J Membrane Biol (2008) 223:49–57
DOI 10.1007/s00232-008-9112-x

The Full-Length Mu-Opioid Receptor: A Conformational Study by Circular Dichroism in Trifluoroethanol and Membrane-Mimetic Environments

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Received: 6 December 2007 / Accepted: 16 May 2008 / Published online: 24 June 2008
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Abstract The secondary structure content of the recombinant human mu-opioid receptor (HuMOR) solubilized in trifluoroethanol (TFE) and in detergent micelles was investigated by circular dichroism. In both conditions, this G protein–coupled receptor adopts a characteristic α-helical structure, with minima at 208 and 222 nm as observed in the circular dichroism spectra. After deconvolution of spectra, the α-helix contents were estimated to be in the range of 50% in TFE and in sodium dodecyl sulfate at pH 6. These values are in accordance with the predicted secondary structure content determined for the mu-opioid receptor. A pH-dependent effect was observed on the secondary structure of the receptor solubilized in detergents, which demonstrates the essential role of ionic and hydrophobic interactions on the secondary structure. Circular dichroism spectra of EGFP–HuMOR, a fusion protein between the enhanced green fluorescent protein (EGFP) and the mu-opioid receptor, and EGFP solubilized in TFE were also analyzed as part of this study.

Keywords G protein–coupled receptor · Mu-opioid receptor · Circular dichroism · Detergent · Trifluoroethanol · Folding

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Introduction

The mu-opioid receptor belongs to the G protein–coupled receptor (GPCR) superfamily, which constitutes the largest and the most diverse class of transmembrane proteins. Human genome sequencing revealed that ~30% of the genes code for membrane proteins (Stevens and Arkin 2000), and 15% of them have been classified as GPCRs (Zhang et al. 2006). The common structural features shared by all members of the GPCR family include an extracellular N-terminal domain, seven membrane-spanning domains connected by loops and a cytoplasmic C-terminal tail responsible for the interaction with G proteins and other intracellular signaling proteins. It is estimated that 30% of the clinically marketed drugs are modulators of GPCR function. Moreover, these drugs have therapeutic benefits on a broad spectrum of human pathologies including cardiovascular and gastrointestinal diseases, central nervous system and immune disorders, cancer and pain (Schlyer and Horuk 2006; Tyndall and Sandilya 2005). Pain-relieving and euphorigenic feelings are the two main effects resulting from opioid system activation. Opiate receptor activation occurs as a natural response to injury resulting from the increased release of their cognate ligands in response to injury, infection, trauma and surgery (Molina 2006). At the molecular level, ligand binding on opiate receptors results in inhibition of adenyl cyclase through inhibitory G proteins ($G_{i/o}$), a decrease of intracellular cyclic adenosine monophosphate (cAMP) levels and a decrease of protein kinase A activity. The human mu-opioid receptor (HuMOR) is the unique receptor for the alkaloid drug extracted from opium poppy, namely, morphine, and for its diacetylated form, heroin, and is therefore responsible for drug addiction (Matthes et al. 1996). Despite the fundamental biological and therapeutic

