. and Berzétei, I. (1977) FEBS Lett. 76, 91–92.
[19] IUPAC-IUB Commission on biochemical nomenclature (1974) Pure Appl. Chem. 40, 293–307.