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Induction of secondary structure in the peptide hormone motilin by interaction with phospholipid vesicles

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Abstract

Motilin is an intestinal peptide hormone that binds to a membrane bound receptor located in the gut tissue. Circular dichroism (CD) was used to study the interaction between either porcine or rabbit motilin or a 1–16 fragment of porcine motilin, with model systems of lipid membranes: sodium dodecyl sulphate (SDS), 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol (DOPG) and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC). The CD measurements show significant induction of secondary structure in both motilins and the fragment when negatively charged vesicles (DOPG) or negatively charged micelles (SDS) were present. In contrast, neutral DOPC vesicles did not induce any change in the secondary structure compared to water, in which a random-like secondary structure dominates. The induced secondary structure in the presence of DOPG vesicles is very close to that induced by a mixed aqueous solution containing 30% hexafluoroisopropanol, in which previous NMR-studies have resulted in a three-dimensional solution structure of porcine motilin. In both porcine and rabbit motilin the α-helix content is about 50%. This is in agreement with the presence of an amphipathic helix in the C-terminal half of motilin interacting with phospholipid membranes. The interaction appears to be mainly electrostatic in nature, and does not induce any significant alterations in the vesicle, as monitored by EPR studies of spin labels located at the fifth carbon atom of the backbone in a stearic acid molecule. In the 1–16 fragment the α-helical content induced by DOPG and SDS is only about 20%.

Key words: Motilin; Peptide hormone; Secondary structure; Circular dichroism; Phospholipid vesicle

1. Introduction

Peptide hormones can be divided into three categories [1]: the first one consists of small peptides where all amino acids are needed when binding to the receptor. In the second category there are hormones with a very complex structure, containing many disulfide bonds. These hormones are strongly dependent on their tertiary structure when binding to the receptor. The third group consists of peptide hormones about 10–50 amino acids long and usually without disulfide bridges. The secondary structure of these peptides has a strong environment dependence and can assume different conformations in different solvents. This third kind of peptides typically forms amphiphilic secondary structures upon interaction with an amphiphilic environment.

There have been suggested three possible situations where it might be functionally important for a peptide hormone to form an amphiphilic secondary structure [2]. One possibility might be the positioning of other parts of the hormone, so that an active site comes in the right position to interact with the receptor. Another explanation is to guide the peptide to its receptor by attaching it to the membrane and allowing it to move on the membrane surface. A third possibility might be to protect the peptide hormone from proteolysis.

Motilin is an intestinal peptide hormone able to stimulate gastrointestinal motor activity [3]. The hormone binds to a membrane-bound receptor, not yet purified, present in smooth muscle tissue of the gastrointestinal tract in rabbit [4] and in man [5]. Porcine motilin consists of 22 amino acids and seems to be a member of the third category of peptide hormones

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discussed above. As shown in Fig. 1 the first five residues are non-polar and the rest are mainly hydrophilic. Detailed structure-activity studies have shown that the biological activity resides largely in the N-terminal part [6]. The pharmacophore appears to involve residue 1, 2, 4 and 7 [7]. Recently also rabbit motilin has been isolated. It differs from porcine motilin in only three residues [8]. The amino acid sequences of porcine and rabbit motilin are shown in Fig. 1.

Earlier ^{1}H-NMR measurements in a mixed aqueous solvent with 30% HFP have shown [9,10] that in porcine motilin residues 3–6 form a wide turn and a highly dynamic 'hinge-like' region was found around tyrosine, residue 7. In the C-terminal part of the peptide a relatively stable α-helix is formed between residues 9 and 20. The mixed aqueous solvent with 30% HFP was used in the NMR studies to induce more secondary structure to allow a structure determination. It was argued that this structure was relevant for motilin in a more hydrophobic environment, such as the biomembrane holding the receptor. However, CD studies have shown that addition of neutral phospholipid (DOPC) vesicles does not induce any change in the secondary structure of motilin, which is dominated by a random-like structure in pure aqueous solution [11].

