Analytical Biochemistry 427 (2012) 211–218

Contents lists available at SciVerse ScienceDirect

Analytical Biochemistry

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

Vibrational circular dichroism study of polypeptide model–membrane systems

Pavlína Novotná [a], Marie Urbanová [b,*]

[a] Department of Analytical Chemistry, Institute of Chemical Technology, 166 28 Prague 6, Czech Republic
[b] Department of Physics and Measurements, Institute of Chemical Technology, 166 28 Prague 6, Czech Republic

ARTICLE INFO

Article history:
Received 5 January 2012
Received in revised form 25 March 2012
Accepted 26 March 2012
Available online 4 April 2012

Keywords:
Polypeptide–membrane structure
Vibrational circular dichroism
Liposome
Micelle
Polypeptide–surfactant interaction
Polypeptide–lipid interaction

ABSTRACT

In this article, we describe the mutual structural effect of the interaction between the model membranes and polylysine and poly-L-arginine. Vibrational circular dichroism (VCD), a method exceptionally sensitive to the polypeptide structure that has not been established in such studies before, was the primary method of this study. A complementary technique, electronic circular dichroism, was applied to verify the newly obtained results and as a bridge to the previous studies. We used micelles composed of sodium dodecyl sulfate (SDS) as a monolayer membrane model and large unilamellar vesicles composed of phospholipids as a bilayer membrane model. We describe the conformational changes of the polypeptides caused by the interaction with the model membranes. Among others, the presence of the liposomes in the solution generated special conditions for the formation of the α-helical structure of poly-L-arginine; the presence of SDS induced the formation of the β-structure of polylysine. From a methodological point of view, we emphasize the advantages of infrared spectroscopic techniques for the liposomic membrane studies as well as the preference of ultraviolet techniques for smaller micellar systems.

The function of the membrane proteins and biological membranes is closely connected to their structure influenced by their mutual interaction. Moreover, the induction of a specific structure of a peptide on the interaction with a lipid surface has been established as a requirement for lytic and antimicrobial activity to occur [1].

Unfortunately, the protein–membrane systems are complex and not easy to characterize. Hence, many different spectral methods and their combinations have been used to characterize these complexes, including nuclear magnetic resonance [1–7], fluorescence spectroscopy [1,8,9], absorption and attenuated total reflection infrared (IR)[1] spectroscopy [7,9–12], and electronic circular dichroism (ECD) [1–3,8,10,13,14]. Among them, IR spectroscopy and the ECD technique have been used especially for the determination of the peptide secondary structure. Although these techniques are conventionally used, they cannot distinguish among all the types of secondary structure. Furthermore, the unsuitable optical properties of the solutions of the model membranes give rise to scattering by which ECD, using radiation of a shorter wavelength than IR spectroscopy, is more influenced. On the other hand, a much more sensitive method [15] with enormous potential considering the determination of the peptide structure, vibrational circular dichroism (VCD) [16,17], has been scarcely used in these kinds of study [10,12].

In contrast to the widespread ECD spectroscopy, the complementary VCD technique is particularly suited for studying the membrane proteins because nearly no scattering complications persist at the long wavelengths and it does not suffer from the disadvantages characteristic of the ultraviolet (UV)/visible region [18]. Especially, the semiempirical structural studies of polypeptides and proteins represent a distinct group of the VCD applications (for reviews, see Refs. [19–21]). VCD measures the rotational strengths of the vibrational transitions and is sensitive to the short-range ordering, thereby allowing discrimination between the β-sheets and all types of the helices as well as the disordered structures [22]. Here, the analysis of the spectra is usually based on the known correlation between the spectral shape of the amide I vibrational mode (predominantly the C=O stretch of the peptide bond with participation of the NH out-of-plane bend and minor contribution from the C–H stretch) and the secondary structure. The amide I transition is very sensitive to the structure of biopolymers alone but also enables the observance of subtle changes in the local structure caused by biologically important interactions [23,24]. However, this advantage of the VCD technique also brings some limitations. In particular, the range of the conditions acceptable for the measurements is more specific and narrower than in conventional spectroscopies. In addition, the absorption must be neither too high nor too small and is gained

* Corresponding author. Fax: +420 220444334.
E-mail address: marie.urbanova@vscht.cz (M. Urbanová).

[1] *Abbreviations used:* IR, infrared; ECD, electronic circular dichroism; VCD, vibrational circular dichroism; UV, ultraviolet; PLL, poly-L-lysine; PLAG, poly-L-arginine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPG, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) sodium salt; SDS, sodium dodecyl sulfate; PDL, poly-D-lysine; LUV, large unilamellar vesicle; DLS, dynamic light scattering; CMC, critical micellar concentration; PPII, polyproline II.

0003-2697/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ab.2012.03.023

by the optimal combination of the concentration, path length, and solvent.

In this study, the poly-L-lysine (PLL) and poly-L-arginine (PLAG) homopolypeptides were studied as appropriate model systems for antimicrobial and cytolytic peptides. Both PLL and PLAG have positively charged side chain groups at physiological pH, but these groups differ especially in their binding properties. Hence, we studied their interactions with the zwitterionic and negatively charged liposomes and with the negatively charged micelles to clarify their similarities and diversities using the advantages of the VCD method complemented by ECD and IR spectroscopy. We show that VCD spectroscopy provided specific results concerning the structure of both partners in the studied systems and that the use of this spectroscopy is more convenient when compared with ECD spectroscopy because of the optical properties of the model membrane systems. One of the main limitations of VCD spectroscopy, the necessity of high concentrations, was applied as an advantage for modeling in vivo membrane systems with local high concentrations of biomolecules.

