178. Membrane Structure and Biologic Activity of Adrenocorticotropin (ACTH) and Melanotropin (MSH) Peptides[1]). Estimation of Structural Parameters Including the Influence of the Helix Dipole Moment

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Estimation of preferred conformation, orientation, and accumulation of adrenocorticotropin (1–24)-peptide at an aqueous-hydrophobic interface produced a model that agreed with that developed from experimental observations with lipid membranes. Thus, the N-terminal message segment (residues 1–11) was incorporated into the hydrophobic phase as an α-helical, perpendicularly oriented domain with an apparent dissociation constant of *ca.* $5 \cdot 10^{-5}$ M. The C-terminal address segment (residues 12–24) remained in the aqueous phase as a random-coil domain. Three parameters proved sufficient to define the model: the *Gibbs* free energy of hydrophobic association, the molecular amphiphilic moment, and the molecular electric dipole moment. For estimating interactions with biologic membrances (that carry a net negative charge), the *Boltzmann* distribution of charged peptides was also considered. The estimations were extended to adrenocorticotropin (1–10)-peptide and α-melanotropin. In the first case, the prediction agreed with the earlier observations, in the second, it awaits its experimental proof. The estimated membrane structures were compared with available biologic data. As for opioid peptides, it appears that the amphiphilic moment is an important new parameter for determining quantitative structure-activity relationships (QSAR) in receptor selection and biologic potency.

Introduction. – Recently, quite unexpected regio-, conformation-, and orientation-selective interactions between artificial lipid bilayer membranes and peptides such as $ACTH_{1-24}$, $ACTH_{1-10}$, and $dynorphin_{1-13}$ were discovered and studied by capacitance minimization [1b] [2], infrared attenuated total reflection spectroscopy [3] [4], and vesicle-mediated hydrophobic photolabelling [5] [6] (reviews, see [7]). The membrane interactions of $ACTH_{1-24}$ and $dynorphin_{1-13}$ are very similar and are caused by the amphiphilic character of these peptides [3] [4].

On the premises that in molecularly disperse peptides the amphiphilic moment is a valid measure of amphiphilic character and that those chain segments that are in hydrophobic surroundings are stabilized as α-helical structures, the preferred conformation, orientation, and accumulation of $dynorphin_{1-13}$ on the surface of neutral lipid membranes has been estimated and found to agree with the observed parameters [8]. Extension of the calculations to 26 opioid peptides described in the pharmacologic literature allowed the prediction of receptor selection and potency with a high degree of confidence [9]. It thus

[1]) Peptide nomenclature and abbreviations, see IUPAC-IUB Recommendations 1983 on 'Nomenclature and Symbolism for Amino Acids and Peptides' [1a]. Furthermore: $ACTH_{n-m}$ = adrenocorticotropin segments comprising residues *n* to *m*, $dynorphin_{1-13}$ = dynorphin A-(1–13)-tridecapeptide; POPC = 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phosphocholine; CNS = central nervous system; IR-ATR = infrared-attenuated total-reflection spectroscopy.

appears that the lipid phase of target cell membrances catalyzes specific peptide-receptor interactions [10].

Here, I apply similar considerations, including the influence of the helix electric dipole moment, to calculate and predict membrane structures and accumulations of $ACTH_{1-24}$, $ACTH_{1-10}$, and α-MSH. The results are discussed in terms of observed membrane interactions and biologic activity.

General Considerations. – In protein folding and binding of small molecules, hydrophobic interactions [11] usually provide the bulk of the binding enthalpy, whereas H bond and *Coulomb* forces are held responsible for interaction specificity [12]. I assumed the same types of force to cause interactions between peptides and neutral membranes (experimental evidence [1b] [2] [6]). Dipole-dipole and *Coulomb* interactions were also considered.

Energy estimations were based on the concept of *Lee* and *Richards* [13] that the difference between the 'accessible surface areas' (the quantitative description of the extent to which molecules can form contacts with H_2O) in the initial and final states of a reaction is a quantitative measure of the hydrophobic free energy of transfer (ΔG_{tr}°, *ca.* 10.46 kJ/nm^2). In membrane interactions, I assumed the amount of ordered H_2O in membrane hydrophobic layers not to be affected; only the change of accessible surface area of the peptides was taken into account. The individual values for residue contributions were taken from [14].

Contributions to ΔG_{tr}° other than hydrophobic were also considered. Charges cannot be brought into non-polar environments without excessive increase in free energy [15]. A charged group must be neutralized before entering the lipophilic phase, either by adding or removing a proton. On this assumption, the contribution from a charge can be calculated from the dissociation constant of the charged group [14]. Furthermore, each polar atom of an amino-acid residue was assumed to form one H bond in H_2O, this bond being broken upon transfer into the non-polar phase. An unsatisfied H bond is reported to increase the free energy by +10.5 kJ/mol [16]. In hydrophobic environments, α-helical structures of molecularly disperse peptides are favoured [17] because the number of broken ('unsatisfied') H bonds is minimized (β-structures with the same number of residues are less stable).

On a lipid membrane, the separation between the bulk H_2O and hydrocarbon phases is not sharp. The intervening region comprising the H-belt [18] and head-group layers may constitute a separate interphase *ca.* 1 nm thick. In it, the H_2O activity is expected gradually to decrease from the bulk aqueous phase to the hydrocarbon layer [8]. Concomitantly with this 'hydrophobic gradient', the helical conformation of inserted, molecularly disperse peptides will become increasingly favoured. Furthermore, the interphase provides H bond acceptor groups [18] that may react with inserted peptides in a specific manner. Despite such imponderabilities, a model based on the separation of aqueous and hydrophobic phases by an ideally thin interface provides results compatible with the experimental data for dynorphin peptides [8] [9]. It was, therefore, adapted to $ACTH_{1-24}$, $ACTH_{1-10}$, and α-MSH.

Our observation that the relatively hydrophobic 'message' segments of $ACTH_{1-24}$ (segment 1–10) and $dynorphin_{1-13}$ (segment 1–5, [Leu']enkephalin) cannot interact with neutral membranes if not attached to the very hydrophilic, charged 'address' segments ($ACTH_{11-24}$, $dynorphin_{6-13}$) focussed our attention on the importance of the amphiphilic

character of peptides for their regio-, conformation-, and orientation-selective membrane association [3] [4]. Clearly, the concept of segmental or primary amphiphilicity [5] [6] supplemented that of secondary or helical amphiphilicity [19]. Primary amphiphilic character is caused by the segregation of hydrophilic and hydrophobic residues towards the C- and N-termini of a peptide chain, whereas secondary amphiphilicity is due to segregation caused by secondary folding.

