Interaction of Adrenocorticotropin-(11–24)-tetradecapeptide with Neutral Lipid Membranes Revealed by Infrared Attenuated Total Reflection Spectroscopy†

Hans-Ulrich Gremlich, Urs-Peter Fringeli, and Robert Schwyzer*

ABSTRACT: Infrared attenuated total reflection spectroscopy (IR-ATR) revealed that the hydrophilic adrenocorticotropin-(11–24)-tetradecapeptide ($ACTH_{11-24}$, net charge 6+) assumed an irregular secondary structure when incorporated into the aqueous layers between equilibrated multibilayers of planar membranes prepared from 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC). This structure was characterized by a perpendicular orientation of the peptide bonds on the bilayer surfaces, as observed earlier for the corresponding segment of adrenocorticotropin-(1–24)-tetracosapeptide ($ACTH_{1-24}$, 6+). Once incorporated, $ACTH_{11-24}$ was not removed by washing, in agreement with its strong positive charge. In contrast to $ACTH_{1-24}$, $ACTH_{11-24}$ was not measurably adsorbed to the neutral membranes from 0.1 mM aqueous solutions. The more hydrophobic adrenocorticotropin-(1–10)-decapeptide is also not adsorbed. We therefore concluded that adsorption of $ACTH_{1-24}$ to neutral membranes was dependent on its amphiphilic primary (amphipathic primary) structure that resulted from the covalent combination of the hydrophobic $ACTH_{1-10}$ segment with the hydrophilic $ACTH_{11-24}$ segment. This conclusion was consistent with the results obtained by vesicle-mediated hydrophobic photolabeling and equilibrium dialysis.

$ACTH_{1-24}$,[1] (+)SYSME(−)HFR(+)WGK(+)PVGK(+)K-(+)R(+)R(+)PVK(+)VYP(−) (Schwyzer & Kappeler, 1963), is adsorbed to neutral bilayer membranes prepared from phosphatidylcholine. It extends its N-terminal message segment, residues 1–10, into the hydrophobic membrane layers as a helical structure with its axis perpendicular to the surface. Its C-terminal address segment, residues 11–24, remains on the aqueous head-group layer as an irregular secondary structure, with peptide bonds oriented perpendicularly to the interface. This peptide segment specific interaction, observed by IR-ATR (Gremlich et al., 1983), is confirmed by vesicle-mediated hydrophobic photolabeling and equilibrium dialysis (Schwyzer et al., 1983; Gysin & Schwyzer, 1984).

The specific membrane interaction of the relatively hydrophobic segment 1–10 is only possible in covalent combination with the very hydrophilic, strongly charged segment 11–24 but is completely missing for the decapeptide $ACTH_{1-10}$. Is the specific behavior of the segment 11–24 on the head-group layer and vice versa also dependent on the covalent presence of the segment 1–10?

We answered this question with the help of IR-ATR and found that the free tetradecapeptide $ACTH_{11-24}$ was not measurably adsorbed to POPC multibilayers from 0.1 mM aqueous solutions. However, when incorporated into the membrane system and sandwiched into the aqueous phase between the bilayers, it assumed an irregular secondary structure with peptide bonds perpendicular to the interface, as in $ACTH_{1-24}$. The presence of the message segment, residues 1–10, thus appeared to be necessary for the adsorption process but not for the conformational arrangement of the address segment, residues 11–24, in the aqueous head-group layer.

This behavior was consistent with that observed by vesicle-mediated hydrophobic photolabeling and equilibrium dialysis. It appears that the *amphiphilic* (*amphipathic*) *primary structure* of $ACTH_{1-24}$, based on the segmental distribution of hydrophobic and hydrophilic amino acids in the primary structure of the hormone, is essential for the observed hydrophobic peptide–membrane interactions (Gysin & Schwyzer, 1984). The actually realized peptide conformation then depended on the microenvironment of the individual peptide segments on or in the membrane (hydrocarbon layer, hydrogen-bond belt, or head-group layer), as suggested earlier in more general terms (Schwyzer, 1963, 1970, 1977). Whether the polarization observed here exists only between lipid bilayers in the multibilayer system or also on a bilayer surface exposed to bulk water (e.g., on a cell surface) is not known.

