Estimated Conformation, Orientation, and Accumulation of Dynorphin A-(1–13)-tridecapeptide on the Surface of Neutral Lipid Membranes†

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ABSTRACT: Equilibrium thermodynamic and kinetic estimations were used to confirm the rather unusual conformation, orientation, and accumulation of dynorphin A-(1–13)-tridecapeptide (dynorphin$_{1-13}$) on the surface of neutral lipid membranes, as observed by Erne et al. [Erne, D., Sargent, D. F., & Schwyzer, R. (1985) *Biochemistry 24*, 4261–4263]. I started from the premise that the most stable conformation of molecularly disperse peptides in contact with the hydrophobic phase of a membrane is helical [Henderson, R. (1979) *Soc. Gen. Physiol. Ser. 33*, 3–15]. Calculation of the Gibbs free energy difference for the transfer of increasing numbers m of N-terminal residues of dynorphin$_{1-13}$ from their random-coil conformation in water to their α-helical conformation in a hydrophobic phase, with the values provided by Von Heijne and Blomberg [Von Heijne, G., & Blomberg, C. (1979) *Eur. J. Biochem. 97*, 175–181], showed an energy minimum at $m = 9$ that corresponded to the observed apparent association constant of 9×10^4 L/mol. This confirmed our experimental observations. The orientation of dynorphin$_{1-13}$ in the interphase was estimated by calculation of the molecular amphiphilic moment $\vec{A}$. This force vector was defined in analogy to the "helical" and "structural" hydrophobic moments of Eisenberg et al. [Eisenberg, D., Weiss, R. M., & Terwilliger, T. C. (1982) *Nature (London) 299*, 371–374]. It takes into account the segregation of hydrophobic and hydrophilic residues with respect to the center of the α-helix. A peptide located in a hydrophobic–hydrophilic gradient experiences a torque that tends to orient $\vec{A}$ in a direction perpendicular to the surfaces of equal hydrophobicity. The scalar magnitude A is a measure for the tendency of a molecule to accumulate in the interphase (position of minimal energy of both its hydrophobic and hydrophilic domains). In dynorphin$_{1-13}$, the direction of $\vec{A}$ was found to differ from that of the helix axis by only 11°. Thus, the helix was expected to assume an almost perpendicular orientation on the membrane surface, which, again, agreed with the experiment. Rate considerations suggested that some of the initial collision complexes formed between peptide and membrane have lifetimes sufficiently long for the formation of short α-helices. Helix formation increases their lifetimes, and the initial helices become oriented in the interphase according to their $\vec{A}$. This model is a plausible description of the events leading to the observed membrane interaction of dynorphin$_{1-13}$.

Recent discoveries have revealed biologically relevant regioselective, conformation-selective, and orientation-selective interactions of dynorphin$_{1-13}$[1] and ACTH$_{1-24}$ with neutral lipid membranes (Gremlich et al., 1983, 1984; Gysin & Schwyzer, 1983a,b, 1984; Erne et al., 1985). The proposed membrane structures display very unusual features. Typical is the more or less perpendicular insertion of the receptor-triggering, N-terminal message segments as α-helices into the hydrophobic membrane layers. Although the peptides are readily soluble in water as monomeric random coils, hydrophobic interactions and peptide amphiphilic character are among the major driving forces for specific membrane association.

In order to test the validity of the proposed structures, I estimated the Gibbs free energy of dynorphin$_{1-13}$–membrane association by calculating the free energy of transfer of the peptide from its random-coil conformation in water to its membrane-bound conformation on the boundary between an aqueous and a hydrophobic phase. I also estimated the orientation of the peptide in a hydrophilic–hydrophobic gradient by calculating its molecular amphiphilic moment. Despite the obvious differences between the model chosen for the calculations and the situation on a real membrane, the results agreed excellently with our earlier experimental observations (Erne et al., 1985). The model is also supported by kinetic considerations.

Basic Models for Energy Calculations

The interaction of dynorphin$_{1-13}$, Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys (Chavkin & Goldstein 1981), with neutral lipid membranes prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was studied by vesicle-mediated hydrophobic photolabeling (Gysin & Schwyzer 1983), infrared attenuated total reflection spectroscopy (IR-ATR), and membrane capacitance minimization (CM) (Erne et al., 1985). Binding from aqueous solutions containing 10 mM KCl saturates reversibly at a bilayer area of 110 nm^2 per peptide molecule, has an apparent dissociation constant of 11 μM, and has rate constants of 2×10^2 s^{-1} (adsorption) and 2×10^{-3} s^{-1} (desorption). Dynorphin has very little order in water (random-coil conformation). On the membrane, the peptide assumes an α-helical structure oriented perpendicularly to the surface. The N-terminal helical segment is inserted into the hydrophobic membrane layers, whereas the C-terminal, strongly charged segment remains exposed to water as a random coil, Figure 1. Circular dichroism spectra revealed a strong increase of dynorphin$_{1-13}$ helicity in 2,2,2-trifluoroethanol/water mixtures, a solvent known to mimic influences of membranes on peptide conformation.