implications of GPCRs, data on their three-dimensional (3-D) structure and their ligands in interaction are scarce, while this information is essential for understanding their functions at the molecular level. Unfortunately, GPCR structures, like those of other integral membrane proteins, are extremely difficult to determine experimentally for numerous reasons. As membrane proteins, GPCRs are not easy to express and purify, and the two major techniques that provide atomic resolution 3-D structural information, namely, nuclear magnetic resonance (NMR) and X-ray crystallography, require large amounts of purified proteins. At this time, only two GPCR atomic resolution structures have been determined: bovine rhodopsin, which is naturally available nearly pure and in high quantities in retina (Palczewski et al. 2000), and β_2-adrenergic receptor, the structure of which has recently been solved after 20 years of constant determination (Cherezov et al. 2007; Rasmussen et al. 2007). Contrary to rhodopsin, GPCRs are naturally weakly expressed and their direct purification from natural sources is not compatible with the requirements of structural biology experiments. As highlighted by Kobilka's group (Cherezov et al. 2007; Rasmussen et al. 2007), structural information on GPCRs is not easy to obtain and heterologous overexpression is essential (Sarramegna et al. 2003). The utilization of peptide domains solubilized in detergents and organic solvents has been an alternative approach to obtain structural information on GPCRs (Arevalo et al. 2003; Choi et al. 2005; Choi et al. 2002; Ding et al. 2002; Kerman and Ananthanarayanan 2005, 2007; Lazarova et al. 2004; Thevenin et al. 2005; Xie et al. 2004). In this context, a circular dichroism (CD) analysis has been realized on a fragment of the mu-opioid receptor (TM2-3) (Kerman and Ananthanarayanan 2005, 2007), which comprises the second and third transmembrane segments and the extracellular loop that connects them. This study, which describes the effect of a hydrophobic environment and of pH on peptide conformation, was realized on a short fragment of the receptor. We were able to produce and purify milligram amounts of the full-length mu-opioid receptor (alone and in fusion with enhanced green fluorescent protein, EGFP) (Sarramegna et al. 2005). We report here the CD characterization of this receptor solubilized in trifluoroethanol (TFE) and in detergents and the effect of pH on its conformation.

Materials and Methods

Plasmids, Strains and Expression

Plasmid constructs used for the overexpression of EGFP–HuMOR-cmyc-6his and HuMOR-cmyc-6his proteins, *Pichia pastoris* strain, and the conditions employed for receptor expression were as described (Sarramegna et al. 2002a, 2002b, 2005).

Preparation of EGFP–HuMOR and HuMOR Enriched Fractions

Crude extracts were prepared at 4°C according to Sarramegna et al. (2005). Briefly, after induction of expression with 0.5% (v/v) methanol, yeast cells expressing either EGFP–HuMOR or HuMOR proteins were harvested and broken for 30 min with glass beads in a breaking buffer (10 mM Tris–HCl, pH 7.5) supplemented with an anti-protease cocktail. The cell lysate was centrifuged at 1,000 × *g* for 15 min to remove unbroken cells and particulate matter. The supernatant was then centrifuged at 10,000 × *g* for 30 min to harvest a crude fraction named P10K. The resulting pellets were stored at −80°C in the breakage buffer.

Solubilization, Purification and Detection of Receptors

The EGFP–HuMOR and HuMOR proteins were solubilized from the centrifugation pellets of P10K fractions and purification was performed on Ni-chelated Sepharose columns (Amersham Bioscience, Uppsala, Sweden). Crude extracts were diluted in a solubilization buffer [SB, 100 mM NaH_2PO_4, 10 mM Tris–HCl, 20 mM β-mercaptoethanol, 8 m urea, 0.1% sodium dodecyl sulfate (SDS; Anatrace, Maumee, OH), pH 8]. The solubilization was allowed to proceed for 1 h at room temperature with gentle agitation on a rotating wheel. Solubilized receptors were then incubated for 1 h at room temperature with chelating Sepharose (1–2 ml, Amersham Bioscience) charged with 0.3 M Ni acetate. The resin was then poured in an 8-ml plastic column, washed with 50 ml of SB and then with 50 ml of SB without urea and β-mercaptoethanol. Proteins bound to the resin were subsequently eluted with a stepwise imidazole gradient (25, 50, 100, 300 mM; 3 × 4 ml^2) in the elution buffer (100 mM NaH_2PO_4, 10 mM Tris–HCl, 0.1% SDS, pH 8). Exchange of SDS with other detergents was performed by washing extensively the proteins bound onto the nickel column with a buffer (100 mM NaH_2PO_4, 10 mM Tris–HCl, pH 8) containing 2 mM *n*-dodecyl-*N,N*-dimethylamine-*N*-oxide (LDAO; Anatrace, Maumee, OH) or 0.15 mM *n*-dodecyl-β-D-maltoside (DDM; Anatrace, Maumee, OH). Elution of proteins in LDAO or DDM micelles was performed using the same conditions as described above. The purification was followed by SDS-polyacrylamide gel electrophoresis (PAGE) using 10% acrylamide gels and monitored by silver nitrate staining and Western blotting as described (Sarramegna et al. 2002a, 2002b, 2005).

Circular Dichroism Experiments

CD Samples in Trifluoroethanol

Purified receptors were prepared by extended dialysis of the samples against pure water. After lyophilization, the receptors were solubilized in 100% TFE (Sigma-Aldrich, Saint-Louis, MO) and filtrated. A sample of pure recombinant EGFP (Clontech, Palo Alto, CA) was prepared in 100% TFE in parallel. Protein concentrations were determined by ultraviolet absorbance spectroscopy using an extinction coefficient of $\varepsilon_{280} = 60{,}956\ m^{-1}\ cm^{-1}$ for HuMOR and by EGFP fluorescence quantization for EGFP–HuMOR

CD Samples in Detergent Micelles

After purification, samples were concentrated and buffer was exchanged on a vivaspin 15R concentrator (Sartorius, Gottingen, Germany) against a buffer devoid of imidazole, containing the same concentration of detergent (0.1% SDS, 2 mM LDAO or 0.15 mM DDM), at different pH and where NaH_2PO_4 concentration was reduced to 10 mM. Protein concentration was determined with a bicinchoninic acid (BCA) protein assay kit (Interchim, Les Ulis, France).

CD Spectroscopy

CD spectra were recorded at room temperature using the Jobin-Yvon (Edison, NJ) Mark VI or the Jasco (Tokyo, Japan) J-815 circular dicrograph at a scan speed of 0.2 nm/s and an integration time of 1 s. Total absorbance was maintained lower than 1.0 to ensure sufficient light transmission. Corresponding blanks were realized for each assay and subtracted from the raw data. Two spectra were recorded and averaged to increase the signal-to-noise ratio. Protein concentrations were 50–300 μg/ml. The data were recorded in ΔA units (Jobin-Yvon Mark VI) or mdeg (Jasco J-815) and then converted into normalized $\Delta\varepsilon$ values on the basis of an amino acid mean residue mass of 112 Da. The CD data were analyzed with the three programs available in the CDPro software package (http://lamar.colostate.edu/~sreeram/CDPro/main.html; Sreerama and Woody 2000)—CDSSTR (Johnson 1999), ContinLL (Provencher and Glockner 1981) and Selcon3 (Sreerama et al. 1999)—using a reference set of 56 proteins including 13 membrane proteins (SMP56) (Sreerama and Woody 2004). The fractions of regular and distorted α and β structures from CDPro were combined to obtain α-helix and β-sheet fractions. Unless mentioned, the secondary structure fractions are presented as averages, with standard deviation of the results given by the three programs.

For experiments in 100% TFE, a difference spectrum between EGFP–HuMOR and EGFP was calculated to obtain the spectrum of HuMOR alone, according to the following equation:

$$\Delta\varepsilon_{\mathrm{HuMOR}}[(\Delta\varepsilon_{\mathrm{EGFP-HuMOR}}N_{\mathrm{EGFP-HuMOR}})-(\Delta\varepsilon_{\mathrm{EGFP}}N_{\mathrm{EGFP}})]/N$$

with N being the number of amino acids and $N_{\mathrm{EGFP-HuMOR}} = 586$, $N_{\mathrm{EGFP}} = 240$ and $N_{\mathrm{HuMOR}} = 346$.

Radioligand Binding Assays

Binding assays were performed, at different pH, in 0.5 ml final volume of binding buffer containing 50 mM Tris and 10 mM EDTA. Specific binding was determined with 1 nM [^{3}H]diprenorphine (Amersham, 50 Ci/mmol) and nonspecific binding in the presence of 1 μM unlabeled diprenorphine (Sigma-Aldrich, Saint-Louis, MO). Filtration, radioactivity and data analysis were as described (Sarramegna et al. 2002a).

Results and Discussion

Preparation of Recombinant Receptors

Different problems concerning the expression, solubilization and purification of GPCRs can explain the lack of structural and biophysical information on this class of membrane proteins (Sarramegna et al. 2003, 2006). In particular, the presence of seven α-helix transmembrane domains in the structure of GPCRs makes them very hydrophobic and, thus, very difficult to handle. We have circumvented a part of these difficulties as we are able to produce pure milligram amounts of the mu-opioid receptor when it is expressed in the methylotrophic yeast *P. pastoris* (Sarramegna et al. 2005). After induction of expression with methanol, an enriched mu-opioid receptor-containing fraction, designated as yeast inclusion body-like structures, was obtained by mechanical cell breakage and centrifugation. Immobilized metal affinity chromatography was performed after solubilization and purification of the samples with 0.1% SDS. The elution of the bound receptor from the nickel resin was realized with imidazole. Monomeric enriched fractions used for CD were selected after SDS-PAGE analysis of the fractions. As described in Sarramegna et al. (2005), the monomeric form of the receptor was eluted at low imidazole concentration (50 mM), whereas multimeric and/or aggregated species were detected at higher concentration.

In the case of CD spectroscopy in TFE, the samples obtained after imidazole elution from the nickel column (Sarramegna et al. 2005) were dialyzed against ultrapure water. This highly hydrophobic membrane receptor precipitated after removal of the detergent. The mu-opioid

receptor was thus recovered after centrifugation and solubilized in TFE.

In the case of CD spectroscopy in detergent micelles, the preparation of samples was realized on-column. After elution of receptors with imidazole, the solution was filtrated and concentrated. During this step, the NaH_2PO_4 concentration was reduced from 100 to 10 mM to increase spectrum quality and imidazole was eliminated since it interferes with CD spectroscopy.

CD Spectroscopy of EGFP–HuMOR in TFE

Although overexpression of HuMOR in *P. pastoris* generates, mainly, an inactive receptor, it can be used as a model to understand the principles governing the folding and stability of GPCRs and their interaction with detergents and organic solvents. In fact, searching for ligand-binding activity does not reflect the different thermodynamic pathways that lead to complete refolding. As emphasized by Kiefer (2003), a two-stage model can serve as a guideline for in vitro folding. This model, which has been proposed for the folding of helical transmembrane proteins, supposes that, first, helices fold spontaneously after being incorporated into the membrane and, second, they spontaneously assemble to form the native folded state of the membrane protein (Popot and Engelman 1990). Hence, in a lipomimetic environment such as TFE and detergent micelles, α-helices will probably be formed before the establishment of tertiary contacts. We therefore used CD to find conditions in which HuMOR exhibits a high level of secondary structures, especially a high α-helix content (Park et al. 1992; Sreerama and Woody 2004) like rhodopsin (Palczewski et al. 2000) and the β_2-adrenergic receptor (Cherezov et al. 2007; Rasmussen et al. 2007).

To realize this purpose, we first worked on an EGFP-amino-tagged mutant of HuMOR. The secondary structure content of the recombinant receptor was determined in TFE as a solubilizing reagent. TFE is a lipomimetic solvent which is known to induce and stabilize the formation of α-helices in peptides that have the propensity to form an α-helix such as transmembrane domains (Buck 1998). Moreover, TFE does not induce or impose secondary structural constraints on regions that are normally unstructured in proteins (Rigby et al. 1998). Thus, the α-helical content determined for the mu-opioid receptor in TFE should give a reference content. Therefore, comparison of the α-helical content in various detergents with the reference value in TFE should indicate the best α-helix-generating detergent in the context of folding studies. A representative CD spectrum of EGFP–HuMOR in 100% TFE is shown in Fig. 1. This spectrum shows a characteristic pattern of a structured protein with a high content of α-helical secondary structures, with minima at 208 and 222 nm. The α-helical content for the fluorescent receptor was estimated to be 48 ± 7.5% (average of results

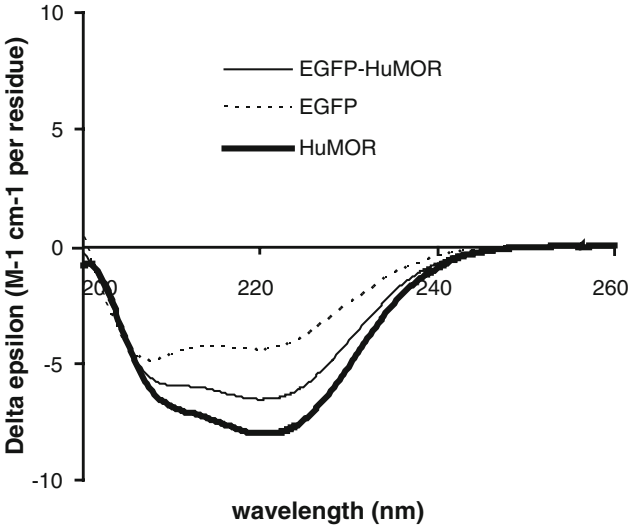

Fig. 1 CD spectra of EGFP–HuMOR and EGFP in 100% TFE and spectrum calculated from the difference between EGFP–HuMOR and EGFP (see section "Materials and Methods")