Experimental Procedures

1-Palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) was purchased from R. Berchtold, Biochemical Laboratory, CH-3007 Bern, Switzerland. All the other materials and the methods, especially IR-ATR, are described by Gremlich et al. (1983).

Results and Discussion

$ACTH_{11-24}$, deposited as a solvent-free film on the ZnSe plate, exhibited nonpolarized amide I and II bands at 1645 and 1545 cm^{-1}, respectively (Figure 1a; Table I), indicative of predominantly random structures of the peptide molecules (Gremlich et al., 1983). In equilibrated membranes prepared from mixtures of POPC and $ACTH_{11-24}$, the amide I band was shifted slightly to larger wavenumbers (1650–1655 cm^{-1}, Figure 1b,c). The dichroic ratio of the amide I band increased from $R = 1.1$ in the absence of lipid to $R = 1.9$ and $R = 2.3$ in fully equilibrated membranes with molar ratios of POPC:$ACTH_{11-24}$ of 75:1 and 100:1, respectively. Simultaneously, the dichroic ratio of the amide II band decreased from

† From the Departments of Molecular Biology and Biophysics (H.-U.G. and R.S.) and Physical Chemistry (U.-P.F.), Swiss Federal Institute of Technology (ETH), CH-8093 Zürich, Switzerland. *Received July 25, 1983.* Supported by project grants of the ETH, the Swiss National Science Foundation, and the Bonizzi-Theler Stiftung.

[1] Abbreviations: ACTH, adrenocorticotropic hormone; $ACTH_{n-m}$, synthetic ACTH peptides comprising the amino acid residues *n* to *m* of the natural sequence; IR-ATR, infrared attenuated total reflection spectroscopy; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine.

0006-2960/84/0423-1808$01.50/0

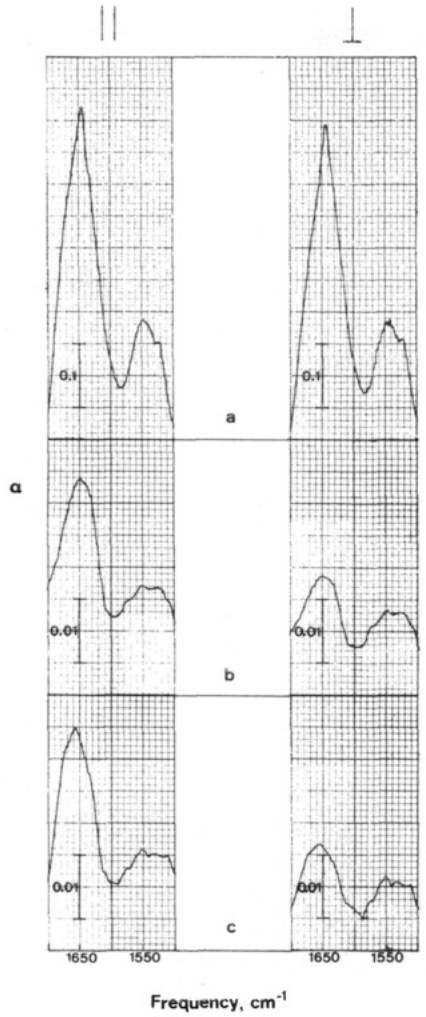

FIGURE 1: Behavior of ACTH$_{11-24}$ in POPC multilayer membranes reflected by amide I and amide II IR-ATR absorption bands (absorbance plot, $\alpha = -\log T$, in which T is transmittance, measured with parallel (||) and perpendicular (⊥) polarized light). (a) Pure ACTH$_{11-24}$, 25 °C, deposited directly on the ZnSe surface by drying a solution in CH_3OH with N_2. (b) Model membrane prepared from CH_3OH solution (7.5 nM POPC, 0.1 mM), after 20-min contact with liquid H_2O, dry N_2, 25 °C, for adequate drying. Spectra of the pure membrane have been subtracted. (c) Model membrane prepared from CH_3OH solution (10 mM POPC, 0.1 mM ACTH$_{11-24}$), after 20-min contact with liquid H_2O, dry N_2, 25 °C, for adequate drying. Spectra of the pure POPC membrane have been subtracted.