The degree of amphiphilic character of a helix is defined by its hydrophobic moment [20]. This is the vector sum of the products of hydrophobicity and position vector of the individual residues. The authors distinguish three possibilities. For 'helical' and 'structural' hydrophobic moments, the position vectors point from the helix axis to the C(α) atoms or to the centres of the residue side chains, respectively. These definitions emphasize the amphiphilicity perpendicular to the helix axis. An alternative definition for the structural hydrophobic moment employs the position vectors from any origin to the centre of the residue side chains [20] and represents amphiphilicity in directions both parallel and perpendicular to a helix axis.

We had pointed out the importance of amphiphilicity parallel to the helix axis for the orientation of $ACTH_{1-24}$ [3] and $dynorphin_{1-13}$ [4] on neutral membranes. I, therefore, adapted the definitions of *Eisenberg* and coworkers to the special problem of peptide-membrane interactions, where a considerable part of the peptide remains exposed to the aqueous phase as a random-coil domain [8]. The centre of the helical part of the molecule was arbitrarily chosen as the origin of the position vectors. These pointed to the C(α)'s of the helix residues, because I assumed the hydrophobic and hydrophilic forces of the quite flexible side chains and the random-coil segment to act on the relatively rigid helix like 'balloons or weights attached with strings' to the C(α) atoms. This and the choice of the hydrophobic free energy of transfer with its usual signs led to the definition of the molecular 'amphiphilic moment' described below.

Another property also is important for orienting helical peptides on membranes: the helix dipole moment (reviews [21]). It arises from the alignment of peptide dipoles parallel to the α-helix axis and derives its orienting force from interaction with membrane-surface dipoles located in the head-group and H-belt layers. The electrostatic effect of the α-helix dipole is roughly equivalent to the effect of two equal partial charges (0.5 to 0.75 e) of opposite sign placed at the ends of the helical segment: positive at the N-, and negative at the C-terminus. Little is known about membrane dipole moments except that they point their negative ends toward the aqueous phase and are in the order of magnitude of 15 debye per lecithin molecule [2]. Thus, perpendicular insertion of the helix N-terminus into the hydrophobic phase will be energetically unfavourable, whereas insertion of the C-terminus will be favourable. Quantitative estimation of dipole-dipole interactions is, for lack of exact membrane data, hardly feasible.

In proteins, residues with positive charges tend to be clustered near the helix C-termini and anionic residues near the N-termini [21]. This is also the case with $ACTH_{1-24}$ and α-MSH. Such a segregation of ionic charges will cause a dipole moment opposing the helix dipole moment. For the estimation of the ionic 'fixed charge dipole moment', the charges were assumed to exert their effect on the C(α) atoms of the helical residues, again because of the relative mobility of the side chains as compared with the helix backbone. Thus, the effective molecular dipole moment was considered to result from a combination of the 'helix dipole moment' [21] and the 'fixed charge dipole moment'.

Methods. – Four parameters were important for estimating conformation, orientation, and accumulation of peptides on a membrane surface [8] [9]. They are briefly described in this section.

Hydrophobic Association. The *Gibbs* free energy of hydrophobic association, $\Delta G^\circ_{ass}(m)$, through m residues at the more hydrophobic end of a peptide chain is calculated from the free energy of transfer, $\Delta G^\circ_{tr}(i)$, of the individual residues from their random-coil conformation in H_2O to their helical conformation in a hydrophobic phase [14].

$$\Delta G^\circ_{tr}(m) = \sum_{i=1}^{i=m} \Delta G^\circ_{tr}(i) + \Delta G^\circ_{tr}(\text{end}) \quad (1)$$

$$\Delta G^\circ_{ass}(m) = \Delta G^\circ_{tr}(m) + \Delta G^\circ_{t+r} \quad (2)$$

The relations are given in *Eqn. 1* and *2*, where ΔG°_{tr}(end) accounts for unsatisfied H bonds at the helix ends, and ΔG°_{t+r} is the free-energy change caused by the loss of two degrees of rotational and one degree of translational freedom of the peptide bound to the membrane [12]. The hydrophobic-association constant and the length of the helix, m, are determined from the position of the energy minimum [8].

Amphiphilic Moment. Segregation of charged and uncharged amino-acid residues into hydrophilic and hydrophobic domains endows peptides with an amphiphilic character. Such peptides will tend to accumulate on aqueous-hydrophobic interphase boundaries and orient themselves in the direction of minimum free energy. The segregation of

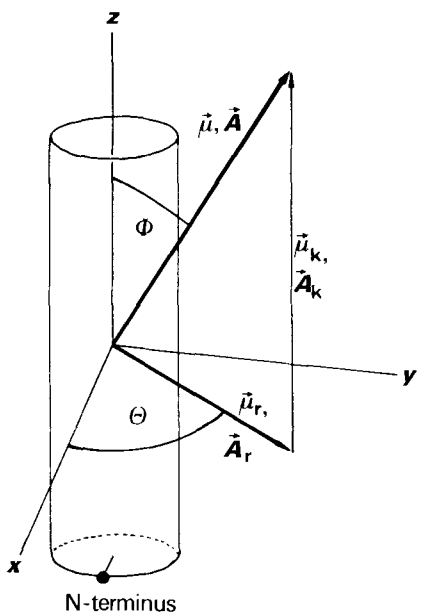

Fig. 1. *Definition of the molecular amphiphilic moment $\vec{A}$ and the effective electric dipole moment $\vec{\mu}$.* See *Eqn. 3* and *4*. The helix axis lies on the z axis of a right-handed spherical coordinate system. The N-terminal residue has a negative z value, and its C(α) atom points in the direction of the x axis. The helix is centered on the origin of the coordinate system. With the present definitions, the amphiphilic-moment vector points in the direction of greatest hydrophilicity, the dipole-moment vector in the direction of greatest positive charge. The component vectors normal and parallel to the helix axis are denoted by subscript r and k, respectively.

hydrophobic and hydrophilic properties may be measured in analogy to the helical hydrophobic moment [20] by the molecular amphiphilic moment A (see *Fig. 1*) defined [8]

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

in *Eqn. 3*, where $\Delta G^{\circ}_{trh}(i)$ is the signed numerical value of the *Gibbs* free energy change for the transfer of the ith residue in its helical conformation from H_2O to a hydrophobic phase (values are taken from *von Heijne* [14]). $\vec{R}_i$ is the position vector from the helix centre to the C(α) of the ith residue measured in units of helix radius, 0.188 nm. Random-coil segments are assumed to exert their action at the helix end. The amphiphilic moment of a peptide located in a hydrophobic gradient produces a torque that tends to orient $\vec{A}$ perpendicular to the surfaces of equal hydrophobicity in the surrounding medium. The greater the scalar magnitude, A, the less pronounced the thermal tumbling of the peptide molecules. Usually, a value of A greater than 150 arbitrary units is necessary to produce biologically relevant membrane associations [8] [9].