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[1] Abbreviations: dynorphin$_{1-13}$, dynorphin A-(1–13)-tridecapeptide; ACTH$_{1-24}$, adrenocorticotropin-(1–24)-tetracosapeptide; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

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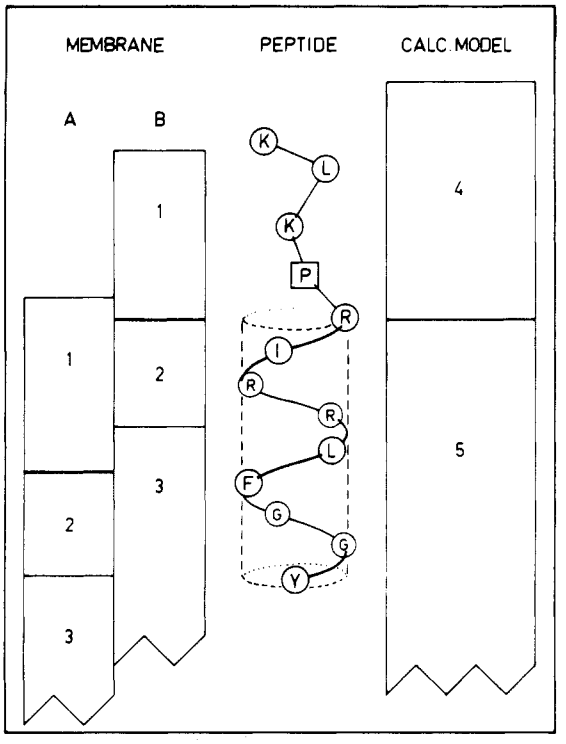

FIGURE 1: Models of dynorphin$_{1-13}$–membrane interaction. The peptide is shown as a helix extending from N-terminal tyrosine-1 (Y) to arginine-9 (R), followed by a random-coil segment consisting of proline-10 (P) to lysine-13 (K). The residue symbols indicate approximate positions of the α-carbon atoms. The helical segment contacts the hydrophobic membrane layers; the random coil is exposed to water. The membrane (left) consists of three chemically distinct layers with progressively lower water concentrations: (1) the head group layer with its phosphocholine residues strongly exposed to water, (2) the more hydrophobic H belt containing the ester carboxy groups as H-bond acceptors, and (3) the very hydrophobic hydrocarbon layer. (A) and (B) show two (extreme) situations of the membrane with respect to the inserted peptide. The model used in the calculations (right) is based on a system of two immiscible solvents with an idealized, sharp interphase boundary: 4 (water) and 5 (an organic solvent such as 1-octanol) with insertion of up to nine N-terminal residues into the hydrophobic phase.

In protein folding and binding of small molecules (e.g., coenzymes), hydrophobic interactions (Kauzmann, 1959) usually provide the bulk of the binding enthalpy, whereas H-bond and Coulomb forces are held responsible for interaction specificity [see Janin & Chothia (1978)]. These considerations were applied, mutatis mutandis, to the problem of dynorphin$_{1-13}$/membrane interaction: hydrophobic interactions were assumed to be the major driving force for association with neutral membranes, whereas H-bond and Coulomb forces within the peptide molecules and between them and the lipid head group and H-belt (Huang, 1976; Chatterjie & Brockerhoff, 1978) layers of the membrane were assumed to contribute to the interaction specificity by influencing helix formation and orientation.

Estimation of hydrophobic interaction energies was based on the concept of Lee and Richards (1971) that the difference between the "accessible surface areas" (the quantitative description of the extent to which molecules can form contacts with water) in the initial and final states of a reaction is a quantitative measure of the hydrophobic free-energy difference. In my case, I assumed the amount of ordered water in the hydrophobic layers of the membrane to be so small as not to contribute significantly to the change, although the issue, particularly for the H belt, can be argued. The change of accessible surface area was thus believed mainly to be due to changes in the state of the peptide and not of the lipid molecules.