obtained with CDSSTR and CONTIL/LL programs; SELCON3 failed to give results). The contribution of EGFP was eliminated by calculating the CD difference spectrum between the EGFP–HuMOR spectrum and the pure EGFP spectrum in 100% TFE. Compared to the EGFP–HuMOR CD pattern, difference spectrum analysis presented an α-helical content of 50 ± 7.5% for HuMOR (average of results obtained with CDSSTR and CONTIL/LL programs; SELCON3 failed to give results). If we assume that EGFP and HuMOR behave independently from each other, the dichroic content of EGFP can be subtracted from that of EGFP–HuMOR to obtain the spectrum of HuMOR. Independent behavior by different domains of proteins has been considered previously by Arevalo et al. (2003) and Venyaminov and Yang (1996). This method gives an α-helical content (approximately 50 ± 7%) similar to that obtained with HuMOR alone (approximately 57.5 ± 3%, see next paragraph). Nevertheless, we cannot exclude that the secondary structure of HuMOR is affected by EGFP when the fusion protein is solubilized in TFE.

CD Spectroscopy of HuMOR in TFE

When the lyophilized mu-opioid receptor was solubilized in 100% TFE, the α-helical content was estimated to be 57.5 ± 3.3% (Fig. 2). Compared to spectra from the GPCR literature, the HuMOR spectrum present strong qualitative similarities with the CD profiles obtained for other purified GPCRs: BLT1 receptor (Baneres et al. 2003), porcine m2 muscarinic acetylcholine receptor (Peterson et al. 1995), β_2-adrenergic receptor (Lin et al. 1996), an olfactory receptor (Kiefer et al. 1996), NK1 receptor (Bane et al.

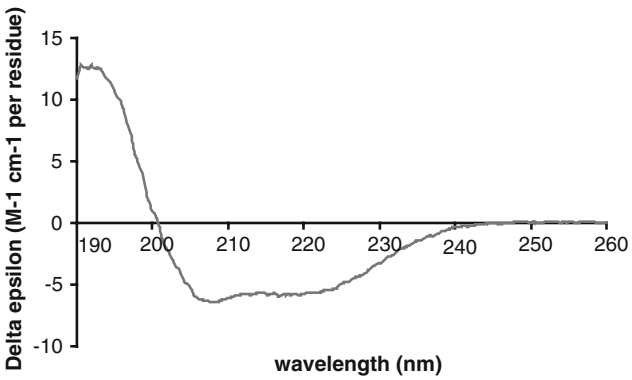

Fig. 2 CD spectrum of HuMOR in 100% TFE

2007), adenosine A2a receptor (Fraser 2006; O'Malley et al. 2007) and serotonin 5-HT4(a) receptor (Baneres et al. 2005). The α-helical contents from CD experiments were compared with the value determined from a homology model (Fowler et al. 2004a, 2004b) based on the crystal structure of rhodopsin (Palczewski et al. 2000). The predicted secondary structure of the mu-opioid receptor was based on the coordinates of the models from the Mosberg laboratory website (http://mosberglab.phar.umich.edu/resources/) and determined using the PROMOTIF program (http://www.rubic.rdg.ac.uk/~gail/#Software; Hutchinson and Thornton 1996). This procedure shows that 76% of the residues from the first transmembrane segment to the seventh one are in a helical conformation. If we add to this evaluation the contribution of the N-terminal domain, which is assumed to be unstructured, and the C-terminal domain, which is assumed to contain the so-called helix-VIII (Feng et al. 2003), we obtain a 54.5% α-helical content for the full-length mu-opioid receptor, which is in full agreement with the value obtained from CD experiments. Thus, in the context of refolding experiments, this α-helical content could represent a reference.