Electric Dipole Moment. Alignment of peptide-bond dipoles and asymmetric arrangement of charged amino-acid residues endow peptide helices with a molecular dipole moment. Such helices will tend to orient themselves in the direction of minimum free energy within the surface dipole layer of membranes. The molecular dipole moment $\vec{\mu}$ was measured in analogy to the molecular amphiphilic moment *(Eqn. 3)*, by *Eqn. 4*, where z_i is

$$\vec{\mu} = \sum_{i=1}^{i=m} z_i \vec{R}_i \tag{4}$$

the charge number of the ionic side chain (assumed to be $\pm 1\ e$) of the ith residue or of the partial charges assigned to the helix ends (assumed to be $\pm 0.63\ e$ [21] located on the end residues, $i = 1$ and $i = m$). $\vec{R}_i$ is the position vector from the helix centre to the C(α) of the ith residue *(Fig. 1)* measured in units of 0.1 nm.

Net Charge. Charged peptides will be attracted or repulsed by the fixed charge layer of a membrane surface according to a *Boltzmann* distribution, *Eqn. 5*, where c_b is the surface

$$\frac{c_b}{c_{b\,max} - c_b} = c_o \exp \frac{-zFV_{gc}}{RT} \tag{5}$$

concentration of the peptide (number of adsorbed molecules per unit area), c_{bmax} the surface concentration at saturation, c_o the molar peptide concentration in the bulk phase, z the net charge, V_{gc} the *Gouy-Chapman* fixed charge potential, F the *Faraday* constant, R the universal gas constant, and T the absolute temperature in K. Biologic membranes usually contain excess negatively charged lipid in such an amount that we may assume a characteristic $V_{gc} \approx -40$ mV for their lipid phase [22].

Results. – Inspection of the amino-acid sequences suggested a pronounced primary amphiphilicity of $ACTH_{1-24}$,

Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-
Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro,

and a less pronounced one of α-MSH,

Ac-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH_2.

The radial distribution of residues in assumed helical structures of both peptides ('*Edmundson* wheels' [19]) indicated a minor secondary amphiphilic moment normal to the helix axis, whereas the segregation of hydrophilic and hydrophobic residues into C- and N-terminal segments indicated a strong, parallel amphiphilic moment. Moreover, the more hydrophobic N-terminal parts, residues 1–11, are devoid of helix-breaking proline. This suggested that $ACTH_{1-24}$ and α-MSH may become oriented on an interface and their N-termini transferred from random coils in H_2O to α-helices in the more hydrophobic phase. The distribution of charged residues indicated a favourable direction of the electric dipole moments.

On this assumption, application of known principles of helix-membrane [14] and side-chain [23] interactions led to scenarios (see 1) to 7)) for the estimation of hydropho-

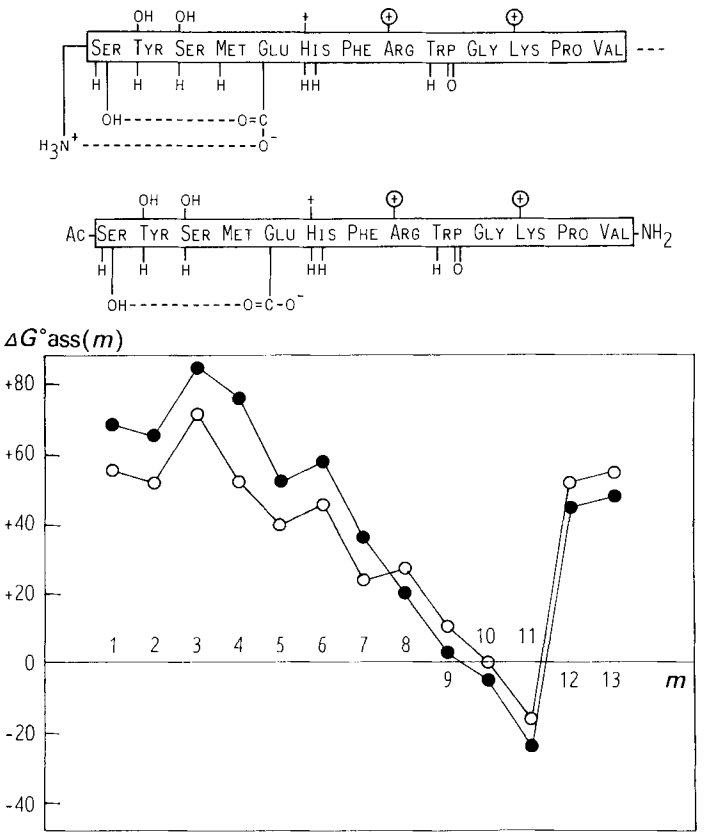

Fig. 2. *Estimated* Gibbs *free-energy differences* ΔG°_{ass}(m) [kJ/mol] *for association with neutral membranes through increasing numbers* m *of N-terminal residues in their* α*-helical conformation* $ACTH_{1-24}$ (●), *α-MSH* (○). See *Eqn. 2.* Abbreviated amino-acid sequences of ACTH (upper) and α-MSH (lower) are symbolized in their state of lowest energy in contact with the aqueous-hydrophobic interface: broken H bonds are indicated by H (–NH– groups of the helix end, His and Trp side chains), =O (helix end carbonyl), and –OH (side-chain hydroxy). '*Maxfield-Sheraga*' contacts leading to intramolecular elimination of broken H bonds and neutralization of charges are indicated by broken lines connecting the corresponding groups. Positive charges exposed to H_2O (Arg, Lys) are in circles, charges that must be eliminated by protonation (Glu in α-MSH) or deprotonation (His) for contact with the hydrophobic phase are indicated by $^-$ and $^+$.

bic association constants (*Eqn. 1* and *2*), and of molecular amphiphilic moments (*Eqn. 3*) similar to those used for predicting the membrane structure of dynorphin$_{1-13}$ [8]: 1) Simulation of the progressive transfer of residues at the N-terminus into the hydrophobic phase accompanied by α-helix formation, leaving the untransferred residues in the aqueous phase (pH 7.0) as random coils.

