Interaction of porphyrin/oligonucleotide complex with liposomes studied by drop coating deposition Raman spectroscopy

E. Kočiřová *, M. Procházka, J. Štěpánek and P. Mojzeř

Faculty of Mathematics and Physics, Institute of Physics, Charles University, Prague, Czech Republic

Abstract. Drop coating deposition Raman (DCDR) microspectroscopy was used to investigate interaction of the complexed cationic copper 5,10,15,20-tetrakis(1-methyl-4-pyridyl) porphyrin (CuP) and phosphorothioate analog of dT₁₅ oligonucleotide with liposomes, the lipid composition of which imitated the natural plasmatic membrane. Great advantage of dried drops on DCDR plates over a solution sample is that the specific drying process on the special hydrophobic surface efficiently separates liposomes from small species in the solvent. In our case, liposomes with bound CuP/oligonucleotide complexes formed a ring at the edge part of the dried drop while dried solution of this complex remained inside this ring. High quality spectra measured from the ring by using Raman confocal microspectrometer revealed unperturbed arrangement of lipid chains by the drying process, partial binding of the CuP/oligonucleotide complexes to liposomes, and a certain reorientation of lipid chains as a consequence of this interaction.

Keywords: Liposome, oligonucleotide, porphyrin, DCDR, Raman spectroscopy

1. Introduction

Synthetic modified oligonucleotides – tailored short strands of nucleic acids – are widely studied as potential selective silencing agents of gene expression [2]. Particular single stranded deoxyribo- and ribo-nucleotides or their modified analogs are designed to inhibit transcription of the gene (antigene strategy), translation of messenger RNA to a protein (antisense strategy) or function of the target protein (aptamer strategy); early enough to block the advance of the viral or malignant diseases. Very important step of a successful oligonucleotide functioning inside the cell is its sufficient uptake and proper intracellular distribution. Since oligonucleotides are large and negatively charged molecules, they possess only a low capacity to penetrate through the cellular membrane. Employment of a suitable “helper” delivery system is usually indispensable. Water-soluble cationic porphyrins are able to bound strongly to oligonucleotides and to compensate their negative charge. They seem thus to be appropriate candidates for this purpose [1,4,7].

The aim of this work was to examine interaction of the porphyrin/oligonucleotide complex with liposomes representing a model of biomembrane by using Raman spectroscopy. Several reasons, mainly

*Corresponding author: E. Kočiřová, Faculty of Mathematics and Physics, Institute of Physics, Charles University, Ke Karlovu 5, Prague 2, CZ-121 16, Czech Republic. Tel.: +420 221 911 471; Fax: +420 224 922 797; E-mail: kocisova@karlov.mff.cuni.cz.

available sample quantities, achievable concentrations and optical properties of the liposome suspension in water, prevented us, though, from measuring standard Raman spectra.

We tried instead the drop coating deposition Raman (DCDR) technique [9], which is based on a deposition of a small droplet of the sample on a Teflon-coated stainless-steel surface. The hydrophobic surface enables drying of the sample by the “coffee ring effect” when the flow of a liquid in the evaporating droplet carries the dispersed material to the edge of the droplet where it forms a ring. The sample inside the ring is highly concentrated forming a “glassy” environment with minimum amount of free water and provides a strong and highly reproducible micro-Raman signal. This technique allows to record Raman spectra of proteins in original concentrations down to 1 μM without considerable loss of their solution conformation [3], but no application for liposome systems has been so far reported.

2. Experimental section

Phosphorothioate analog of dT₁₅ oligothymidylate was synthesized in the Laboratory of Plant Molecular Physiology at Masaryk University (Brno, Czech Republic). Copper(II) 5,10,15,20-tetrakis (1-methyl-4-pyridyl) porphyrin (CuP) was purchased from Frontier Scientific, UT, USA.

Liposomes were prepared from asolectin suspension (Asolectin from soybean, