Conformational changes upon binding of a receptor loop to lipid structures: possible role in signal transduction

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Abstract The *mas* oncogene codes for a seven transmembrane helix protein. The amino acid sequence 253–266, from the third extracellular loop and beginning of helix 7, was synthesized either blocked or carrying an amino acid spin label at the N-terminus. Peptide binding to bilayers and micelles was monitored by ESR, fluorescence and circular dichroism. Binding induced tighter lipid packing, and caused an increase of peptide secondary structure. While binding to bilayers occurred only when peptide and phospholipid bore opposite charges, in micelles the interaction took place irrespective of charge. The results suggest that changes in lipid packing could modulate conformational changes in receptor loops related to the triggering of signal transduction.

Key words: Receptor; Loop; Peptide–lipid interaction; Signal transduction; Spectroscopic technique

1. Introduction

A large amount of work has appeared in recent years focusing on functional and structural properties of seven transmembrane helix receptors. Physiological roles have been assigned both to transmembrane portions and to extra- and intra-cellular water-exposed loops [1–4].

Physical techniques have been largely employed in the study of topological and conformational features of membrane proteins and of model peptides containing parts of their sequence [5–7]. While a lot of attention has been devoted to transmembrane helix segments, and to their interaction with model membranes [8–10], few reports have aimed at the investigation of loop sequences [6,11].

Here we describe studies undertaken with a tetradecapeptide containing amino acids 253–266 (EYWSTFGNLHHISL) from the protein expressed by the *mas* oncogene [12]. This orphan receptor was found to behave like a G protein-coupled receptor when stimulated by the peptide hormone angiotensin II [13]. Sequence analysis from a hydrophobicity plot by the Kyte and Doolittle method [14] shows that the protein presents seven transmembrane helices and that amino acids 253–266 encompass part of the extracellular loop 3 and the beginning of helix 7.

The peptide was synthesized containing either an *N*-acetyl blocking group or an amino acid spin label (2,2,6,6-tetramethylpiperidine-*N*-oxyl-4-amino-4-carboxylic acid, TOAC [15,16]) bound to Glu^1 by a peptide bond [15,16].

Spectroscopic techniques (spin labeling electron spin resonance (ESR), circular dichroism (CD), and fluorescence) were employed to examine conformational properties of the peptides in solution and upon their interaction with micellar detergents and phospholipid bilayers. ESR spectra of lipid spin probes also provided information on changes in the lipid environment as a consequence of peptide–lipid interaction.

2. Materials and methods

2.1. Chemicals

Dimyristoyl phosphatidylcholine (DMPC), dimyristoyl phosphatidylglycerol (DMPG), sodium dodecyl sulfate (SDS), polyoxyethylene 23 lauryl ether (Brij-35), *N*-hexadecyl-*N*,*N*-dimethyl-3-ammonium-1-propanesulfonate (HPS), and the spin label 5-doxylstearic acid methyl ester (5-MeSL) were obtained from Sigma Chemical Co. Hexadecyltrimethylammonium bromide and chloride (CTAB and CTAC) came from Aldrich Chemical Co., palmitoyl-oleoyl phosphatidylglycerol (POPG), palmitoyl-oleoyl phosphatidylcholine (POPC), and the spin label 1-palmitoyl-2-stearoyl-(5-doxyl)-*sn*-glycero-3-phosphocholine (5-PCSL) from Avanti Polar Lipids, Inc. All other reagents were of analytical grade.

2.2. Peptide synthesis, spin labeling, and purification

The *N*-acetylated (Ac-14) and the spin labeled (TOAC-14) tetradecapeptides were synthesized by the Merrifield solid-phase method [17,18] using N-terminal *tert*-butyloxycarbonyl (*t*-Boc)-protected amino acids on a Biosearch automatic synthesizer. The stable free radical amino acid 2,2,6,6-tetramethylpiperidine-*N*-oxyl-4-amino-4-carboxylic acid (TOAC) was synthesized and coupled to the peptide-resin at the N-terminal as described by Nakaie et al. [15,16]. The peptides were deprotected and cleaved from the resin by treatment with anhydrous HF. Purification was performed by reverse-phase HPLC on a Vydac C_{18} column eluted with H_2O/0.1% TFA-acetonitrile gradient. The peptide concentration was calculated spectrophotometrically using $\varepsilon_{Y280} + \varepsilon_{W280} = 7565\ M^{-1} \cdot cm^{-1}$.