Those sections of a peptide molecule that enter the lipid phase are stripped of their surface water film. This leads to negative values for the change of the Gibbs free energy of transfer $\Delta G°_{tr}$ caused by the unpolar parts of the molecules and to positive values caused by the polar parts. Polar atom groups are assumed to form H bonds with water that are broken in the hydrophobic environment, thus causing an unfavorable energy change. There are two possibilities for satisfying the ensuing H-bond requirements: intermolecular formation of H bonds with lipid molecules or with residual water in the H-belt and head group layers and intramolecular formation of H bonds by folding of the peptide chain. The α-helix offers good conditions for the latter (Henderson, 1979): only four peptide –NH–groups at the N-terminal and three peptide –CO– groups at the C-terminal ends retain unsatisfied H bonds that may partly be satisfied at the membrane surface by appropriate orientation of the helices. Broken H bonds of side-chain atoms may be satisfied by intramolecular contacts (Maxfield & Scheraga, 1975) between residues situated four positions apart on the helix or intermolecularly as above. Insertion of charged groups into the membrane hydrophobic layers is practically impossible (Parsegian, 1969); they must be decharged by protonation or deprotonation (Von Heijne & Blomberg 1979), be neutralized by "Maxfield–Scheraga contacts" or, then, remain exposed to water.

Figure 1 is a more explicit representation of the model used in the calculations. The α-helix was assumed to run from Tyr-1 to Arg-9, where it is interrupted by Pro-10. The C-terminal random coil comprises residues 10 (Pro) to 13 (Lys). At first sight, the approximately vertical insertion of the helix into the membrane appears to be quite impossible because of the unfavorable free-energy changes caused by the charged α-amino group and the four unsatisfied H bonds at the N-terminus. Further destabilizations are to be expected from the unsatisified H bond of the tyrosine hydroxy group and from the charges on the three arginine side chains. Insertion of the C-terminal helix end is discouraged by the breaking of three =CO···H bonds. An additional, unfavorable energy change of the peptide is caused by the loss of one degree of translational and two degrees of rotational freedom. All these energy gains as well as the free energy of peptide–membrane association (about $\Delta G_{assoc} = -27.6$ kJ/mol) must be accounted for by the free-energy differences for the transfer of dynorphin$_{1-13}$ from its random-coil conformation in water to its membrane-bound conformation and orientation in the membrane–water interphase.

Yet another factor adds to the difficulty of assessing the free energy of transfer: the interphase boundary between bulk water and the membrane hydrocarbon layer is not sharp, Figure 1. The intermediate region comprising the ester (H belt) and phosphocholine (head group) layers is, depending on lipid conformation, about 0.5–1.0-nm deep. It is thus able to accomodate several amino acid residues of an inserted peptide. In the intermediate (or interphase) region, the water activity is expected gradually to decrease from its high value in the bulk phase to its very low value in the hydrocarbon layer. Concomitantly with this hydrophobic gradient, the helical conformation of inserted peptides will become increasingly favored (Henderson, 1979). Thus, variations of helix length and depth of insertion may alter the free energy of transfer to an unknown extent. Stereoselective H bonds may be formed between donor groups of the peptide and acceptor groups of the H belt (Huang, 1976; Chatterjie & Brockerhoff, 1978).

Table I: Dynorphin$_{1-13}$: Estimated Free Energy Difference, $\Delta G°_{tr}(m)$ (kJ/mol), for Its Transfer from a Random-Coil Conformation in Water to a Partly Helical Structure in an Aqueous–Hydrophobic Interphase through Different Numbers, m, of N-Terminal Residues in Their α-Helical Conformation, Equation 1

							$\Delta G°_{tr}$(end)		
m	residue	HPC[a]	H bond	charge	$\Delta G°_{tr}(i)$	$\sum_1^m \Delta G°_{tr}(i)$	N-end	C-end	$\Delta G°_{tr}(m)$
1	Y^+	−24.07	10.5	5.7	−7.87	−7.87	10.5		2.63
2	G	−7.85			−7.85	−15.72	21.0		5.28
3	G	−7.85			−7.85	−23.57	31.5	10.5	18.43
4	F	−21.98			−21.98	−45.55	42.0	10.5	6.95
5	L	−17.79			−17.79	−63.34	42.0	10.5	−10.84
6	R^+	−16.2[b]			−16.2	−79.54	42.0	10.5	−27.04
7	R^+	−16.2[b]			−16.2	−95.74	42.0	10.5	−43.24
8	I	−18.32			−18.32	−114.06	42.0	10.5	−61.55
9	R^+	−16.2[b]			−16.2	−130.26	42.0	10.5	−77.76
10	P	−15.18	52.5[c]	31.3	68.6	−61.66	42.0	10.5	−9.16
11	K^+	−17.8[d]	63.0[e]	62.6	107.8	46.1	42.0	10.5	98.6

[a] Hydrophobic contribution calculated from the accessible area. [b] Contribution of the arginine residue without its guanidinium group (see text). [c] Including contributions of Arg-6. [d] Contribution of the lysine residue without its side-chain ammonium group. [e] Including contributions of Arg-7.