Analysis of the Secondary Structure of EGFP in TFE

A strange CD behavior of EGFP was observed when it was solubilized in 100% TFE. Indeed, EGFP, which is known to display a high β-sheet content (Phillips 2006), presented in TFE two minima at 208 and 222 nm, which are characteristic of a α-helical conformation. In this case, the α-helical content was estimated to be 45.4 ± 2.12% and the β-sheet content was only 12.3 ± 0.5%. The PROMOTIF program, which identifies structural motifs in proteins, was used to determine percentages of secondary structure present in the green fluorescent protein. Atomic coordinates obtained from X-ray crystallography of wt GFP (PDB 1gfl) (Yang et al. 1996), GFP (S65T mutant, PDB 1ema) (Ormo et al. 1996) and GFP (F64L mutant, PDB 1 emm) (Palm et al. 1997) were used to perform this study since data were not available for EGFP (F64L, S65T mutant). GFP and its mutants display a high β-strand contribution (50%) and a low amount of α-helical structures (7.3–11.8%), which correspond to an internal helix in the protein. Moreover, in an aqueous buffer at pH 7.5, CD spectroscopy performed on wt GFP revealed 52% β-sheet and 20% α-helical contents (Visser et al. 2002). This suggested that TFE can induce conformational changes in EGFP, as already described for all other β-sheet proteins (Arunkumar et al. 1996).

CD Spectroscopy of HuMOR Solubilized in SDS: Effect of pH

In order to investigate the effect of the charge on the structure of HuMOR, the CD spectra of the receptor solubilized in SDS, at the critical micelle concentration (cmc), were recorded at various pH values after buffer exchange. Among the variety of detergents, SDS, a so-called harsh anionic detergent, is known to favor helical conformations of peptides or proteins (Booth 2003; Kiefer 2003). The receptor samples solubilized in SDS micelles at different pH values exhibited CD spectra characteristic of predominantly α-helical structure, as seen by the presence of two distinct absorbance bands at 208 and 222 nm (Fig. 3A). A higher helical content was observed when pH was decreased from 9 to 6 (Table 1), and the pH 6 value was in accordance with the α-helical content determined for the receptor in TFE. These results indicate that a slightly acidic medium is a proper condition to favor α-helical folding of HuMOR in 0.1% SDS. Protonation in SDS (Hamed et al. 1983) and other detergents (Schievano et al. 2004) at negatively charged glutamate and aspartate side chains is known to favor α-helix formation in proteins. That was already observed for the TM2-3 domain of the mu-opioid receptor (Kerman and Ananthanarayanan 2007). The secondary structures observed for the receptor were stable over 1 week at 4°C. In light of our results, one can conclude that SDS can solubilize HuMOR from inclusion body-like structures while inducing and preserving helical structures. The SDS-solubilized receptor was tested for its ability to bind [^{3}H]-diprenorphine (an opioid receptor antagonist), but no specific binding could be detected. According to Kiefer (2003), GPCRs in SDS may be in a molten, globule-like state that contains considerable secondary α-helical structure but not in the native tertiary fold, which explains how their functions are impaired.

CD Spectroscopy of HuMOR Solubilized in DDM and in LDAO: Effect of pH

In another set of experiments, SDS was exchanged, on the Ni-chelated Sepharose column, with two types of detergents at their respective critical micellar concentrations and