2.3. Sample preparation

Lipid multibilayers were prepared by evaporating stock chloroform solutions of the phospholipids under a stream of N_2. For ESR studies of the lipid environment a stock chloroform solution of 5-PCSL was added in order to obtain 1% (mol/mol) of the phospholipid. The samples were dried under vacuum for 2 h. Multilamellar liposomes were obtained upon addition of water or peptide solution and vortexed; the pH was adjusted with 0.1 N HCl or NaOH. For CD studies, small unilamellar vesicles (SUV) were prepared by sonication of multilamellar dispersions with a titanium microtip at 0°C, under nitrogen, for 75 min, in a 1 min on, 1.5 min off cycle, at a nominal power of 75 W. The peptide was added to already prepared SUV.

Stock solutions of the detergents were prepared in deionized water. For ESR measurements incorporation of the spin probe was done by adding the detergent solution to a film prepared from a stock chloro-

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form solution of 5-MeSL to obtain a 1:100 spin label:detergent molar ratio.

2.4. Equipment

ESR spectra were obtained on a Bruker ER 200D-SRC, X band spectrometer, using flat quartz cells for aqueous solution manufactured by James Scanlon, Costa Mesa, California. Temperature control was performed with a Bruker B-ST 100/700 variable temperature device.

CD spectra were recorded on Jasco J-720 and CD6 Jobin Yvon spectropolarimeters. Spectra were baseline corrected by using control samples of similarly prepared solutions. The ellipticity (θ) is reported as mean residue molar ellipticity.

Steady-state fluorescence spectra were carried out on a Hitachi model 3100 spectrofluorimeter, with 1 cm path length quartz cells using the excitation wavelength at 295 nm.

3. Results

3.1. ESR studies – spin labeled peptide

In aqueous solution the spin labeled peptide (TOAC-14) yielded ESR spectra indicative of motional restriction due to binding of the TOAC moiety to the peptide backbone. The rotational correlation time (τ_C) [19] increased from 0.32×10^{-10} s for TOAC to 2.72×10^{-10} s when bound to the peptide at pH 3.5 and room temperature.

The ESR spectra of TOAC-14 are sensitive to binding to detergents and to bilayers. Fig. 1 displays spectra in aqueous media and in the presence of SDS and DMPG at pH 3.5. While binding to micelles was found to occur irrespective of detergent

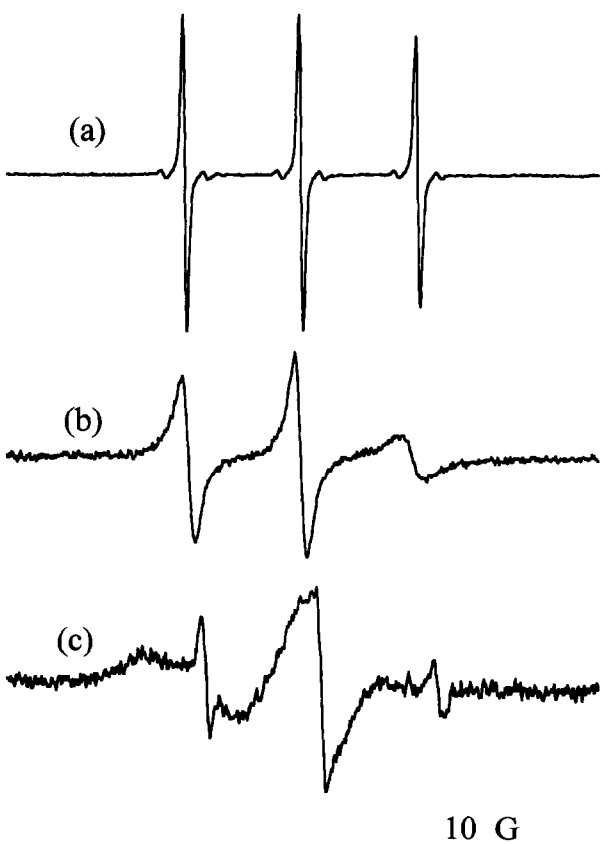

Fig. 1. ESR spectra at pH 3.5 of 5×10^{-5} M TOAC-14 in aqueous solution at 22°C (a) and in the presence of 0.1 M SDS micelles at 22°C (b) and of 0.074 M DMPG liposomes at 45°C (c).