We have now used CD spectroscopy to investigate the effect on motilin secondary structure of other solvent systems. Since biological membranes usually are negatively charged we particularly chose negatively charged phospholipid (DOPG) vesicles, as well as the detergent SDS, which also contains a negative charge at neutral pH. The results show a quite significant interaction accompanied by induction of secondary structure, similar to the one in 30% HFP, between motilin and these solvent systems with negatively charged phospholipid or detergent components. This result strongly suggests a biological significance of the solution structure determined previously by NMR. The CD studies include a 1–16 fragment of porcine motilin, which showed a similar pattern of structure induction albeit to a lesser extant. The investigation also includes EPR studies of spin labeled phospholipid membrane vesicles to monitor the state of the vesicles during their interaction with motilin.

2. Materials and methods

Materials

Porcine motilin was kindly provided by professor Viktor Mutt, Karolinska Institutet in Stockholm (Sweden). Synthetic rabbit motilin was generously provided by Dr. P. Durieux, Novabiochem, Laufelfingen, Switzerland. For the CD studies both motilin peptides were diluted in pure water to a concentration of about $1.9 \cdot 10^{-5}$ M. A synthetic 1–16 fragment of porcine motilin with Met-13 changed to Leu was kindly provided by Dr. M. Macielag, British Oxygen Corporation, Murray Hill, NY (USA). A five amino acid peptide, corresponding to the N-terminal fragment of motilin, was synthesized by UCB-Bioproducts (Braine L'Alleud, Belgium).

Sodium dodecyl sulphate (SDS) was obtained from BDH. 1,1,1,3,3,3-Hexafluoro-2-isopropanol ($(CF_3)_2$ CHOH, HFP) was obtained from Sigma.

1,2-Dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and the sodium salt of 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol (DOPG) were purchased from Avanti Polar Lipids (USA). The purity of the two lipids was better than 99% as checked by thin-layer chromatography at our laboratory. The spin label 4-oxo-2,2,6,6-tetramethyl piperidine-1-oxyl (TEMPONE) was purchased from Molecular Probes (USA) and was used directly without any further purification. Potassium trioxalatochromate ($K_3Cr[C_2O_4]_3$), was synthesized according to the procedure outlined by Malati and Azim (1959). The purity of the product was checked by light absorption measurements. The spectrum and the calculated molar absorptivity of the compound were in agreement with those previously reported.

Preparation of phospholipid vesicles

The vesicles were prepared by sonication according to the following procedure (cf. Ref. [12]). Appropriate amounts of the dry powder of DOPC or DOPG were dissolved in a mixture of chloroform/methanol (2:1, v/v). The solvent was evaporated and dried at 320 K and 0.1 torr during at least 2 h. 2 ml of an aqueous solution (143 mM KCl) was added and the suspension was sonicated four times in intervals of 10 min. During the sonication the sample was cooled at about 283 K. The sonicator was a Soniprep 150 (MSE Scientific Instruments, UK) supplemented with an exponential microprobe. The level of the amplitude used was 10–14 mm. Between the different sonication steps the lipid/water mixture was frozen in liquid nitrogen and thawed several times in order to obtain as large aggregates as possible. The result was an optically clear solution of vesicles.

Procedure to determine the internal volume of the vesicles

Vesicles consist of lipid bilayers enclosing a volume which is possible to determine by EPR measurements. The method uses a combination of a spin label (TEMPONE) permeable to membranes and paramagnetic ions impermeable to membranes. The latter ones effectively quench the EPR signal from spin labels exterior to the vesicle by dipolar spin-spin broadening. The method is based on the fact that the chemical exchange over the membrane is slow on the EPR time scale and that the paramagnetic ions do not penetrate the vesi-

cles. From the experimentally determined enclosed volume, and the length and the area of the polar headgroup of the lipid it is possible to calculate the radius of the vesicle [13–16].

To determine vesicle volume the solution of vesicles was equilibrated for 30 min at room temperature with a suitable amount of TEMPONE dissolved in aqueous solution (143 mM KCl). The spin label penetrates the membrane very rapidly and full equilibration is obtained during this time. The equilibrated vesicle-TEMPONE solution was thereafter divided into two solutions A and B. Solution A was diluted with a suitable 143 mM KCl solution giving a final TEMPONE concentration equal to 1.89 mM (A′). Solution B was thoroughly mixed with another water solution containing the spin-broadening agent (stock solution of 300 mM $K_3Cr[C_2O_4]_3$) chromium oxalate). This latter diluted solution is denoted B′ (1.89 mM TEMPONE, 119 mM KCl, 50 mM $K_3Cr[C_2O_4]_3$). The concentration of the different salt species was carefully chosen in order to prevent disruption of the vesicles. The pH of the vesicle solutions was determined to be about 6.