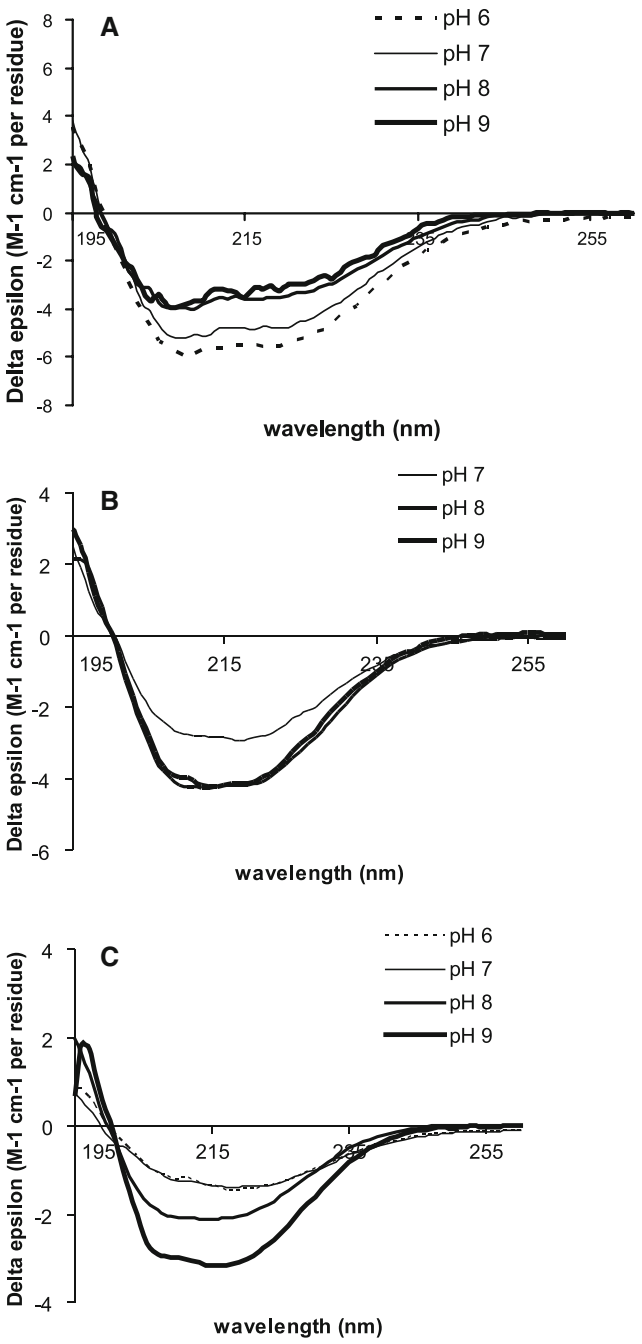

Fig. 3 CD spectra of HuMOR in 0.1% SDS (**A**), 0.15 mM DDM (**B**) and 2 mM LDAO (**C**) at various pH values

at various pH values. The detergents used were DDM, a non-ionic detergent, and LDAO, a zwitterionic detergent which displays non-ionic detergent behavior. These two detergents are considered mild and have the same alkyl chain length (12 carbons). In the course of selecting detergents for this CD study, octylglucoside (OG), a non-ionic detergent which contains an eight-carbon alkyl chain, was also tested for its on-column SDS-exchange capability. It was not considered as the EGFP-tagged mu-opioid receptor was not stable in this detergent and remained aggregated in the nickel phase. The CD spectra of HuMOR in DDM and LDAO are presented in Fig. 3B and C, and the deconvolution results are presented in Table 1. In DDM, the α-helical content represented approximately 33–35% of the secondary structural components when HuMOR was studied in pH 8 and pH 9 buffers; it represented only 22% at pH 7. In the same way, in LDAO the fraction of α-helix was very low compared to what was obtained when the receptor was solubilized in SDS. Moreover, lowering the pH had a dramatic effect on the receptor structure since the α-helical content droped from approximately 22% at pH 9 to approximately 8% at pH 6. This helical content does not match the expected value (~50%) at any pH, and the effect of pH for LDAO was opposite to the effect of pH in SDS micelles. When tested for their ability to bind specifically [^{3}H]-diprenorphine, the receptors in DDM or LDAO micelles appeared to be nonfunctional as observed in SDS, showing the occurrence of an unfolding state of the receptor under these conditions.