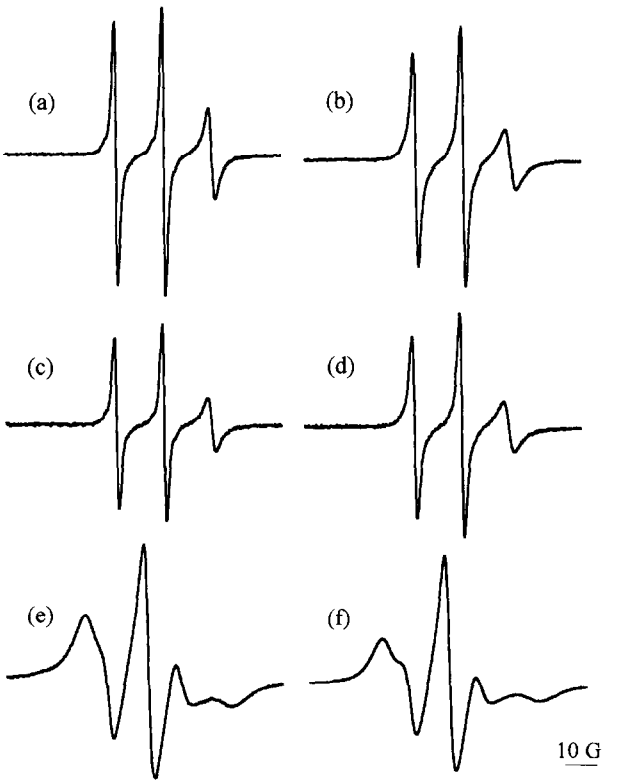

Fig. 2. ESR spectra of 5-MeSL in 0.01 M SDS micelles (a,b,c,d) and of 5-PCSL in 0.01 M DMPG multibilayers (e,f), in the absence (a,c,e) and in the presence (b,d,f) of 5×10^{-4} M Ac-14 at pH 3.5 (a,b,e,f) and 7.5 (c,d). The temperature was 22°C in a,b,c,d, and 45°C in e,f.

head group and peptide charge, no interaction took place with bilayers unless peptide and phospholipid bore opposite charges. Thus, TOAC-14 bound to SDS, CTAB, HPS and Brij-35 micelles both at pH 3.5 and 7.5. In contrast, the spin labeled peptide was found to interact with DMPG only at low pH, both in the gel and in the liquid crystalline phase. Little or no binding to DMPG was detected above pH 5.5 or to DMPC in the pH range 2.0 to 12.0, between 5°C and 50°C.

3.2. ESR studies – spin labeled micelles and bilayers

Changes in spectra of micelle or bilayer-incorporated lipid spin probes, indicative of peptide binding, followed the same pattern found for the labeled peptide, i.e. a decreased degree of motion was observed for the incorporated spin probe 5-MeSL in the presence of Ac-14 in all detergent systems both at low (3.5) and high (7.5) pH, whereas an increased degree of order was measured in the spectra of 5-PCSL only in DMPG bilayers at low pH, in the liquid crystalline phase. Again the spectral changes were negligible for DMPG and DMPC at pH 7.5, as well as for DMPC at pH 3.5. Fig. 2 illustrates the effect of Ac-14 on the molecular organization of SDS micelles (pH 3.5 and 7.5) and DMPG bilayers at pH 3.5, 45°C. Line broadening resulting from slower motion is seen in all spectra of Ac-14-containing micelles. In DMPG bilayers, the order parameter S, calculated from the outer and inner extrema (with polarity correction) in the spectra of 5-PCSL [20] increased from 0.53 to 0.60 upon addition of 0.5 mM Ac-14.