Glass capillary tubes (o.d. = 1.4 mm, length = 75 mm) were filled with solution A′ and thereafter flame-sealed. EPR-spectra were recorded at 298 K with a Bruker model ESP 300E X-band spectrometer (9 GHz). EPR-spectra were registered for at least three different capillary tubes. A typical spectrum recorded for solution A′ is shown in Fig. 2a. In a second run EPR-spectra for three different glass capillaries filled with solution B′ were registered. An EPR spectrum typical for these samples is shown in Fig. 2b.

Preparation of phospholipid vesicles spin labeled with 5-doxylstearic acid

5-Doxylstearic acid (5-NS) was purchased from Molecular Probes (USA). The lipid and the spin label were thoroughly mixed in a chloroform/methanol solution (2:1, v/v). Thereafter the same sonication procedure as described earlier was used to produce vesicles labeled with the 5-NS probe. The label/lipid ratio for the vesicle sample was 1/50 on a molar scale.

Amino acid analysis

For determination of an extinction coefficient for rabbit motilin, quantitation of the peptide was performed by light absorption and amino acid analysis. Aliquots of 30 μl containing about 10 μg of peptide were transferred to amino analysis tubes and frozen. These samples were subsequently hydrolysed in 6 M constant boiling HCl, at 110°C for 24 h, and lyophilized. The amino acids were quantitated by using a Biotronic LC 5000 liquid chromatography equipment, with a Shimadzu C-R2AX data module. The mass of the sample was calculated using the mean abundance of the following amino acids: Val, Met, Ile, Leu, Tyr, which varied around an average within ±5%.

Light absorption

Light absorption measurements were carried out in a CARY 4 spectrophotometer using cuvettes with 2 mm light path. Spectra were baseline corrected.

Circular dichroism

Circular dichroism (CD) measurements were carried out using a JASCO J-700 spectro-polarimeter with 2 mm light path cuvettes at 22°C. Spectra were baseline corrected and corrected for dilution during titration with HFP, SDS, DOPG or DOPC using the spectrometer software. The mean residue molar ellipticity (Θ) at 222 nm was calculated as (modified from the equation in Ref. [17])

$$\Theta_{222} = \frac{\Theta_{OBS}}{C \cdot L \cdot N} \quad (1)$$

where Θ_{OBS} is the measured ellipticity at 222 nm, C is the molar concentration, L is the light path in mm and N is the number of amino acids in the peptide.

The amount of α-helical structure was determined using the assumption that only random coil and α-helix secondary structures were present. This gives the following equation:

$$\%\ \alpha\text{-helix} = \left(\frac{B - \Theta_\lambda}{B - A}\right) \cdot 100 \quad (2)$$

where A is mean residue molar ellipticity for a model α-helix at wavelength λ, B is the mean residue molar ellipticity for a model random coil at wavelength λ, and Θ_λ is the mean residue molar ellipticity of the sample at wavelength λ. Our calculations were performed using the ellipticity at 222 nm where the random coil contribution to ellipticity is small (3900 deg cm^2 dmol^{-1}) and where the α-helical contribution to ellipticity has its most negative value (−38 000 deg cm^2 dmol^{-1}) [18].

3. Results

Light absorption

The extinction coefficient of rabbit motilin was determined with light absorption measurements and amino acid analysis (data not shown). The results of the amino acid analysis were in agreement with the sequence shown in Fig. 1. For rabbit motilin we found that $\epsilon_{275} = 1800\ M^{-1}\,cm^{-1}$ at 22°C, which is lower than for porcine motilin ($\epsilon_{275} = 2300\ M^{-1}\,cm^{-1}$) but higher than for tyrosine alone in solution ($\epsilon_{275} = 1450\ M^{-1}\,cm^{-1}$) [19,11].