2) The α-NH_3^+ group of Ser-1 in $ACTH_{1-24}$ is deprotonated at the interface and the uncharged NH_2 group is transferred. In α-MSH, Ser-1 is acetylated and requires no deprotonation.

3) Four residues at the N-terminal helix end acquire unsatisfied -NH···H bonds during transfer. In α-MSH, the Ac group at the N-terminus was considered to form an H bond with the NH group of Met-4, reducing the number of N-terminal unsatisfied -NH···H bonds to three.

4) Three residues at the C-terminal end of the helix acquire unsatisfied -CO- bonds upon complete transfer to the hydrophobic phase. However, with an almost perpendicular orientation of the helix on the interface suggested by the amphiphilic moment (see below), two to three -CO- groups may remain in contact with H_2O while the C(α)'s, the N-atoms at C(α), and the side chains are transferred to the hydrophobic phase. Nevertheless, I considered the H bond of the third group from the end to be broken.

5) According to three-dimensional models, Glu-5, after transfer to the hydrophobic phase, can easily form a *'Maxfield-Scheraga'* [22] contact with Ser-1, leading to formation of two intramolecular H bonds and charge neutralization (*Fig. 2*). In α-MSH, internal neutralization of the Glu-5 charge is not possible. However, an H bond between the Ser-1 OH and the Glu-5 COO^- groups is still feasible.

Table 1. *Adrenocorticotropin (1–24)-Tetracosapeptide: Estimated Free-Energy Difference* ΔG_{tr}° (m) [kJ/mol] *for its Transfer from a Random-Coil Conformation in* H_2O *to a Partly Helical Structure on an Aqueous-Hydrophobic Interface through Increasing Numbers* m *of N-Terminal Residues in their* α*-Helical Conformation.* See *Eqn. 1.*

m	Residue	HPC[a])	H Bond	Charge	ΔG_{tr}° (i)	$\sum_{i=1}^{m} \Delta G_{tr}^{\circ}(i)$	ΔG_{tr}° (end)		$\Delta G_{tr}^{\circ}(m)$
							N-End	C-End	
1	Ser^+	− 12.04	+ 10.50	+ 4.56	+ 3.02	+ 3.02	+ 10.5	–	+ 13.52
2	Tyr	− 24.07	+ 10.50	–	− 13.57	− 10.55	+ 21.0	–	+ 10.45
3	Ser	− 12.04	+ 10.50	–	− 1.54	− 12.09	+ 31.5	+ 10.5	+ 29.91
4	Met	− 19.36	–	–	− 19.36	− 31.45	+ 42.0	+ 10.5	+ 21.05
5	Glu^-	− 19.89	+ 21.0	+ 15.7	− 24.45[b])	− 55.90	+ 42.0	+ 10.5	− 3.40
6	His^+	− 20.41	+ 21.0	+ 5.69	+ 6.28	− 49.62	+ 42.0	+ 10.5	+ 2.88
7	Phe	− 21.98	–	–	− 21.98	− 71.6	+ 42.0	+ 10.5	− 19.1
8	Arg^+	− 16.2[c])			− 16.2	− 87.8	+ 42.0	+ 10.5	− 35.30
9	Trp	− 26.69	+ 10.5	–	− 16.19	− 103.99	+ 42.0	+ 10.5	− 51.49
10	Gly	− 7.85	–	–	− 7.85	− 111.84	+ 42.0	+ 10.5	− 59.34
11	Lys^+	− 19.0[d])			− 19.0	− 130.84	+ 42.0	+ 10.5	− 78.38
12	Pro	− 15.18	+ 21.0	+ 62.78[e])	+ 68.60	− 62.24	+ 42.0	+ 10.5	− 9.78
13	Val[f])	− 19.00	+ 21.0	–	+ 2.00	− 60.24	+ 42.0	+ 10.5	− 7.78

[a]) Hydrophobic contribution calculated from the accessible area (*von Heijne* and *Blomberg* [14]).

[b]) After compensation for the satisfaction of two H bonds and neutralization of the charge through interaction with Ser-1.

[c]) Contribution of the Arg residue without its guanidinium group (see text).

[d]) Contribution of the Lys residue without its side-chain NH_3^+ group (see text).

[e]) Charge and H bond contribution of the Arg-8 guanidinium group (see text).

[f]) Calculated as valine amide (as in α-melanotropin).

Table 2. *α-Melanotropin: Estimated Free-Energy Difference* ΔG°_{tr}(m) [kJ/mol] *for its Transfer from a Random-Coil Conformation in* H_2O *to a Partly Helical Structure on an Aqueous-Hydrophobic Interface through Increasing Numbers* m *of N-Terminal Residues in their α-Helical Conformation.* See *Eqn. 1.*

m	Residue	HPC[a])	H Bond	Charge	$\Delta G^\circ_{tr}(i)$	$\sum_{i=1}^{m} \Delta G^\circ_{tr}(i)$	ΔG°_{tr} (end)		$\Delta G^\circ_{tr}(m)$
							N-End	C-End	
0	Ac	− 5.4	–	–	− 5.4	− 5.4	–	–	− 5.4
1	Ser	− 12.04	+ 10.5	–	− 1.54	− 6.94	+ 10.5	–	+ 3.56
2	Tyr	− 24.07	+ 10.5	–	− 13.57	− 20.51	+ 21.0	–	+ 0.49
3	Ser	− 12.04	+ 10.5	–	− 1.54	− 22.05	+ 31.5	+ 10.5	+ 19.95
4	Met	− 19.36	–	–	− 19.36	− 41.41	+ 31.5	+ 10.5	+ 0.59
5	Glu^-	− 19.98	[10.5][b])	[15.7][b])	− 12.69[c])	− 54.10	+ 31.5	+ 10.5	− 12.10
6	His^+	− 20.41	+ 21.0	+ 5.69	+ 6.28	− 47.82	"	"	− 5.82
7	Phe	− 21.98	–	–	− 21.98	− 69.80	"	"	− 27.80
8	Arg^+	− 16.2[d])	–	–	+ 2.80[e])	− 67.00	"	"	− 25.00
9	Trp	− 26.69	+ 10.5	–	− 16.19	− 83.19	"	"	− 41.19
10	Gly	− 7.85	–	–	− 7.85	− 91.04	"	"	− 49.04
11	Lys^+	− 19.0[f])	–	–	− 19.0	− 110.04	"	"	− 68.04
12	Pro	− 15.18	+ 21.0	+ 62.78[g])	+ 68.60	− 41.44	"	"	+ 0.56
13	Val[h])	− 19.00	+ 21.0	–	+ 2.00	− 39.44	"	"	+ 2.56

[a]) Hydrophobic contribution calculated from the accessible surface areas, taken from [14].
[b]) Not effective until $m = 8$ (Arg.)
[c]) HPC of Glu without its side chain COO^- group.
[d]) HPC of Arg without its guanidinium group.
[e]) Including HPC, H bond, and charge contributions of the Glu-5 carboxylate H-bonded to Ser-1.
[f]) Lys without its side chain NH_3^+ group.
[g]) Charge and H bonds of Arg-8.
[h]) Valine amide.