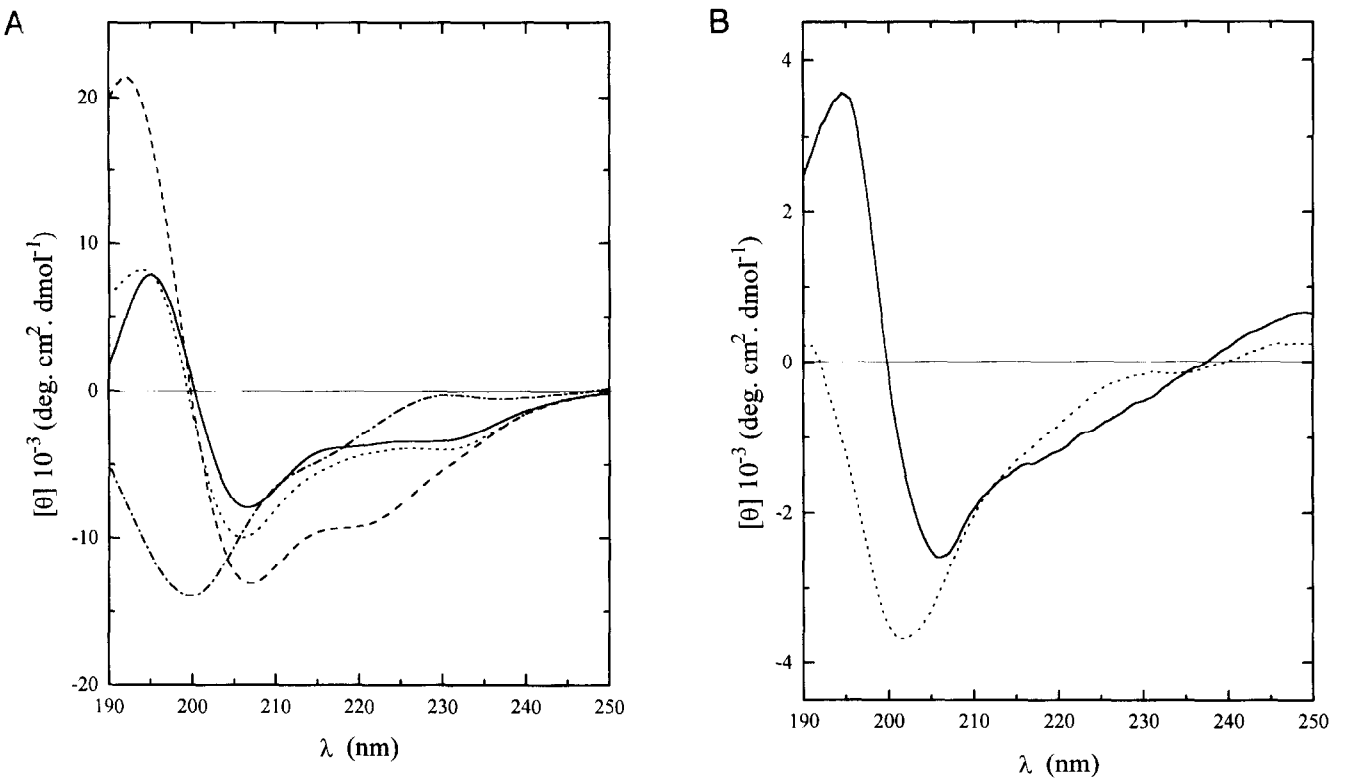

Fig. 3. CD spectra of 1×10^{-4} M Ac-14. (A) in aqueous phase, pH 3.5 (-·-·-), in TFE (----), and in the presence of 0.1 M SDS, pH 3.5 (—) and 7.5 (···). (B) in the presence of 2.8×10^{-3} M POPG, pH 3.5 (—) and 7.5 (···).

3.3. CD studies

Fig. 3 displays the CD spectra of Ac-14 in aqueous solution, in an α-helix-inducing solvent (TFE), in the presence of micellar SDS and of POPG SUV. The spectra clearly show that the peptide tends to acquire secondary structure when moving from aqueous phase to the other media. Once again, the behavior was parallel to that seen in the previous studies, namely, the increase in secondary structure was independent of peptide and micellar surface charge, being observed for all detergents at pH 3.5 and 7.5, and was only detected when the peptide and POPG carried positive and negative charges, respectively.