The presence of water in the diffuse interphase is expected to decrease hydrophobic interaction but to stabilize unsatisfied H bonds and charges. The fluidity of the membrane may allow rocking and pumping movements of the peptide and alleviate constraints on rotational and translational degrees of freedom. The balance of these forces will determine degree of helicity, depth of insertion, association constant, and orientation of the helix.

Estimation of Gibbs Free Energy of Dynorphin$_{1-13}$–Membrane Association $\Delta G°_{assoc}(m)$ through N-Terminal α-Helices with Different Numbers m of Amino Acid Residues. As a first approximation to the problem, I ignored the described imponderabilities of a diffuse membrane interphase and calculated the free-energy differences $\Delta G°_{tr}(m)$ for the transfer of dynorphin$_{1-13}$ molecules from their random-coil conformation in water to their partly helical conformations in an idealized, sharp interphase boundary between an aqueous and a hydrophobic phase (Figure 1). Known thermodynamic parameters for the transfer of individual amino acid residues $\Delta G°_{tr}(i)$ (Von Heijne & Blomberg 1979) were used. The binding was studied for hypothetical molecules of which different numbers, m, of N-terminal residues are transferred from their random-coil conformations in water to their α-helical conformations in the hydrophobic phase, assuming that the remaining C-terminal residues remain exposed to water in their random-coil conformations and do not contribute to the free energy of transfer. Thus

$$\Delta G°_{tr}(m) = \sum_{i=1}^{i=m} \Delta G°_{tr}(i) + \Delta G°_{tr}(\text{end}) \qquad (1)$$

where $\Delta G°_{tr}$(end) accounts for the unsatisfied H bonds at the N- and C-terminal helix ends. Given the increase of translational and rotational free energy $\Delta G°_{t+r}$ (Janin & Chothia 1978), upon transfer into the interphase boundary, the Gibbs free energy of dynorphin$_{1-13}$–interphase association (as a first approximation to membrane binding) is

$$\Delta G°_{assoc}(m) = \Delta G°_{tr}(m) + \Delta G°_{t+r} \qquad (2)$$

The scenario for calculations with eq 1 and 2 was as follows: (1) The progressive transfer of residues into the hydrophobic phase, starting with Tyr-1, was simulated accompanied by simultaneous, progressive α-helix formation and the leaving of the untransferred residues in the aqueous phase (pH $\simeq$ 7.0) in their random-coil conformations. (2) The α-ammonium group (p$K \simeq$ 8) is deprotonated at the aqueous–hydrophobic boundary, and the uncharged amino group is transferred. (3) Four residues at the N-terminal helix end acquire unsatisfied –NH– H bonds during transfer (loss of water molecules). (4) The side-chain charges of arginine residues (guanidinium groups) remain in contact with water and, despite the transfer of the uncharged part of the residue, are not transferred until the arginine is three positions away from the C-terminal helix end (Von Heijne, 1981). (5) The three residues at the C-terminal helix end acquire unsatisfied –CO– H bonds upon complete transfer to the hydrophobic phase. According to the physical model (Figure 1), two to three –CO– groups may remain in contact with water while the α-carbons, α-nitrogens, and side chains of the residues are transferred to the hydrophobic phase. However, I considered the H bond of the third –CO– group from the end to be broken. (6) The translational and rotational free energies of a spherical particle with specific volume v = 0.75 cm^3/g and molecular weight 1600 in the standard state were calculated according to Janin and Chothia (1978) and found to be practically equal, 49.8 kJ/mol each. This value was assumed to represent the loss of one translational and two rotational degrees of freedom of dynorphin$_{1-13}$ upon binding to the interphase.