6) The side-chain charges of Glu-5, Arg-8, and Lys-11 remain in contact with H_2O and, despite the transfer of the uncharged part of the residue, are not transferred until the glutamic acid is two, the arginine three, and the lysine four positions away from the helix end [14].

7) The free-energy changes for the loss of one translational and two rotational degrees of freedom of $ACTH_{1-24}$, $ACTH_{1-10}$, and α-MSH bound to an interface were calculated according to *Janin* and *Chothia* [12].

Tables 1 and *2* list the contributions of individual residues and N-terminal segments with $m = 1$–13 to the free energies of transfer of $ACTH_{1-24}$ and α-MSH *(Eqn. 1)*. The development of the *Gibbs* free energy of hydrophobic association with increasing helix length *(Eqn. 2)*, is shown in *Fig. 2*. Energy minima of −24.66 and −17.53 kJ/mol for $ACTH_{1-24}$ and α-MSH, respectively, were reached with residues 11. For $ACTH_{1-10}$, a minimum of −10.54 kJ/mol at $m = 10$ was calculated, assuming the COO^- group of Gly-10 to remain in contact with H_2O. *Fig. 2* also shows, schematically, the H bond and charge systems of $ACTH_{1-24}$ and α-MSH in their states of lowest energy on the interface. Transfer of further residues was unfavourable, mainly because Pro-12 contributed two unsatisfied H bonds, and the guanidinium group of Arg-8 was transferred to the hydrophobic phase. Therefore, residues 12–24 were assumed to remain in the aqueous phase as parts of the original random coil, without any energy contribution. Their only effect was considered to be on ΔG°_{t+r} through molecular size and weight.

Table 3. *Elements Used for Estimating the Amphiphilic Moments of ACTH$_{1-24}$ and α-MSH*[a])

i	Residue	HPC	H Bond	Charge	End	$\Delta G^\circ_{trh}(i)$	Θ	$\vec{k}_i$	$\Delta G^\circ_{trh}(i)\vec{k}_i$
1	Ser^{+b})	− 4.2	+ 10.5	[4.56]	+ 10.5	+ 16.8	0	− 3.99	− 67.03
2	Tyr	− 16.3	+ 10.5	–	+ 10.5	+ 4.7	100	− 3.19	− 14.99
3	Ser	− 4.2	+ 10.5	–	+ 10.5	+ 16.8	200	− 2.39	− 40.15
4	Met^{c})	− 11.3	–	–	+ 10.5	− 0.8	300	− 1.60	+ 1.28
5	Glu^{-d})	− 12.2	[21.0]	[15.9]	–	− 12.2	400	− 0.8	+ 9.76
6	His^{+}*	− 12.6	+ 21.0	+ 5.9	–	+ 14.3	500	0	0
7	Phe	− 14.2	–	–	–	− 14.2	600	0.80	− 11.36
8	Arg^{+}	− 15.5	+ 31.5	+ 31.4	–	+ 47.4	700	+ 1.60	+ 75.84
9	Trp	− 18.9	+ 10.5	–	+ 10.5	+ 2.1	800	+ 2.39	+ 5.02
10	Gly	0.0	–	–	+ 10.5	+ 10.5	900	+ 3.19	+ 33.5
11	Lys^{+e})	− 13.0	+ 10.5	+ 20.1	+ 10.5	+ 28.1	1000	+ 3.99	+ 112.12

a) Energy differences in kJ/mol; HPC = hydrophobic contribution; $\Delta G^\circ_{trh}(i)$ is the *Gibbs* free energy of transfer of the ith residue from its α-helical conformation in H_2O to its α-helical conformation in a hydrophobic phase (*von Heijne* [14]). Angle Θ in degrees; k_i relative to r_i measured in units of helix radius, *Fig. 1*. Helix center (*) at residue 6 ($x = y = z = 0$).

b) N-Terminal charge not taken into account: in ACTH, Ser-1 is assumed to interact with the side chain COO^- group of Glu-5 with H bonding and charge neutralization, in α-MSH the Ser NH_2 group is acetylated; $\Delta G^\circ_{tr}(Ac) \approx -5.4$ kJ/mol.

c) End group contribution of Met-4 is missing in α-MSH because of internal H bonding with acetyl C=O.

d) Glu-5 H bond and charge contributions not taken into account for ACTH because of interaction with Ser-1; in α-MSH the charge and one H bond of the side chain COO^- group are assumed to be effective.

e) In ACTH$_{1-24}$, $\Delta G^\circ_{trh}(12-24) \approx 104$ kJ/mol, in α-MSH, $\Delta G^\circ_{trh}(12-13) \approx 2.8$ kJ/mol. In ACTH$_{1-10}$, Lys is missing and replaced by the negatively charged O-atom of the COO^- group of Gly-10, $\Delta G^\circ_{trh} \approx 18$ kJ/mol. All of these estimated contributions are assumed to be effective at $\Theta = 1000°$ and $\vec{k}_i = 3.99$.

Amphiphilic and electric dipole moments of ACTH$_{1-24}$, ACTH$_{1-10}$, and α-MSH were calculated for the molecules in their states of minimal energy *(Fig. 2)*. *Table 3* lists the individual elements for estimating the amphiphilic moment. Vectors $\vec{A}$ and $\vec{\mu}$ were computed from their component vectors normal and parallel to the helix axis *(Fig. 1)*. The estimated parameters are assembled in *Table 4*.

Table 4. *Estimated Amphiphilic and Electric Dipole Moments of ACTH Peptides and α-MSH*[a])

Moment	ACTH$_{1-24}$		α-MSH		ACTH$_{1-10}$	
$A(\Phi, \Theta)$	538	(15°, 289°)	132	(20°, 2°)	64	(3°, 343°)
$\mu(\Phi, \Theta)$	124	(14°, 289°)	16	(38°, 280°)	80	(178°, 70°)

a) See *Eqn. 3 and 4 and Fig. 1*; A is given in arbitrary units, μ in debye.