Analysis of the CD spectra according to Perczel et al. [21] showed that the α-helical content of Ac-14 increased from 0% in aqueous solution to ca. 14.8% in SDS and ca. 10.2% in HPS micelles (averages of values for pHs 3.5 and 7.5).

No attempt was done to analyze the spectra obtained for phospholipid systems due to possible artifacts [22,23]. Visual inspection suggests that the spectrum obtained for Ac-14 bound to POPG (Fig. 3B) differs from those in the presence of micelles (Fig. 3A).

Table 1
λ_{max} for Ac-14 in various media[a]

Medium	λ_{max} (nm)	
	pH 3.5	pH 7.5
H_2O	353.0	354.0
SDS	337.0	338.0
CTAC	339.5	242.5
HPS	339.5	341.0
MeOH	339.8	
TFE	341.0	

[a] concentrations (M): Ac-14 1×10^{-5}, SDS 0.1, CTAC 0.09, and HPS 0.04.

3.4. Fluorescence studies

The Ac-14 tryptophan intrinsic fluorescence was also used to monitor peptide binding. The fluorescence intensity increased and the wavelength of maximum emission (λ_{max}) decreased in the presence of micellar detergents, indicating that the peptide experiences an environment of lower polarity. Table 1 displays λ_{max} for Ac-14 in the various media studied. The data corroborate the results found in the previous sections. It is seen that λ_{max} reflects a polarity similar to that of methanol and TFE, suggesting that the tryptophan resides at the polar head group-water interface.

4. Discussion

The present results provide clear evidence that the peptide under study, containing amino acids 253–266 of the *mas* oncogene receptor, is capable of interacting with lipid structures. In doing so, conformational changes are detected both at the peptide and at the lipid level. The ESR spectra of lipid spin probes indicate that the lipid environment becomes more tightly packed as a result of peptide binding (Fig. 2).

The maximum wavelength of tryptophan fluorescence suggests that the polarity experienced by the peptide in micelles corresponds to the water-micelle interface (Table 1).

An increase in peptide secondary structure upon binding to micelles and POPG bilayers (pH 3.5) was evidenced by CD spectra (Fig. 3). Analysis of the spectra in the presence of

micelles revealed an increase in α-helical content. The fluorescence and CD data are in agreement with the predicted conformation for the whole receptor [12], where amino acids 253–266 are located in the third extracellular loop and at the beginning of transmembrane helix 7. These data suggest that the tetradecapeptide is capable of displaying a behavior similar to the one it has in the whole protein.

Whereas binding to lipid bilayers is clearly modulated by electrostatic interaction, this is not a requirement in less tightly packed micellar aggregates. The fact that binding occurs even when peptide and detergent bear the same charge clearly points to the fact that other forces (hydrophobic interaction, hydrogen bonding) contribute to binding.

Differences in binding ability as a function of lipid packing have been shown to occur when a signal sequence peptide was exposed to monolayers at different surface pressure: at high pressure the peptide was found to be essentially in a β-sheet conformation at the water-monolayer interface; a predominantly α-helical conformation was seen at lower pressure, when the peptide had the possibility of being inserted in the less tightly packed monolayer [24]. Seelig [25] showed that in bilayers and micelles the surface pressure is equivalent to 32 and 10 N/cm, respectively. Therefore, the different behavior of the peptide under study towards bilayers and micelles is analogous to that observed for the signal sequence in monolayers at different surface pressure.

The lipid environment has been shown to affect the properties of membrane bound receptors such as rhodopsin [26], the nicotinic acetylcholine receptor [27], the β-adrenergic receptor [28], and the insulin receptor in a variety of ways [29–32]. In particular, curvature has been implied in receptor function [26,29,33]. A conformational change, presumably required for signal transduction, was observed for the insulin receptor upon binding of the hormone [34,35]. Ligand-induced receptor conformational changes are probably involved in triggering the signal transduction process in the case of G protein-coupled receptors [36].

Changes in lipid packing (caused by ligand interaction with lipid and/or with protein) could play a role in inducing and propagating conformational changes at receptor loops that would modulate loop-surface, loop-loop, and loop-helix interactions leading to the G protein-coupling receptor conformation.

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