Materials and methods

Materials

The 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) sodium salt (DPPG) phospholipids were purchased from Avanti Polar Lipids (USA). The sodium dodecyl sulfate (SDS) surfactant and the PLL hydrobromide (~219 amino acid units), poly-D-lysine (PDL) hydrobromide (~283 amino acid units), and PLAG hydrochloride (~184 amino acid units) polypeptides were purchased from Sigma–Aldrich (USA). All of the chemicals were used without further purification. See Fig. S1 in supplementary material for the structures of the polypeptides, lipids, and SDS.

Preparation of large unilamellar vesicles

The liposomes were prepared by standard procedures [25]. The appropriate amount of dried lipid was weighed and dissolved in chloroform or a chloroform/methanol mixture (2:1, v/v) and vortexed for 5 min. The sample was dried under low pressure to form a thin film on the vial wall, after which it was left under high vacuum for 4 h. The film was then hydrated via the addition of 10 mM phosphate buffer with 150 mM NaCl (pH 7.2) and vortexed extensively. The resulting multilamellar liposome suspension was then reduced to uniform large unilamellar vesicles (LUVs) by passing 17 times through a polycarbonate membrane with a mean pore diameter of 100 nm using a Mini-Extruder (Avanti Polar Lipids). The dynamic light scattering (DLS) method confirmed the quite narrow size distribution of the LUVs with the maximum at 110 ± 3 nm. After the extrusion, the LUVs were allowed to equilibrate for at least 2 h before use. The final lipid concentration was calculated based on the weight of the dried lipid [26–30]. For the VCD measurements, a deuterated phosphate buffer was used. During the liposome preparation, the D–H exchange, observed in the mid IR, took place.

Samples for spectral measurements

The spectral measurements were done in the solutions of a phosphate buffer (deuterated for VCD). The polypeptides were dissolved in 10 mM phosphate buffer with 150 mM NaCl (pH 7.2). The polypeptide stock solution was then slowly added to the solution of the lipid LUVs or SDS micelles. Afterward, the mixture was mildly vortexed and allowed to equilibrate. After the sample preparation, the DLS measurements were used to confirm the size distribution of LUVs.

For the VCD measurements, the polypeptide concentration was 6.5 g/L, which represented the molar concentrations of 5.1 and 4.2×10^{-2} M for PLL and PLAG, respectively. Within the article, the polypeptide molar concentration is expressed per amino acid residuum. For the ECD measurements, the concentrations were 7.8 and 6.4×10^{-4} M for PLL and PLAG, respectively.

For the VCD measurements, the concentration of the lipids was 1.4×10^{-1} M and that of the surfactant was 5.6×10^{-1} M. For the ECD measurements, the concentration of the lipids was 2.8×10^{-3} M and that of the surfactant was 1.1×10^{-2} M, which is 48% above the critical micellar concentration (CMC) of SDS (7×10^{-3} M).

For the VCD measurements, the lipid-to-peptide residue molar ratios (lipid/peptide) were 2.8 and 3.4 for PLL and PLAG, respectively, and the surfactant-to-peptide residue molar ratios were 11 and 13 for PLL and PLAG, respectively. These values were obtained by search ECD experiments performed for the lipid/peptide residue molar ratios 0 to 9 and the DPPG/(DPPG + PMPC) ratios 0 to 1 (see example in Fig. S2 of supplementary material). The lipid/peptide residue ratio restricted the polypeptide concentration used, which was approximately five times lower than that usually employed for the VCD study of peptides and proteins. The DPPG-to-peptide residue molar ratio *Ri* (DPPG/peptide residue) was varied during the measurements from 0 to 3.4 at the constant lipid/peptide ratio.

Electronic circular dichroism

The ECD spectra were measured in a quartz cuvette with an optical path length of 1 mm (Starna, USA) using a J-810 spectropolarimeter (Jasco, Japan). The conditions of the measurements were as follows: a spectral region of 200 to 260 nm, a scanning speed of 5 nm/min, a response time of 16 s, a resolution of 0.5 nm, a bandwidth of 1 nm, and a sensitivity of 100 m deg. The final spectrum was obtained as an average of five accumulations. The spectra were corrected for a baseline by subtracting the spectra of the corresponding polypeptide-free solutions. The ECD measurements were conducted at room temperature (25 °C).

VCD and transmission Fourier transform IR spectroscopy

The VCD and IR absorption spectra in the 1800 to 1400 cm^{-1} region were measured on a Fourier transform IR (FTIR) IFS 66/S spectrometer equipped with a PMA 37 VCD/IRRAS module (Bruker, Germany).

The samples were placed in a demountable cell (A145, Bruker) composed of CaF_2 windows separated by a 50-μm Teflon spacer that was suitable for the used low-peptide concentration.

All of the VCD spectra were recorded at a spectral resolution of 8 cm^{-1} and are the averages of the 10 blocks of 3686 scans. OPUS 6.5 software (Bruker) was used for the VCD spectra calculations. The VCD and IR spectra were corrected for a baseline, which was obtained as a spectrum of the polypeptide-free solution measured under the same experimental conditions. The composition of the mixture of deuterated and nondeuterated phosphate buffer used as a solvent for the baseline correction corresponded to the H-to-D exchange during the extrusion and was obtained from the IR absorption spectra. An example of the impact of the baseline correction and the standard deviation of the VCD spectra is demonstrated in Fig. S3.