The individual residue contributions $\Delta G°_{tr}(i)$ and the contributions of the N-terminal segments with m = 1 to m = 11 are calculated in Table I. Conceivably, the greatest possible length of N-terminal helix is m = 9, because residue 10 is proline, a very strong helix breaker. Indeed, the energy minimum is reached with residue 9. Transfer of residues 10 and 11 is very unfavorable, mainly because now the guanidinium groups of Arg-6 and Arg-7 must be accommodated in a hydrophobic environment. Therefore, residues 10–13 are assumed to remain in the aqueous phase as parts of a random coil, without contributing to the free energy of transfer. Their only effect is on $\Delta G°_{t+r}$ through molecular size and weight. Figure 2 shows the values of $\Delta G°_{assoc}(m)$ for dynorphin$_{1-13}$ calculated from eq 2 with $\Delta G°_{t+r}$ = 50 kJ/mol. The observed membrane binding energy of −27.6 kJ/mol (Erne et al., 1985) was reached with the incorporation of residue 9 into the helix and the hydrophobic phase. Hydrophobic binding through a helix of only eight residues is expected to be about 1000 times weaker than through a helix of nine ($K_{diss} \simeq$ 8.3 mM instead of 11 μM). Extension of the transfer to residues 10, 11, and beyond leads to strongly positive values of $\Delta G°_{assoc}$ (not shown in Figure 2). Thus, calculation of the energy minimum predicted the experimentally observed association constant and the helix length.

Orientation of Dynorphin$_{1-13}$ on a Membrane: Estimation of Molecular Amphiphilic Moment $\vec{A}$. Amphiphilic helices (Perutz et al., 1965) have attracted a great deal of attention because they appear to be responsible for interactions of certain peptides and proteins with lipid membranes [see Eisenberg et al. (1982) and Kaiser & Kézdy (1983)]. The asymmetric

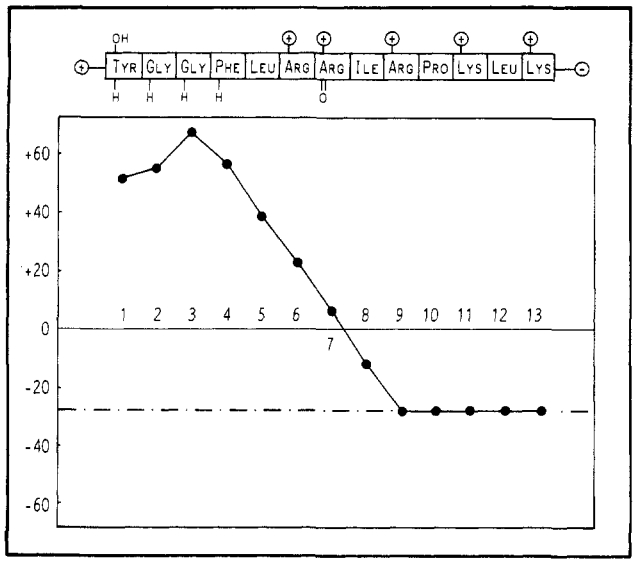

FIGURE 2: Dynorphin$_{1-13}$: estimated free energy differences for association to an aqueous–hydrophobic interphase, $\Delta G°_{assoc}(m)$ (kJ/mol), eq 2 (ordinate), through an increasing number of up to nine N-terminal amino acid residues (abscissa) in their helical conformation. Residues 10–13 were assumed to remain in the aqueous phase and not to contribute to the enthalpy change. The broken line, −27.6 kJ/mol, indicates the experimentally observed value for dynorphin$_{1-13}$ binding to flat POPC bilayer membranes. Charges and unsatisfied H bonds are shown next to the residues with which they are associated.

distribution of hydrophobic and hydrophilic amino acid side chains around the axis causes one side of the helix to be more hydrophobic than the other. This becomes apparent by viewing the helix from one end and projecting the α-carbon atoms onto a plane perpendicular to the axis (Schiffer & Edmundson, 1967) or by calculating the helical hydrophobic moment perpendicular to the helix axis (Eisenberg et al., 1982). This type of amphiphilicity is called secondary or helical amphiphilicity because it is a consequence of secondary folding. It is expected to orient the helix axis more or less parallel to an interphase boundary.

The N-terminal helix of dynorphin$_{1-13}$ exhibits pronounced helical amphiphilicity (Figure 1; Schwyzer, 1985a). The observed perpendicular orientation of the helix axis on the membrane surface is therefore quite unexpected and can only be explained by the presence of another, strong hydrophobic moment parallel to the helix axis (Erne et al., 1985). This force component arises from the unequal distribution of hydrophobic and hydrophilic amino acid side chains between the N-terminal and C-terminal segments of the peptide chain. Such amphiphilicity is called primary or segmental amphiphilicity (Gysin & Schwyzer, 1984).