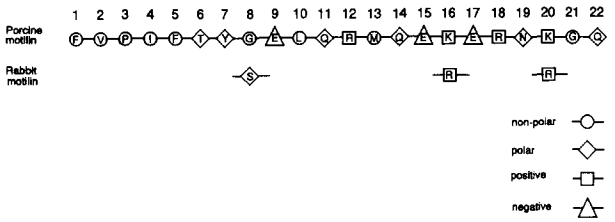

Fig. 1. The sequences of porcine and rabbit motilin. Rabbit motilin has three exchanged amino acids at residues 8, 16 and 20. Different categories of amino acids are labeled with different signs.

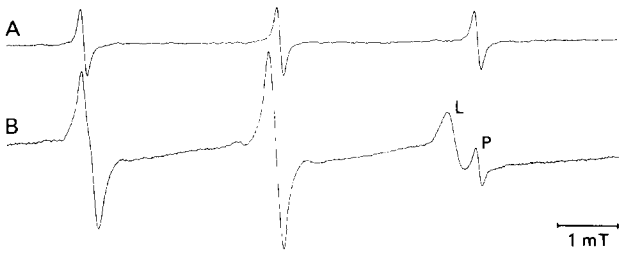

Fig. 2. (A) EPR spectrum of TEMPONE dissolved in an aqueous vesicle suspension of DOPG (15 mg/ml). (B) The corresponding spectrum (amplified 125 times) upon addition of chromium oxalate. P denotes the signal of TEMPONE solubilized in the interior volume of the vesicles, and L denotes the signal from the label solubilized in the lipid bilayer. The total magnetic field scan range was 6 mT.

Characterization of phospholipid vesicles

The EPR spectrum of spin labelled vesicles without addition of the spin broadening agent consists of three hyperfine lines (Fig. 2a). The corresponding EPR spectrum for vesicles with addition of chromium oxalate is displayed in Fig 2b. Addition of chromium oxalate results in a tremendous decrease in signal intensity due to effective spin-spin broadening between chromium oxalate ions and TEMPONE molecules distributed exterior of the vesicles. Since the hyperfine coupling constant of the spin label is affected by the polarity of the medium, the high-field line is split into two due to a distribution of TEMPONE between the interior of the vesicles and the lipid bilayer. The line at the highest field (denoted by P in Fig. 2b) arises from spin labels located in the aqueous phase inside the vesicles and the other line (denoted L in Fig. 2b) is due to those solubilized in the lipid bilayer. The intravesicular volume, V_{in} (μl/mg lipid) can be calculated from

$$V_{in} = C^{-1} \cdot 10^3 \cdot \left[I'_p \cdot I_p^{-1} \right] \quad (3)$$

where C is the concentration of the lipid (mg cm^{-3}), I'_p, is the normalized intensity of the high-field line of the quenched system (i.e., vesicles with addition of chromium oxalate) and I_p is the normalized intensity of the high-field line in the unquenched system [13].

From these measurements it was found that DOPC and DOPG vesicles prepared by sonication were characterized by a radius of 120 Å and 110 Å, respectively. The pH of the solutions were measured to be about 6. DOPC is a zwitterion at neutral pH. DOPG has a

Table 1
Mean residue molar ellipticity (deg cm^2 dmol^{-1}) at 222 nm and the amount of α-helical structure for porcine, rabbit and 1–16 motilin in different solvents (SDS, DOPG, DOPC, HFP) at room temperature

Solvent		Porcine motilin		Rabbit motilin		1–16 motilin	
		Θ_{222}	% α-helix	Θ_{222}	% α-helix	Θ_{222}	% α-helix
Pure water	0	−6800	26	−8200	29	−1900	14
SDS (mM)	1	−12400	41	−14700	48	−3000	16
	2	−13300	41	−14700	44	−3700	18
	5	−13100	40	−13200	41	−3600	18
	10	−11300	36	−12000	38	−3500	18
DOPG (mg/ml)	0.05	−15200	46	−16400	48	−5200	22
	0.1	−17000	50	−15700	47	−6000	24
	0.2	−16100	48	−15900	47	−5000	21
	0.5	−16600	49	−15600	46	−4900	21
	1.0	−15900	47	−15000	45	−4900	21
	2.0	−16500	49	−15900	47	−4600	20
DOPC (mg/ml)	0.05	−8200	29	−8200	29	−1600	13
	0.1	−7600	28	−8800	30	−1400	13
	0.2	−8000	28	−8500	30	−1600	13
	0.5	−9200	31	−8300	29	−1500	13
	1.0	−8700	30	−9600	32	−1800	14
	2.0	−8400	29	−9000	31	−2200	15
HFP	30%	−14400	44	−15900	47		–

The motilin concentration was $1.9 \cdot 10^{-5}$ M. The uncertainty in each value of Θ_{222} is ±10% estimated from repeated measurements.

reported pK_a of 2.9 [20]. Therefore we expect the DOPG vesicles to carry a net negative charge under the experimental conditions used.