Table I: Dichroic Ratio R of the ACTH$_{11-24}$ Amide I and Amide II Absorption Bands at 25 °C

sample	$R_{\text{amide I}}$	$R_{\text{amide II}}$
ACTH$_{11-24}$	1.1 ± 0.1	1.0 ± 0.1
POPC–ACTH$_{11-24}$ (75:1)	1.9 ± 0.1	0.9 ± 0.1
POPC–ACTH$_{11-24}$ (100:1)	2.3 ± 0.1	0.8 ± 0.1

R = 1.0 to R = 0.9 and R = 0.8 (Figure 1b,c; Table I).

Hydrogen–deuterium exchange studies indicated that the amide hydrogen was completely exchanged for deuterium after 16 h in an atmosphere of N_2 saturated with ^2H_2O both in the ACTH$_{11-24}$ films and the POPC–ACTH$_{11-24}$ (100:1) model membranes (Figure 2). In both cases, the ACTH$_{11-24}$ molecules must have been quite freely accessible to the deuterated water. For model membranes this meant that the peptide was most probably situated in the aqueous phase between the head groups of adjacent lipid bilayers.

We interpreted the observed shift of the amide I band and the polarizations as indicating an irregular, extended secondary structure that was ordered with respect to the lipid–water

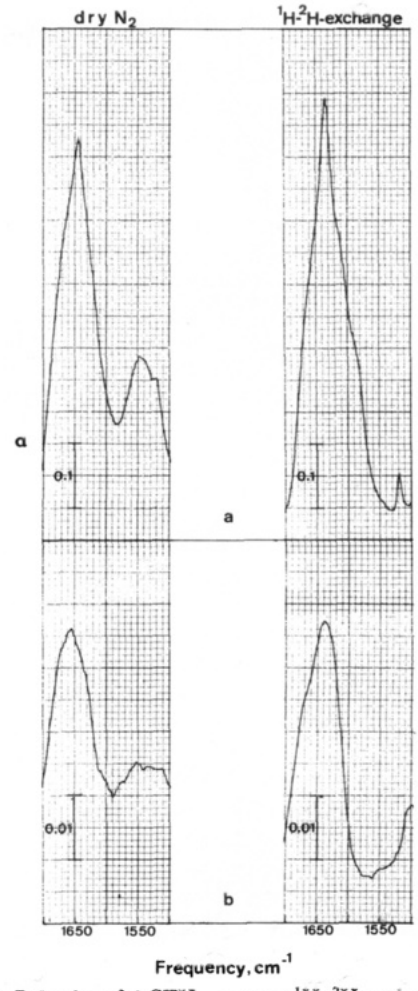

FIGURE 2: Behavior of ACTH$_{11-24}$ upon ^1H–^2H exchange reflected by amide I and amide II absorption bands (absorbance plot, $\alpha = -\log T$, in which T is transmittance). Samples were either dried with dry N_2 or flushed with ^2H_2O-saturated N_2 (^1H–^2H exchange) for 16 h. All spectra were recorded with parallel polarized light. (a) Pure ACTH$_{11-24}$, 25 °C, deposited directly on the ZnSe surface by drying a solution in CH_3OH with N_2. (b) Model membrane prepared from CH_3OH solution (10 mM POPC, 0.1 mM ACTH$_{11-24}$), after 20-min contact with liquid H_2O, dry N_2, 25 °C, for adequate drying. Spectra of the pure POPC membrane have been subtracted.

interface in the sense that the planes of the peptide bonds were oriented perpendicularly to the membrane surface. If we accept the same order parameter (S = 0.6) for ACTH$_{11-24}$ as is calculated for the corresponding segment, residues 11–24, of ACTH$_{1-24}$ (Gremlich et al., 1983), then the measured dichroic ratios indicated a 90° angle between the planes of the peptide bonds and the membrane interface. Thus, the behavior of ACTH$_{11-24}$ sandwiched in between the model membranes was indistinguishable by IR-ATR from that of the corresponding segment of ACTH$_{1-24}$. Again, the ordering effect was tentatively explained by distinct electrostatic (mainly Coulombic and dipole–dipole) interactions between charged side chains and peptide bonds of ACTH$_{11-24}$ and the polar head groups of the phosphatidylcholine molecules.