Table 1 CD estimates of secondary structure fractions of HuMOR in different detergents at various pH values

	α-helix	β-sheet	Turns	Unordered
HuMOR in 0.1% SDS				
pH 6	50 ± 6.8	9.1 ± 1.9	15.4 ± 2.6	26.6 ± 2.9
pH 7	40.6 ± 2.6	12.9 ± 1.7	18.9 ± 1.5	28.6 ± 0.3
pH 8	39.9 ± 4.6	12.4 ± 1.9	18 ± 2.1	29.9 ± 2.4
pH 9	36.1 ± 1.4	16.1 ± 1.2	20 ± 0.8	28.3 ± 0.3
HuMOR in 0.15 mM DDM				
pH 6	n.d.	n.d.	n.d.	n.d.
pH 7	20.6 ± 1	28.3 ± 0.6	22 ± 0.2	29.6 ± 1.5
pH 8	34.6 ± 4.7	18.7 ± 0.6	20.6 ± 2.5	23.4 ± 6
pH 9	32.9 ± 5	18.7 ± 1.7	20.7 ± 3.3	28.2 ± 3.1
HuMOR in 2 mM LDAO				
pH 6	8 ± 1.8	38.4 ± 1	22.8 ± 2.1	29.7 ± 3.1
pH 7	6.3 ± 1.5	40.3 ± 3.7	23.7 ± 0.8	29.2 ± 4.7
pH 8	15.7 ± 0.9	32.2 ± 0.3	22.4 ± 0.4	29 ± 0.8
pH 9	21.9 ± 2.7	20.5 ± 6.3	23.2 ± 2	34.6 ± 5.4

CD estimates of secondary structure fractions from CDPro are given as averages (±standard deviation) of the results obtained from the three programs (CDSSTR, CONTIN/LL and SELCON3)

n.d.— not done

Analysis of Detergent Effects

As the mu-opioid receptor was expressed in *P. pastoris* inclusion body-like structures under an aggregated form, we used the harsh detergent SDS to solubilize and purify the proteins. SDS micelles provide an anionic, membrane-mimetic environment with a hydrophobic core and a polar head. SDS was the best solubilizing detergent, whereas

neutral detergents such as DDM or LDAO were unable to solubilize the receptor from inclusion body-like structures (Sarramegna et al. 2005). Moreover, contrary to TFE, it was impossible to solubilize the receptor with any detergents used directly on the purified and lyophilized sample. For membrane proteins, it is generally reported that hydrophobic domains penetrate deeply into the hydrophobic core of the SDS micelles and adopt an α-helical structure due to hydrophobic interactions (Montserret et al. 2000). However, electrostatic interaction between the sulfate group of SDS and positively charged amino acids inside the membrane protein can play an important role in the formation and/or stability of the SDS-induced α-helix conformation, as demonstrated for lysozyme (Montserret et al. 2000). It was suggested that anionic groups of SDS first bind to the cationic groups of certain amino acids like lysine while additional detergent molecules bind to hydrophobic domains through hydrophobic interactions (Wu et al. 1981; Wu and Yang 1978). The increase in ellipticities in acidic SDS solutions appears to correlate well with this explanation. In the absence of an electrostatic anchor such as SDS, the binding of hydrophobic C_{12} tails of the other detergents is not sufficient to form stable protein–detergent complexes that allow formation of a hydrophobic environment required for α-helix hydrogen bond network stabilization. Moreover, we observe a dramatic effect on helix formation upon acidification of protein–DDM and protein–LDAO complexes. Positive repulsive electrostatic interaction at low pH could play a role in the destabilization of the detergent-dependent helical conformation of the membrane receptor.

Conclusion

In this study, we determined experimental conditions for which HuMOR adopts a high α-helical secondary structure content. Indeed, we have shown that the HuMOR secondary structure is essentially the same in TFE and in SDS micelles at pH 6 with an α-helical content consistent with the one expected. The HuMOR preparations are still not functional and have to be totally refolded. Nevertheless, the SDS/HuMOR micelle solutions are stable over weeks and contain an appropriate proportion of α-helices. The present results establish these conditions as suitable starting points for the complete refolding of the receptor by modifying the buffer composition, by exchanging SDS with other detergents and/or by transferring the receptor into other environments such as lipid bilayers, amphipols (Popot et al. 2003) or fluorinated surfactants (Palchevskyy et al. 2006).

Acknowledgment We are grateful to Dr. Monique Erard and Dr. Magali Blaud for their help in the circular dichroism experiments and to Dr. Virginie Gervais for the utilization of the PROMOTIF program. This work was supported by the Centre National de la Recherche Scientifique and by the University Paul Sabatier (Toulouse III).

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