CD studies on motilin with different solvent systems

Motilin in water ($1.9 \cdot 10^{-5}$ M) was titrated with the negatively charged vesicles (DOPG) and monitored by CD. The results are shown in Fig. 3. Already with the first addition of DOPG the CD spectrum changes in shape and the mean residue molar ellipticity at 222 nm decreases. After the second addition of DOPG ($1.3 \cdot 10^{-4}$ M) the CD spectrum did not change any further. The amount of α-helical structure in motilin was estimated by using equation 1 and 2 as described in Materials and methods. The results are shown in Table 1 together with the mean residue molar ellipticity for porcine and rabbit motilin in different solvents. The results for both motilins show that the amount of α-helical structure increases from about 25–30% to about 50% when DOPG is added. As a comparison, a 1–16 fragment of porcine motilin was also investigated. The corresponding increase in α-helical content induced by DOPG for the 1–16 fragment is from about 14% to about 20% (Table 1).

The quantitative aspects of the interaction between motilin and the phospholipid vesicles shows that the structuring effect of the interaction is complete when the motilin/DOPG ratio is $1.9 \cdot 10^{-5}/1.3 \cdot 10^{-4} = 1/7$. An average DOPG vesicle has a radius of 110 Å and surface area of $15.2 \cdot 10^4$ Å^2. The surface area of a phospholipid headgroup is 70 Å^2 [21]. The outer surface of the vesicle therefore contains 2200 DOPG molecules. Assuming a bilayer thickness of 38 Å [22] a simple geometric calculation shows that each vesicle contains about 930 DOPG molecules on the inside. The ratio of DOPG molecules on the inside and outside of the vesicle is therefore 1/2.4. Given the ratio of motilin/DOPG of 1/7, each motilin molecule has about 5 DOPG partners, with a total surface area of about 350 Å^2 to interact with. The dimensions of the motilin molecule determined by NMR shows that the largest dimension is about 35 Å and the smallest about 10–20 Å [10]. Obviously the structuring interaction of motilin with the phospholipid vesicles under these conditions suggests a very crowded membrane surface. Comparing CD spectra of motilin in different solvents (water, SDS, DOPG, DOPC and HFP) (Fig. 4) shows that the spectrum of motilin in water and in DOPC have almost the same shapes, whereas the shape changes and the mean residue molar ellipticity at 222 nm decreases in SDS, DOPG and HFP. The amount of α-helical structure in all solvent systems under investigation is presented in Table 1. Comparing the different results for pure aqueous solution in the different titration series, we estimate an overall accuracy better than $\pm 10\%$ in each CD measurement. The present values of about $-14\,000$ to $-18\,000$ (deg cm^2 dmol^{-1}) in 30% HFP (Tables 1 and 2) compare reasonably well with the one previously reported for porcine motilin at room temperature ($-18\,000$ deg cm^2 dmol^{-1}) [9]. Our results show that motilin forms slightly more α-helical structure in DOPG (48%) than in SDS (40%) and that the amount of α-helical structure (44–52%) in 30% HFP is similar to that in DOPG. In these experiments the concentration of SDS was 10 mM, well above the