In order to be consistent with the energy calculations presented above, I have chosen the signed numerical value of the Gibbs free energy of transfer as a basis for amphiphilicity estimations and not one of the hydrophobicity scales (with opposite signs) used in the calculation of the helical hydrophobic moment (Eisenberg et al., 1982). This choice stresses the fact that both the hydrophilic contribution of residues (arbitrarily defined as positive $\Delta G°_{tr}$) and the hydrophobic contribution (negative $\Delta G°_{tr}$) are important for determining the amphiphilic character of a peptide [see Table II and Eisenberg et al. (1982)]. The system used here differs from that based on a hydrophobicity scale only by the opposite direction of the force vectors. The orienting hydrophilic and hydrophobic forces of the amino acid residues and their side chains were assumed to act on the helix via their α-carbon atoms, which occupy well-defined positions relative to the helix axis

Table II: Gibbs Free Energy Differences, $\Delta G°_{trh}(i)$ (kJ/mol), for Transfer of N-Terminal Residues of Dynorphin$_{1-13}$ from Their Helical Conformation in Water to Their Helical Conformation in a Hydrophobic Phase

i	residue[b]	HPC[a]	H bond	charge	$\Delta G°_{trh}(i)$
9	R^{e+}	−15.5	42.0	31.4	57.9
8	I^{e}	−10.5	10.5		0
7	R^{e+}	−15.5	42.0	31.4	57.9
6	R^{+}	−15.5	31.5	31.4	47.4
5	xLx	−10.1			−10.1
4	F^{e}	−14.2	10.5		−3.7
3	G^{e}		10.5		10.5
2	G^{e}		10.5		10.5
1	Y^{e+}	−16.3	21.0	5.7	10.4

[a] HPC means hydrophobic contribution. [b] An "e" indicates an "end" residue with potentially unsatisfied H bonds; xLx is the "central" leucine residue with $z = 0$, $\theta = 400°$, and $\vec{r} = 0.188$ nm.

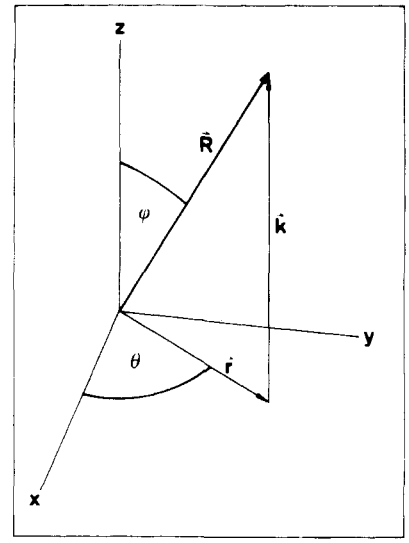

FIGURE 3: Definition of position vectors used in calculation of molecular amphiphilic moment and its component vectors perpendicular and parallel to the z axis. The molecular helix axis lies on the z axis of the right-handed spherical coordinate system. The α-carbon of the N-terminal residue ($i = 1$) is assigned a negative value with $\theta = 0°$; $\vec{r}_i$ and $\vec{k}_i$ are the component vectors of $\vec{R}_i$, normal and parallel to the helix axis, respectively.

[this is consistent with the idealized models of Eisenberg et al. (1982)].

Thus, I define the degree of amphiphilic character of dynorphin$_{1-13}$ by its molecular amphiphilic moment:

$$\vec{A} = \sum_{i=1}^{i=m} \Delta G°_{trh}(i)\vec{R}_i \qquad (3)$$

where $\Delta G°_{trh}(i)$ is the Gibbs free energy change for the transfer of the ith residue from its helical conformation in water to its helical conformation in a hydrophobic phase (Von Heijne, 1981), taking into account the end-group effects. $\vec{R}_i$ is the position vector from the center of the helix to the α-carbon of the ith residue, Figure 3.