Accumulation of peptides on the fixed charge layer of anionic membranes ($V_{gc} \approx -40$ mV) was calculated with *Eqn. 5*, assuming the His residue either to carry one or no positive charge. The apparent molar dissociation constants were $1.8 \cdot 10^{-5}$ or $8.7 \cdot 10^{-5}$ for ACTH$_{1-24}$ (7+ or 6+), $4.4 \cdot 10^{-2}$ or $2.1 \cdot 10^{-1}$ for α-MSH (2+ or 1+), and $2.1 \cdot 10^{-1}$ or 1 for ACTH$_{1-10}$ (1+ or 0), respectively. Refinements for the influence of deposited discrete charges (modified *Stern* equation [2]) were not included, because the approximation used here was sufficient for the following discussion of biologic data.

Discussion. – *Fig. 3* shows a model for the interaction of $ACTH_{1-24}$ with an aqueous-hydrophobic interphase boundary that was derived from the estimated parameters. It corresponded closely to the model obtained from experimental data:

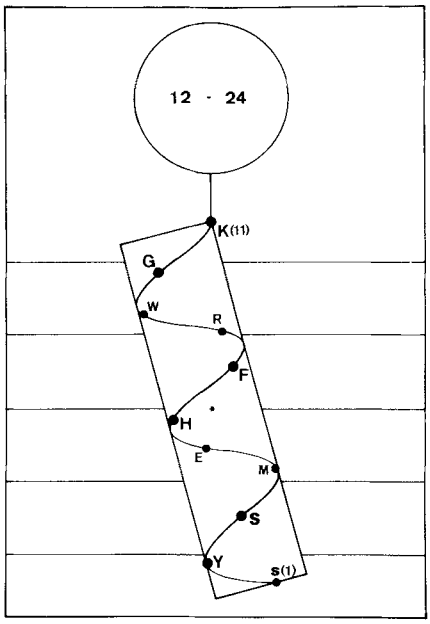

Fig. 3. *Predicted conformation and orientation of $ACTH_{1-24}$ in a hypothetical hydrophobic gradient or on a lipid membrane* (side view). Residue symbols (one-letter symbols) are given for the helical segment only, large ones in front of, smaller ones behind the axis. Residues 12–24 are in a random-coil segment exposed to H_2O and exerting its upward pull on the helix through the C(α) of Lys-11 (= K(11)). The assumed pivot point of the helix axis (*) lies on the origin of the right-handed polar coordinate system (see *Fig. 1*). The horizontal lines indicate surfaces of equal hydrophobicity in a hydrophobic gradient increasing from top to bottom. Directions of the molecular amphiphilic and electric dipole moments are perpendicularly upward from*.

ACTH peptides are flexible molecules in aqueous solutions (review [24]), but may assume preferred conformations in special environments. In 2,2,2-trifluoroethanol, $ACTH_{1-24}$ adopts a partially helical structure with an α-helix located near the N-terminus [25]. In contact with neutral lecithin (POPC) membranes, the N-terminal decapeptide segment is inserted into the hydrophobic phase [6] as an α-helix [3], but the C-terminal part remains in contact with H_2O. IR-ATR studies with polarized radiation indicate an approximately perpendicular orientation of the helix axis on the membrane [3]. These phenomena are particularly clear with very small peptide-lipid ratios (1:120). In *Fig. 3*, the amphiphilic moment (not shown) points towards the aqueous phase (top) and is oriented normally to the surfaces of equal hydrophobicity. The angle Φ between the amphiphilic moment vector and the helix axis (see *Fig. 1*) is considerably smaller than 45°, and is compatible with the observed dichroic ratio [3]. The predicted angle Θ was not accessible to experimentation. The estimated electric dipole moment vector *(Table 4)* points in the same direction as the amphiphilic moment. If the helix penetrates into the membrane surface dipole layer, the electric dipole moment will reinforce the action of the amphiphilic moment on molecule orientation shown in *Fig. 3*.

Position and shape of the IR amide II band of $ACTH_{1-24}$ on POPC membranes (1660 cm^{-1}) [3] indicate a helix length of 11–12 residues [26]. This agrees well with the estimated value of 11 residues (see *Fig. 2*). However, because of the labelling data, we had assumed a helix length of about 10 residues [3] [6]. It now appears that 11 is a more likely figure. This view is supported by the presence of helix-breaking proline as the next residue, as in the case of $dynorphin_{1-13}$ [4] [8]. The exposition of residues 12–24 to the aqueous phase (see *Fig. 3*) is supported by IR-ATR and labelling experiments, which exclude interaction of this segment with neutral (but not with anionic) membranes [3] [6].

The estimated dissociation constant $K_d = 5 \cdot 10^{-5}$ M for the hydrophobic association of $ACTH_{1-24}$ with an aqueous-hydrophobic interface agreed with the experimental data obtained from neutral membranes. Capacitance minimization studies indicate that $ACTH_{1-24}$ is reversibly adsorbed from dilute aqueous solutions containing 10 mM KCl onto 'black lipid membranes' prepared from egg-yolk lecithin [1]. The observed K_d for lecithin bilayers containing hexane equals $3.83 \pm 2 \cdot 10^{-5}$ M, if changes in membrane surface dipole moment caused by peptide adsorption are taken into account, and $7.5 \pm 0.8 \cdot 10^{-5}$ M without considering such changes [2]. Interaction with anionic membranes was estimated to be considerably stronger. This is qualitatively reflected in the results of labelling and equilibrium dialysis experiments [6] where increases of up to several orders of magnitude are indicated.

The membrane area occupied by one molecule of $ACTH_{1-24}$ at saturation is found to be 41 nm^2 with and 75 nm^2 without accounting for changes of dipole moment. From IR-ATR studies [3], we may assume that saturation is reached at peptide/lipid ratios of $\leqslant 1{:}120$. If we assume a lipid mean area on membranes of *ca.* 0.3 nm^2/molecule [27], the experimental value from IR-ATR indicates saturation at $\leqslant 36$ nm^2 per molecule of $ACTH_{1-24}$. The estimation of such areas was clearly beyond the scope of the methods applied here.

In $ACTH_{1-10}$, the direction of the amphiphilic moment vector was favourable for hydrophobic interaction of the N-terminus *(Table 4)*. However, its scalar magnitude was much too small to prevent thermal tumbling of the molecules on an interphase boundary. In order to orient molecules and to allow hydrophobic interactions to manifest themselves, values of 100–150 arbitrary units appear to be necessary [9]. Moreover, its relatively strong electric dipole moment is oriented in the wrong direction and is expected to prohibit N-terminal penetration into the membrane surface dipole layer. Even assuming ideal orientation of the molecules on the interface, the estimated molar dissociation constant of $1 \cdot 10^{-2}$ was too large to allow appreciable hydrophobic interaction. As $ACTH_{1-10}$ has no net charge, I concluded that it will interact neither hydrophobically with neutral membranes nor hydrophobically and electrostatically with anionic membranes. This agrees with the experimental observations [3] [6].