With IR-ATR, we were not able to detect any adsorption of ACTH$_{11-24}$ from 0.1 mM solutions onto preformed POPC multibilayers under the conditions under which it is readily observed for ACTH$_{1-24}$ (Gremlich et al., 1983). This confirms the importance of the covalent combination of ACTH$_{1-10}$ with ACTH$_{11-24}$ into a primary (or segmental) amphiphilic structure for the adsorption process. This is true not only for neutral but also for anionic membranes, as shown in a study of the hydrophobic and electrostatic interactions between ACTH

peptides and liposomes by vesicle-mediated hydrophobic photolabeling and equilibrium dialysis (Gysin & Schwyzer, 1984). The more hydrophobic decapeptide, $ACTH_{1-10}$, is not adsorbed to neutral and anionic liposomes, but in $ACTH_{1-24}$ it is this segment that establishes the hydrophobic contacts in both types of liposome. The very hydrophilic $ACTH_{11-24}$ is not attracted to neutral liposomes but does interact with anionic vesicles (equilibrium dialysis); however, it displays no hydrophobic contacts to the membranes. This is in complete analogy to the findings presented here.

No significant loss of $ACTH_{11-24}$ was observed by contacting fully equilibrated POPC–$ACTH_{11-24}$ (100:1) model membranes with water at 25 °C up to 24 h. This behavior was consistent with that of $ACTH_{1-24}$ and other charged peptides [see Gremlich et al. (1983)].

Conclusions

The studies presented here complete our picture of the interaction between $ACTH_{1-24}$ and model lipid membranes in an essential point: Neither the hydrophobic message segment, residues 1–10 (that is responsible for triggering the receptor), nor the hydrophilic address segment, residues 11–24 (that greatly potentiates the message activity and conveys target-cell specificity), is alone capable of being adsorbed to and interacting with model phosphatidylcholine membranes. It is only their covalent combination, as in $ACTH_{1-24}$, that gives rise to distinct membrane interactions accompanied by membrane-induced, specific conformation transitions and unique topological arrangements in the anisotropic, amphipathic environment. The interaction is not through an amphipathic secondary structure, such as an α-helix, that has often been invoked as being responsible for membrane interactions of apolipoproteins, peptide toxins, and other peptide hormones [see Kaiser & Kézdi (1983) and Epand (1983)] but through a novel type, the *amphipathic primary structure* (primary or segmental amphiphilic structures). In our case, the message provides the hydrophobic contact by entering the membrane as a perpendicularly oriented helix, and the positively charged address provides electrostatic contacts to the head-group layer resulting in a perpendicular orientation of the peptide bond planes with respect to the membrane surface.

Primary amphiphilicity, as we may call the amphiphilicity caused by amphipathic primary structures, is also the cause for the membrane interactions of $ACTH_{1-24}$ and dynorphin-(1–13)-tridecapeptide observed with vesicle-mediated hydrophobic photolabeling (Gysin & Schwyzer, 1983a, 1984). Such interactions appear to correlate well with the biologic potency of several peptide agonists and antagonists and therefore to be biologically relevant (Gysin & Schwyzer, 1983b; Schwyzer et al., 1983).

Registry No. $ACTH_{11-24}$, 4237-93-8; POPC, 26853-31-6.

References

Epand, R. M. (1983) *Mol. Cell. Biochem.* (in press).
Gremlich, H.-U., Fringeli, U.-P. & Schwyzer, R. (1983) *Biochemistry 22*, 4257–4263.
Gysin, B., & Schwyzer R. (1983a) *Arch. Biochem. Biophys. 225*, 467–474.
Gysin, B., & Schwyzer, R. (1983b) *FEBS Lett. 158*, 12–16.
Gysin, B., & Schwyzer, R. (1984) *Biochemistry* (following paper in this issue).
Kaiser, E. T., & Kézdy, F. J. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 1137–1143.
Schwyzer, R. (1963) *Pure Appl. Chem. 6*, 265–295.
Schwyzer, R. (1970) *Proc. Int. Congr. Pharmacol., 4th, 1969 5*, 196–209.
Schwyzer, R. (1977) *Ann. N.Y. Acad. Sci. 297*, 3–26.
Schwyzer, R., & Kappeler, H. (1963) *Helv. Chim. Acta 46*, 1550–1572.
Schwyzer, R., Gremlich, H.-U., Gysin, B., & Fringeli, U.-P. (1983) in *Peptides 1982* (Bláha, K., & Maloň, P., Eds.) pp 55–71, de Gruyter, Berlin and New York.