The force vectors $\Delta G°_{trh}(i)\vec{R}_i$ act along the line on which $\vec{R}_i$ is situated. For hydrophilic residues (positive $\Delta G°_{trh}$), the force vector points in the same direction as the position vector; for hydrophobic residues (negative $\Delta G°_{trh}$), its direction is 180° away from it. The same holds for the component force vectors of $\vec{A}$:

$$\vec{\mu}_r = \sum_{i=1}^{i=m} \Delta G°_{trh}(i)\vec{r} \qquad (4)$$

$$\vec{\mu}_k = \sum_{i=1}^{i=m} \Delta G°_{trh}(i)\vec{k} \qquad (5)$$

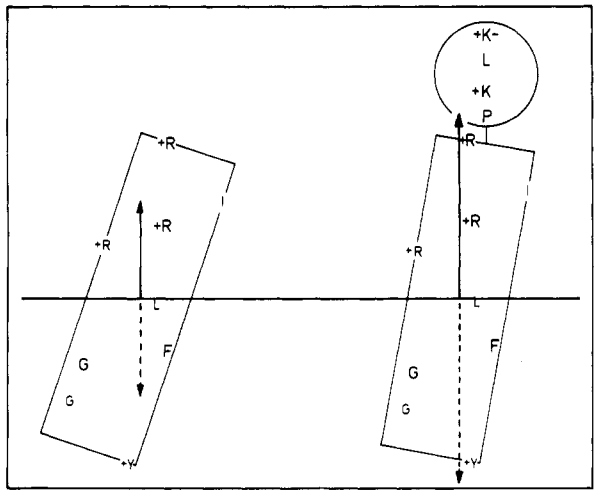

FIGURE 4: Estimated orientation of dynorphin$_{1-13}$ nonapeptide helix in a hypothetical hydrophobic gradient (side view): (left) calculated without the influence of the C-terminal random-coil segment, 10–13; (right) with 10–13 acting on the helix end. The vectors $\vec{A}$ point up, and their lengths indicate their relative magnitudes. The horizontal line through the centers of the helices lies on a surface of equal hydrophobicity, which is though to increase from the top to the bottom of the figure.

Values of $\Delta G_{trh}(i)$, Table II, were calculated according to Von Heijne (1981), and the lengths of the position vectors were measured in units of helix radius with the unit vectors $\vec{r} = \vec{k} = 0.188$ nm. The magnitude of the force vectors was not defined in physical units, e.g., newton × meter, N m, because the point $\Delta G°_{tr} = 0$ is arbitrarily related to concentration and cannot be used to define the forces acting on a couple (in the sense of mechanical statics).

The amphiphilic moment of dynorphin$_{1-13}$ was calculated for two different models. In the first, the contribution of the random-coil segment 10–13 was not taken into account (calculation for dynorphin$_{1-9}$ methyl ester); in the second, it was considered to contribute its free energy of transfer as a whole onto the C-terminal end of the 1–9 helix axis, like a balloon acting through a string. The following results were obtained (values for the second model in parentheses):

$$\theta = 144° \ (132°); \quad A = 277 \ (464); \quad \Phi = 17° \ (11°)$$

In both cases, the angle Φ is considerably smaller than 45°, indicating a preference for the perpendicular orientation on the membrane over the parallel orientation. This confirms our experimental observations.

The amphiphilic moment of a peptide located in a hydrophobic gradient or on an interphase boundary produces a torque that tends to orient $\vec{A}$ perpendicular to the surfaces of equal hydrophobicity (Figure 4). Its scalar magnitude, A, is a measure for the tendency of a molecule to accumulate in the interphase and assume a position of minimal energy for both its hydrophobic and hydrophilic domains. The Gibbs free energy of association, $\Delta G°_{assoc}$, determines the depth of intrusion and the strength of the hydrophobic membrane interaction [see also Eisenberg et al. (1982)].

Rate Considerations

Let dynorphin$_{1-13}$, in its random-coil conformation in water (D), collide with the surface of a neutral lipid membrane (M) to give an initial, loose collision complex (D,M) before acquiring its membrane-bound, partly helical structure (D′,M):

$$D + M \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} D,M \xrightarrow{k_2} D',M \qquad (6)$$

D′,M can be reached if the lifetime τ of D,M is compatible with k_2, a rate constant that I assume, in a first approximation, to be governed mainly by helix formation and only negligibly by translational and rotational movements that orient the molecule in the interphase.

The elementary step of α-helix growth is estimated to proceed with a rate constant of about 10^8 s^{-1} (Zana, 1975). Establishment of a helix with 10 residues would require $\tau \geq 10^{-7}$ s. Assuming the collision to be diffusion-controlled with $k_1 \simeq 2 \times 10^9$ M^{-1} s^{-1} (Von Heijne & Blomberg, 1979), the minimal stability requirement of D,M to assure 10 helix-forming steps would be $K_d = k_{-1}/k_1 = 5$ mM or $\Delta G°_{assoc} \simeq -13$ kJ/mol.

