Biochimica et Biophysica Acta 1838 (2014) 34–42

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Ligand- and drug-binding studies of membrane proteins revealed through circular dichroism spectroscopy☆

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ARTICLE INFO

Article history:
Received 15 March 2013
Received in revised form 13 June 2013
Accepted 14 June 2013
Available online 26 June 2013

Keywords:
Circular dichroism (CD) spectroscopy
Synchrotron radiation circular dichroism (SRCD)
Membrane proteins
Ligand interactions
Two-component signal transduction
k_d determination

ABSTRACT

A great number of membrane proteins have proven difficult to crystallise for use in X-ray crystallographic structural determination or too complex for NMR structural studies. Circular dichroism (CD) is a fast and relatively easy spectroscopic technique to study protein conformational behaviour. In this review examples of the applications of CD and synchrotron radiation CD (SRCD) to membrane protein ligand binding interaction studies are discussed. The availability of SRCD has been an important advancement in recent progress, most particularly because it can be used to extend the spectral region in the far-UV region (important for increasing the accuracy of secondary structure estimations) and for working with membrane proteins available in only small quantities for which SRCD has facilitated molecular recognition studies. Such studies have been accomplished by probing in the near-UV region the local tertiary structure of aromatic amino acid residues upon addition of chiral or non-chiral ligands using long pathlength cells of small volume capacity. In particular, this review describes the most recent use of the technique in the following areas: to obtain quantitative data on ligand binding (exemplified by the FsrC membrane sensor kinase receptor); to distinguish between functionally similar drugs that exhibit different mechanisms of action towards membrane proteins (exemplified by secretory phospholipase A_2); and to identify suitable detergent conditions to observe membrane protein–ligand interactions using stabilised proteins (exemplified by the antiseptic transporter SugE). Finally, the importance of characterising in solution the conformational behaviour and ligand binding properties of proteins in both far- and near-UV regions is discussed. This article is part of a Special Issue entitled: Structural and biophysical characterisation of membrane protein–ligand binding.

Contents

Abbreviations: DDM, *n*-dodecyl-β-D-maltoside; SRCD, synchrotron radiation circular dichroism; CD, circular dichroism; GBAP, gelatinase biosynthesis-activating pheromone; UV, ultraviolet; TM, transmembrane domain; k_d, dissociation constant; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPE, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine

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http://dx.doi.org/10.1016/j.bbamem.2013.06.019

1. Introduction

Circular dichroism (CD) is a well-known spectroscopic technique to study chiral molecules, in particular proteins in solution as well as in thin amorphous dry films [1–4]. Through pattern recognition of the CD spectral features, proteins can be classified in terms of their folding and secondary structure composition. A unique strength of CD spectroscopy is its high sensitivity to sample perturbations allowing proteins to be investigated qualitatively and quantitatively as a function of temperature, solvent composition, chemical agents, detergents, pH, and ligand binding interactions [5–9]. SRCD measured at Diamond B23 beamline shares the same advantage of other SRCD beamlines worldwide, by having higher photon flux and extended vacuum UV region down to 125 nm. This is the technique of choice for the characterisation of membrane protein property in solution. SRCD spectra measured at Diamond B23 beamline provide additional information content than those from bench-top CD instruments because of the beamline's higher photon flux and extended vacuum UV region down to 125 nm [8,11,12]. Also a unique feature of B23 is its highly collimated and small cross section beam light that allows measurements using very small volume capacity cells of a wide range of pathlengths, from a few microns to 10 cm [8,11,12] otherwise unattainable with bench-top instruments.

CD spectroscopy has proved highly useful for studies of ligand binding by soluble proteins [5–12], particularly as it is a relatively quick and easy spectroscopic measurement, requiring no extensive sample preparation, and it has the potential for use in rapid throughput technologies [13]. However, there have been fewer examples of its use in studies of ligand and/or drug binding to membrane proteins (see Section 2). CD and SRCD have most frequently been used to determine secondary structural content and integrity of membrane proteins such as histidine kinases [14–17], membrane transport proteins [18], protein fragments [19] and in membrane unfolding studies [16,18]. Sreerama and Woody [20] found that the CD analysis of secondary structure content of membrane proteins using soluble protein reference sets was slightly inferior to that obtained for soluble proteins. The inclusion of membrane proteins in the soluble protein reference sets – now common practise – has since improved the CD analysis for both membrane and soluble proteins [21–23]. It is not our intention here to provide a detailed review of all these aspects, but rather to describe recent developing advances in CD- and SRCD-based approaches that offer promising routes for progressing knowledge of membrane protein–ligand interactions in the future.