α-MSH was expected to interact with membranes in a manner intermediate between that of $ACTH_{1-24}$ and $ACTH_{1-10}$. Although the direction of its amphiphilic-moment vector favoured a 'perpendicular' orientation on an interface, the scalar magnitude was just between effectiveness and ineffectiveness. The not very favourably oriented electric dipole moment was relatively weak. Thus, tumbling of the molecules on the interface was expected to preclude hydrophobic interactions. Estimated *Coulomb* attraction to an anionic interface ($V_{gc} = -40$ mV) was quite weak. I concluded that α-MSH may accumulate slightly on the negative fixed charge layer of membranes, but that its orientation,

helix formation, and hydrophobic interactions will be negligible. These predictions await their experimental test.

Correlations between Membrane Structure and Biologic Activity. – ACTH and MSH peptides act on adrenal cell steroidogenesis [28], melanophore darkening [29], and behaviour ('learning' in active and passive avoidance tests with rats [30], cognitive functions, and interpersonal and environmental awareness in psychologic tests with human beings [31]). Their presence in certain areas of the brain [32] supports the view of direct action on the central nervous system (CNS). Different rank orders of peptide potency in different tissues indicate different receptor subtypes that are all triggered to the same degree (all these peptides are full agonists) by the same 'message' domain, but with a different probability, depending on the 'address' domains [24].

Rank orders and relative potencies for steroidogenic, melanotropic, and rat CNS receptors are $ACTH_{1-24} \gg \alpha$-MSH > $ACTH_{1-10}$ ($1:10^{-6}:10^{-7}$), α-MSH > $ACTH_{1-24}$ > $ACTH_{1-10}$ ($1:10^{-2}:10^{-4}$), and $ACTH_{1-10} \approx \alpha$-MSH $\approx ACTH_{1-24}$ (*ca.* 1:1:1), respectively. Although the biologic data are not nearly as accurate and complete as for opioid peptides, they suggest a similar molecular mechanism of receptor selection as is established for opioid κ-, μ-, and δ-receptors [9].

Opioid κ-receptor selectivity is determined by A and Φ of the opioid peptides; potency at this site is a function of $A \cdot \exp(z)^2$) [9]. For productive interaction, the κ-receptor requires the message domain of the peptide to be present in a relatively hydrophobic membrane compartment as a perpendicularly oriented helix. Lack of data points prevented such a rigorous correlation between ACTH/MSH peptides and steroidgenic receptor interaction. However, the membrane structure of $ACTH_{1-24}$ suggested that the main steroidogenic receptor has similar requirements as the opioid κ-site. This view was supported by the extremely low steroidogenic potency of α-MSH and $ACTH_{1-10}$, that did not interact with membranes in the same manner as $ACTH_{1-24}$. The somewhat stronger activity of α-MSH compared with $ACTH_{1-10}$ may tentatively be explained by the difference in A causing a difference in the probability of inserting the message domain into the membrane, as in the case of opioid peptides [9].

Rank order and relative potencies for melanotropic activity are reminiscent of the interactions of opioid peptides with μ-receptors [9]. The μ-site appears to be exposed to the aqueous phase surrounding the target cells, and to experience electrostatic accumulation close to the anionic fixed charge layer. μ-Potency follows $\exp(z)$, but is strongly reduced for κ-agonists, that insert their message domains as helices into relatively hydrophobic membrane compartments [9]. This might also explain the difference in melanotropic potency between α-MSH(1+) and $ACTH_{1-24}$(6+). However, the charge difference between α-MSH and $ACTH_{1-10}$ could only explain roughly one order of magnitude of potency difference. The rest must be attributed to the lack of structural elements in $ACTH_{1-10}$ that are specifically necessary for productive receptor interactions (receptor requirements such as the acetyl and amide groups and the important domain 11–13 [29]).

If we accept the behavioural data as indicating an almost equal interaction of all three peptides with specific CNS receptors, we may conclude that the CNS sites are exposed to the aqueous phase and have no requirement for membrane-induced conformation and

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[2]) z is the effective net charge on the peptide, *Eqn. 5*.

orientation of the message domains [3]. In this respect, they certainly resemble the melanotropic receptors. More complete and more precise data, including receptor-specific binding, would be necessary for a valid analysis that might reveal more subtle differences.

Conclusion. – On the premises that peptide segments adopt α-helical structures in hydrophobic environments [17] and that amphiphilic [20] [8] [9] and electric-dipole [21] character are responsible for orienting peptides on aqueous-hydrophobic interphase boundaries, a model was developed that allows the prediction of preferred conformation, orientation, and accumulation of peptides on lipid bilayer membranes. The parameters estimated for dynorphin$_{1-13}$ [8], ACTH$_{1-24}$, and ACTH$_{1-10}$ agree well with those derived from experimental observations [3–6]. The main orientation parameter appears to be the amphiphilic moment as shown by its excellent correlation with biologic activity and receptor selection of opioid peptides [9]. The electric dipole moment apparently tends to reinforce or attenuate the influence of amphiphilic character on orientation if the peptide penetrates the membrane surface dipole layer. Unfortunately, too little is known about magnitude, exact location, and orientation of membrane surface dipoles to permit more quantiative predictions based on peptide dipole moment.

A prediction was made of the membrane interaction of α-MSH, the validity of which is currently being evaluated. The predicted membrane structures and membrane interactions of ACTH$_{1-24}$, ACTH$_{1-10}$, and α-MSH and their correlation with the available biologic data suggest a molecular mechanism of receptor selection similar to that proposed for opioid receptors [9]. Biologic action of peptides is quite generally thought to depend on 'receptor requirements' that have to be met by peptide structures in order to produce a productive, 'complementary' fit between peptide message domains and receptor recognition sites [33] [34]. In the present model, the membrane screens peptides for their ability to interact with different membrane compartments by making use of their effective net charge, amphiphilic and electric dipole moments, and hydrophobic association. After partitioning into the appropriate membrane compartment(s), the peptide message domain interacts with the receptor subsites exposed to this compartment. This is made possible by a set of 'membrane requirements' which supplement the 'receptor requirements' in an effective manner. Thus, the membrane appears to catalyze specific peptide receptor interactions [7] [10] in much the same manner as micelles catalyze chemical and biochemical reactions [35].