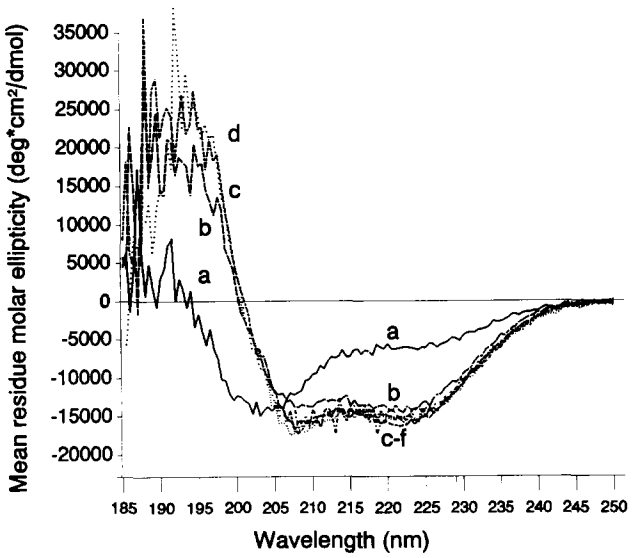

Fig. 3. Circular dichroism spectra of motilin at a concentration of $1.9 \cdot 10^{-5}$ M, titrated with DOPG vesicles at 22°C and pH of about 6. The concentration of the vesicles was gradually varied from 0 to 2 mg/ml (a to f). The latter corresponds to a molar concentration of 1.1 μM of vesicles with an average radius of 100 Å.

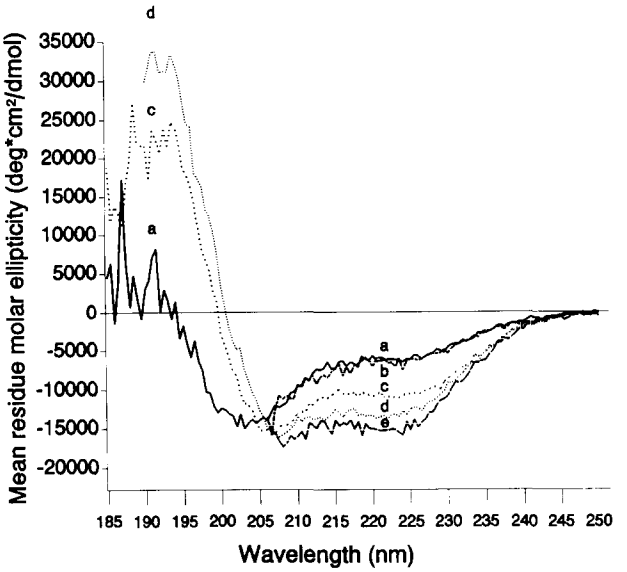

Fig. 4. Circular dichroism spectra of motilin, $1.9 \cdot 10^{-5}$ M, in water (a), DOPG (e), DOPC (b), SDS (c) and 30% HFP (d). The DOPG and DOPC concentrations were 0.5 mg/ml and the SDS concentration was 10 mM. The temperature was 22°C.

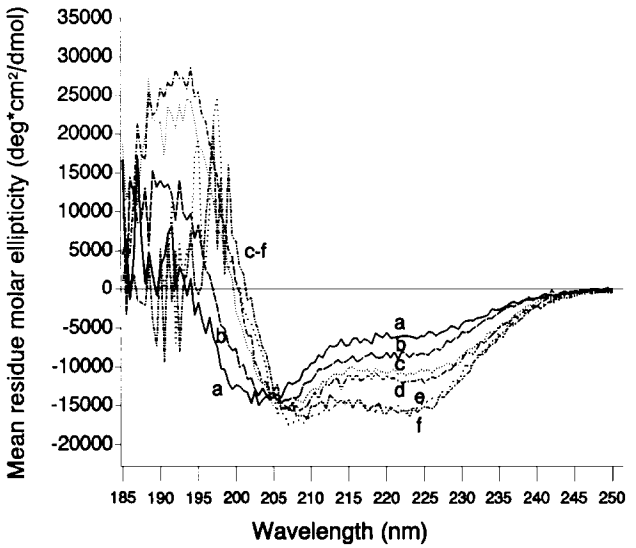

Fig. 5. Circular dichroism spectra of porcine and rabbit motilin, $1.9 \cdot 10^{-5}$ M and $2.0 \cdot 10^{-5}$ M, in water (a and b), DOPG (e and f) and SDS (c and d). The DOPG concentration was 0.5 mg/ml and the SDS concentration was 10 mM.

critical micellar concentration, 8.2 mM, [23] and corresponding to a molar ratio motilin/SDS of 1/526. Similar results were, however, also observed with SDS concentration as low as 1.0 mM, below the critical micellar concentration.