Estimation of $\Delta G°_{assoc}$ for the first stage of eq 6 is strongly biased by assumptions. In a first approach, I accounted only for the transfer of the side chains of Tyr-1, Phe-4, Leu-5, Ile-8, Pro-10, and Leu-12 from water to a hydrophobic phase, leaving the rest of the molecule exposed to water. This assumption agrees with observations on Met- and Leu-enkephalin that suggest membrane binding through Tyr, Phe, and Met/Leu side chains (Deber & Behnam 1984). Part of the population of D,M would then be characterized by $\Delta G°_{tr} \simeq -60$ kJ/mol, balancing $\Delta G°_{t+r} \simeq 50$ kJ/mol and leaving $\Delta G°_{assoc} \simeq -10$ kJ/mol, which is in the required range. The association may be stronger if less than the supposed three degrees of freedom are lost or if other parts of the molecule participate in the hydrophobic interaction (e.g., two α-carbons would contribute about 4 kJ/mol). On the other hand, it may be weaker if not all of the mentioned six side chains are transferred. Thus, in the assumed model, the formation of a partly helical structure is kinetically possible, and the results may be extrapolated to the conditions on the surface of a neutral membrane.

The random-coil peptide D in D,M is in surroundings with reduced water activity. It is therefore expected to balance its loss of external H bonds by helix formation. This tends to increase the lifetime of the peptide–membrane complexes (every incorporated residue will tend to increase $\Delta G°_{assoc}$ by about 8 kJ/mol; Von Heijne, 1981). The helix in statu nascendi would initially be oriented with its axis roughly parallel to the interphase surface. Such an orientation is compatible with helical secondary amphiphilicity (Erne et al., 1985) and with partial satisfaction of helix–terminal unsatisfied H bonds by water. A rough estimation of the free energy of transfer of the N-terminal nonapeptide segment from a random coil in water to an amphiphilic helix lying on the membrane surface indicated a strong preference of the helical over the random-coil structure. However, the orientation was considered to be unfavorable with respect to a perpendicularly inserted helix mainly because of the strong difference between the amphiphilic moments parallel and perpendicular to the helix axis.

Conclusions

On the premise that the α-helix is the most stable conformation of peptide domains interacting with a membrane hydrophobic phase, calculation of the Gibbs free energy of transfer of increasing numbers, m, of N-terminal residues from their random-coil conformation in water to their α-helical conformation in a hydrophobic phase and of the molecular amphiphilic moment, $\vec{A}$, provided the parameters necessary and sufficient for predicting preferred conformation, orientation, and accumulation of dynorphin$_{1-13}$ on the surface of neutral lipid membranes.

On cell plasma membranes or on membranes doped with anionic lipid (Gysin & Schwyzer, 1984), a further accumulating effect was expected and observed. It is due to a Boltzmann distribution of the (positively) charged peptide between the bulk phase and the (negatively) charged membrane surface. Assuming a Gouy–Chapman fixed-charge

surface potential of $V_{gc} \simeq -40$ mV (in biologic systems) and a net charge of dynorphin$_{1-13}$ of 5+, the enhancement of the surface concentration over the bulk concentration would amount to a factor of $\exp[-zFV_{gc}/(RT)] \simeq 3000$ (z is the peptide net charge, F is the faraday constant, and RT has its usual meaning).

The model of dynorphin$_{1-13}$–membrane interaction presented here displays characteristics closely resembling those held responsible for the catalysis of bimolecular chemical reactions by detergent micelles (Jencks, 1975; Schwyzer, 1985b). The binding energy between the peptide and the membrane may be utilized to overcome the entropy requirements involved in bringing the reacting groups, peptide and receptor, together (catalysis by induced association). The free energy from the binding interaction between the membrane and part of the peptide may be utilized to force the reacting portion of the peptide into an environment in which it is destabilized and can reach the transition state more easily (catalysis by substrate destabilization). These two mechanisms may influence both the reaction rates and the equilibrium constants of the peptide–receptor interaction. In dynorphin$_{1-13}$, the message Tyr-Gly-Gly-Phe- (Chavkin & Goldstein, 1981) is destabilized through loss of a proton and of five H bonds (Figure 2). The amino and hydroxy functions of Tyr-1 that are known to be essential for opiate receptor interaction are forced into a very unfavorable environment. Interactions of such destabilized groups to form H bonds or salt bridges with receptor recognition sites exposed in the hydrophobic membrane compartment (Gremlich et al., 1983) are expected to be rapid and strong. Influences of membrane-induced catalysis of peptide–receptor interactions on macroscopic binding parameters and kinetics were examined by Sargent and Schwyzer (submitted for publication).

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