Perhaps the main reason why the application of CD and SRCD spectroscopy methods has proved more limited for ligand binding studies of membrane proteins in the past is largely due to the technical challenges associated with working with hydrophobic membrane proteins. Here we review some examples of the very recent advances in the use of CD and SRCD spectroscopy for qualitative and quantitative ligand- and drug-binding interactions by membrane proteins. A number of technical but addressable considerations have also been reported in these studies; they address some of the technical challenges, and have resulted in the successful use of the techniques for: (i) obtaining quantitative data on ligand and inhibitor binding to membrane proteins (FsrC) [24,25], (ii) identifying functionally similar but mechanistically distinct drugs that target membrane proteins (PLA_2) [26]; and (iii) characterising suitable detergent conditions for observing ligand interactions in membrane proteins [27–33]. A more detailed account is now described.

2. Quantitative data on membrane protein–ligand binding

CD methods have been used qualitatively to identify and characterise conformational changes in membrane proteins induced upon ligand binding. Examples of studies investigating ligand-induced changes on secondary structure and α-helical content, utilising the far-UV region, include: the studies of Fuertes et al. (2001) on the kinetoplastid membrane protein-11 of *Leishmania infantum*, in which pH- and temperature-dependent Ca^{2+} binding, was revealed [34]; successful application of CD techniques to immobilised protein membranes to investigate changes in α-helical content in response to hormone challenges [35]; and the studies of Dudzik et al. [36] on the neurological α-synuclein protein implicated in Parkinson's disease, in which far-UV CD spectroscopy was used to confirm that there was no change in the helicity of membrane-bound α-synuclein upon binding of its Cu^{2+} ligand [36]. Measurements in the visible regions involving time-resolved CD spectroscopy have also been used; for example, this approach was used to verify the assembly kinetics of the light harvesting chlorophyll *a*/*b* protein complex, in which it was shown that chlorophyll *a* binding possesses a fast kinetic phase whilst that of chlorophyll *b* was slower [37].

The first quantitative data of membrane protein–ligand interactions revealed through the use of SRCD spectroscopy was reported during our studies of the intact membrane sensor kinase FsrC [24] using Diamond beamline B23 [38,39]. FsrC belongs to the histidine protein kinase family of predominantly membrane proteins that constitute the sensory components of bacterial two-component signal transduction systems. These systems are the main mechanism by which bacteria sense and respond to environmental change [40,41]. FsrC is the sensory component of the Fsr signal transduction pathway involved in quorum-driven gene regulation of several virulence factor genes in the hospital-acquired infection agent *Enterococcus faecalis* and other enterococci (Fig. 1) [42–44]. FsrC senses changes in cell density or quorum by responding to changes in the external level of the gelatinase biosynthesis-activating pheromone (GBAP), a small cyclic peptide of 11 amino acid residues: H_3N^+-QNSPNIFGQWM-COO^- (lactone-linked between Ser-3 and Met-11). Following interactions between the GBAP ligand and the FsrC receptor, the pathway is activated through auto-phosphorylation of FsrC, followed by signal transduction to FsrA and culminating in activation of virulence genes such as gelatinase (GelE) and serine protease (SprE) and autoregulation of the *fsr* genes (Fig. 1; [44]). Production of purified intact FsrC protein that is active and responsive to the GBAP ligand was demonstrated in a wide-ranging study designed to evaluate the overexpression and purification of the genome complement of intact membrane sensor kinases of *E. faecalis* V583 [45]. In this study, the conditions required to successfully overexpress in *Escherichia coli* and to purify intact FsrC (including its transmembrane and sensing domains) were reported. Using in vitro activity assays, it was also shown that full-length purified FsrC was isolated as an active protein and that it responds to its GBAP signal in vitro, which is important information for confirming a link between conformational changes observed using CD and changes in membrane enzyme activity [45].

The initial ligand–receptor interactions of quorum sensing systems such as Fsr are suggested to be promising targets for the design of novel antibacterial drugs that might prove more effective against antibiotic-resistant strains of bacteria [46]. To assess the feasibility of novel drug design for disrupting GBAP–FsrC interactions, we employed SRCD spectroscopy as a means for observing, investigating and quantifying GBAP interactions with this detergent-solubilised membrane protein. Several technical considerations were established during these studies:

1) Reproducible SRCD spectra using beamline B23 [38,39] were obtained only when a sufficient incubation period was included for FsrC stabilisation following sample dilution and preparation from concentrated stocks [24]. For example, for FsrC in 0.02% DDM repeated scan measurements in the far-UV region under conditions that did not promote UV protein denaturation showed significant CD spectral changes that were used to determine the amount of incubation time needed to measure reproducible CD spectra. The incubation time required to equilibrate the structural