In Fig. 5 the CD spectra of porcine and rabbit motilin in different solvents are compared. These CD spectra show that both peptides react in the same way in SDS, DOPG and HFP, whereas they are somewhat different in pure water. Calculating the amount of α-helical structure (Tables 1 and 2) shows that rabbit motilin contains about 30% α-helical structure while porcine motilin contains about 25% α-helical structure in water. In the solvents containing SDS, DOPG or HFP the amount of α-helical structure is approximately the same in the two species of motilin. Measurements on porcine and rabbit motilin were also performed at different temperatures, with and without HFP. These studies show that porcine and rabbit motilin change in about the same way, in both solvents (Table 2).

Interaction between motilin and spin labeled phospholipid vesicles studied by EPR

To investigate the interaction between motilin and the DOPG vesicles, further spin labelling experiments were performed. In these experiments 5-doxylstearic acid (5-NS) was used as spin label. The nitroxide moiety of the label is attached to the fifth carbon atom of the backbone in the stearic acid molecule. At acid pH it is known that this label is located close to but not at the lipid/water interface. In an early investigation [24] the interaction between the peptide melittin and

Table 2
Mean residue molar ellipticity at 222 nm and the amount of α-helical structure for porcine and rabbit motilin in 20 mM acetic acid with and without 30% HFP at different temperatures

Motilin from:	Solvent	Temp. (°C)	Θ_{222} (deg cm^2 dmol)$^{-1}$	% α-helix
Pig	20 mM HAc	3	−7500	27
		23	−4900	21
		60	−3500	18
	20 mM HAc	3	−21100	60
	and 30%	23	17800	52
	HFP	60	−12800	40
Rabbit	20 mM HAc	3	−9000	31
		23	−6500	25
		60	−3800	18
	20 mM HAc	3	−18300	53
	and 30%	23	−15900	47
	HFP	60	−11700	37

The motilin concentration was $1.9 \cdot 10^{-5}$ M. The uncertainty in each value of Θ_{222} is ±10% estimated from repeated measurements.

model membranes containing 5-NS was studied. It was found that the interaction between the protein and the membrane significantly disordered the multibilayers and restricted the mobility of the probe molecule. In a recent investigation [25] one of us has found that the ordering as well as the dynamic properties of the 5-NS label dissolved in DOPC vesicles were drastically changed when the water medium was replaced with mixtures of glycerol and water. The reason for this change is likely to be due to changes in the packing properties of the lipids in the bilayer of the vesicles due to strong interaction between the lipid and the glycerol molecules. Hence if motilin would interact strongly with the lipidic bilayer of the DOPG vesicles a drastic change of the lineshape of the inserted spin label should be observed.

Fig. 6 shows the EPR spectra from DOPG vesicles spin labeled with 5-NS in the absence as well as the presence of motilin with a ratio of motilin/phospholipid about 1/7 (cf. the CD studies described above). We could not observe any significant differences between the two spectra. This indicates first of all that the vesicles are intact and secondly that motilin does

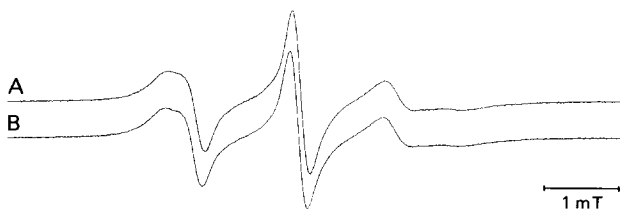

Fig. 6 (A) EPR spectrum of 5-NS solubilized in DOPG vesicles with addition of motilin at a motilin/DOPG ratio of 1/7. (B) EPR spectrum of 5-NS solubilized in DOPG without any motilin present. The temperature was 318 K. The total magnetic field scan range was 10 mT.

not change the packing properties of the lipid double layer of DOPG vesicles upon interaction under the present conditions.

4. Discussion

The careful characterization of the two types of phospholipids prepared by sonication of either DOPC or DOPG showed that they do not differ significantly in terms of size, but only in charge. Both have a diameter with an average value of about 200 Å evaluated from the EPR spin label experiments. For comparison the largest dimension of the motilin molecule is about 35 Å [10]. This is an important observation for understanding the results of the next part of our studies.