REFERENCES

[1] a) IUPAC-IUB, *Pure Appl. Chem.* **1984,** *56,* 595; b) P. Schoch, D. F. Sargent, R. Schwyzer, *J. Membr. Biol.* **1979,** *46,* 71; *Biochem. Soc. Trans.* **1979,** *7,* 846; H.-U. Gremlich, D. F. Sargent, R. Schwyzer, *Biophys. Struct. Mech.* **1981,** *8,* 61.

[2] P. Schoch, 'Kapazitätsminimalisierung: eine neue Methode zur Messung von Oberflächenpotentialen von künstlichen Lipidmembranen und ihre Anwendung auf Lipid/Peptid Wechselwirkungen', Dissertation ETH No. 6699, Zürich, 1980; P. Schoch, D. F. Sargent, *Biochim. Biophys. Acta* **1980,** *602,* 234.

[3] H.-U. Gremlich, U.-P. Fringeli, R. Schwyzer, *Biochemistry* **1983,** *22,* 4257; *ibid.* **1984,** *23,* 1808.

[4] D. Erne, D. F. Sargent, R. Schwyzer, *Biochemistry* **1985,** *24,* 4261.

[5] B. Gysin, R. Schwyzer, *Arch. Biochem. Biophys.* **1983,** *225,* 467.

[6] B. Gysin, R. Schwyzer, *Biochemistry* **1984,** *23,* 1811.

[7] R. Schwyzer, H.-U. Gremlich, B. Gysin, D. F. Sargent, H.-P. Fringeli, in 'Peptides 1982', Eds. K. Blaha and P. Malon, Walter de Gruyter & Co., Berlin, 1983, pp. 55–71; R. Schwyzer, H.-U. Gremlich, B. Gysin, D. F. Sargent, H.-P. Fringeli, in 'Peptides: Structure and Function', Eds. V. J. Hruby and D. H. Rich, Pierce Chem. Co., Rockford, IL, 1983, pp. 657–664; R. Schwyzer, in 'Natural Products Chemistry 1984', Eds. R. I. Zalewski and J. J. Skolik, Elsevier Sci. Publ. B. V., Amsterdam, 1985, pp. 591–598; in 'Natural Products and Biological Activities', Eds. H. Imura, T. Goto, T. Murachi, and T. Nakajima, University of Tokyo Press, Tokyo, 1986, pp. 197–207.

[8] R. Schwyzer, *Biochemistry* **1986,** *25,* 4281–4286.

[9] R. Schwyzer, *Biochemistry* **1986,** *25,* in press.

[10] D. F. Sargent, R. Schwyzer, *Proc. Natl. Acad. Sci. USA* **1986,** *83,* 5774–5778.

[11] W. Kauzmann, *Adv. Protein Chem.* **1959,** *14,* 1.

[12] J. Janin, C. Chothia, *Biochemistry* **1978,** *17,* 2943.

[13] B. Lee, F. M. Richards, *J. Mol. Biol.* **1971,** *55,* 379; F. M. Richards, *Ann. Rev. Biophys. Bioeng.* **1977,** *6,* 151.

[14] G. von Heijne, C. Blomberg, *Eur. J. Biochem.* **1979,** *97,* 175; G. von Heijne, *ibid.* **1980,** *103,* 431; *ibid.* **1981,** *116,* 419.

[15] A. Parsegian, *Nature (London)* **1969,** *221,* 844.

[16] C. Chothia, *Nature (London)* **1974,** *248,* 338.

[17] R. Henderson, *Soc. Gen. Physiol.* **1979,** *33,* 3.

[18] C.-H. Huang, *Nature (London)* **1976,** *259,* 242; N. Chatterjie, H. Brockerhoff, *Biochim. Biophys. Acta* **1978,** *511,* 116.

[19] M. F. Perutz, J. C. Kendrew, H. C. Watson, *J. Mol. Biol.* **1965,** *13,* 669; M. Schiffer, A. B. Edmundson, *Biophys. J.* **1967,** *7,* 121.

[20] D. Eisenberg, R. M. Weiss, T. C. Terwilliger, *Nature (London)* **1982,** *299,* 371.

[21] A. Wada, *Adv. Biophys.* **1976,** *9,* 1; W. G. J. Hol, *Prog. Biophys. Molec. Biol.* **1985,** *45,* 149.

[22] T. P. Seltzmann, F. M. Finn, C. C. Widnell, K. Hofmann, *J. Biol. Chem.* **1974,** *250,* 1193.

[23] F. R. Maxfield, H. A. Scheraga, *Macromolecules* **1975,** *8,* 491.

[24] R. Schwyzer, *Ann. N. Y. Acad. Sci.* **1977,** *297,* 3–26; *Proc. R. Soc. London, [Ser.] B* **1980,** *210,* 5.

[25] M. Löw, L. Kisfaludy, S. Fermandjian, *Acta Biochim. Biophys. Acad. Sci. Hung.* **1975,** *10,* 229; D. Greff, F. Thoma, S. Fermandjian, M. Löw, L. Kisfaludy, *Biochim. Biophys. Acta* **1976,** *439,* 219; E. Nabedryk-Viala, C. Thiery, P. Calvet, S. Fermandjian, L. Kisfaludy, J. M. Thiery, *Biochim. Biophys. Acta* **1978,** *536,* 252.

[26] N. A. Nevskaya, Yu. N. Chirgadze, *Biopolymers* **1976,** *15,* 637.

[27] G. B. Robinson, in 'Biological Membranes', Ed. D. S. Parsons, Clarendon Press, Oxford, 1975, pp. 33–54.

[28] S. Seelig, B. D. Lindley, G. Sayers, *Methods Enzymol.* **1975,** *39(D),* 347.

[29] A. N. Eberle, R. Schwyzer, *Helv. Chim. Acta* **1975,** *58,* 1528.

[30] D. de Wied, A. Witter, H. M. Greven, *Biochem. Pharmacol.* **1975,** *24,* 1463.

[31] C. A. Sandmann, B. B. Walker, C. A. Lawton, *Peptides* **1980,** *1,* 109.

[32] D. T. Krieger, A. Liotta, M. D. Brownstein, *Proc. Natl. Acad. Sci. USA* **1977,** *74,* 648.

[33] R. Schwyzer, *Pure Appl. Chem.* **1963,** *6,* 265.

[34] P. W. Schiller, in 'The Peptides', Eds. E. Gross and H. Meienhofer, Academic Press, New York, 1984, Vol. 6, pp. 219–268.

[35] W. P. Jencks, *Adv. Enzymol. Rel. Areas Mol. Biol.* **1975,** *43,* 219.