These results (Figs. 3–5) show that porcine and rabbit motilins change their secondary structures when negatively charged vesicles (DOPG) or negatively charged micelles (SDS) were present, but not when neutral vesicles (DOPC) were added. Obviously it is the charge and not the size of the vesicles that are responsible for the presence and absence of interaction with DOPG and DOPC, respectively. When DOPG or SDS were added more α-helical structure was formed. In the DOPG case the structure changed dramatically already when an addition corresponding to 3 DOPG/motilin was made. When more DOPG was added the structure hardly changed any further, indicating that motilin has reached its most stable structure, at a ratio of motilin/DOPG of 1/7. As was commented on in Results, the ordering effect needs a very small surface area (350 $Å^2$) per motilin molecule. A similar secondary structure of motilin was stabilized by SDS at a ratio of motilin/SDS of about 1/50. Somewhat more α-helical structure was stabilized by DOPG than by SDS.

Addition of HFP also induces a similar change in the secondary structure of motilin. The amount of α-helical structure reaches almost the same values as with DOPG. These observations suggest that the earlier NMR measurements on motilin, made in 30% HFP, probably gave a peptide solution structure which should be relevant for a phospholipid environment, as in a biomembrane.

Only the negatively charged vesicles and micelles interact with and change the secondary structure of motilin. Although the motilin molecule itself is neutral under the conditions of this investigation, its C-terminal half has several positively and negatively charged residues. These are part of the α-helix that was found between residues 9–20 in the NMR structure. Considering the charge distribution (Fig. 1) among residues 9–20 we observe that the three positively charged residues occur a multiple of 4 residues apart. It is a reasonable assumption that the DOPG induced α-helix, which should have a pitch of about 3.5 amino acids/turn, turns one side carrying an excess of positive charge towards the membrane. Since motilin forms amphiphilic structures in negatively charged amphiphilic membranes, it is a typical example of the third category of the peptide hormones described in Introduction.

The results show that although the structure inducing effect on motilin by the DOPG vesicles is strong, the reciprocal effect of motilin on the vesicles is not observable, when monitored by 5-doxylstearic acid spin labels close to the lipid/water interface. This suggests that the interaction involves mainly electrostatic effects at the surface of the vesicle.

Porcine and rabbit motilin differ in amino acid composition only at 3 residues. Our results show that there is only a small difference between the CD spectra from porcine and rabbit motilin in water, due to the amino acid composition. Upon titration with SDS, DOPG, DOPC and HFP both porcine and rabbit motilin react similarly. Temperature dependence measurements on porcine and rabbit motilin showed that they also react similarly with temperature. Therefore the structural difference between the two peptides should be considered as negligible.

The induction of α-helix in motilin by phospholipid membranes may play an important role in the interaction between the hormone and its membrane-bound receptor. Indeed fragments of motilin containing the complete pharmacophore but lacking the α-helix, have a biological activity about 10-fold less than that of the full length molecule [26]. Therefore it is possible that the α-helix also interacts with the N-terminal part of the molecule, stabilizing its bioactive conformation. The 1–16 fragment may represent an intermediate case. The moderate α-helix induction by DOPG from about 14% to 20% (Table 1) again shows the importance of the C-terminal for the α-helical secondary structure. In agreement with this, we found that for a small peptide corresponding to the N terminal (1–5) by itself, there was no evidence of any secondary structure significantly deviating from a random one, even in the mixed solvent, SDS or phospholipid vesicle systems (data not shown).

In conclusion, the results presented here provide a direct link between the threedimensional solution structure of motilin determined by NMR in a mixed aqueous/fluorinated alcohol solvent, and the structure induced by negatively charged phospholipid vesicles, mimicking a biological membrane. This finding strongly suggests that the NMR solution structure may be relevant for the environment in which the membrane-bound receptor recognizes the hormone. The results also suggest that well defined negatively charged phospholipid vesicles (as well as detergents) may be useful model

systems for various spectroscopic studies of membrane active peptides (often not even soluble in an aqueous solvent), and that the amphiphilic environment may also to some extent be mimicked by a certain fraction of fluorinated alcohols in the aqueous solvent.

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6. References

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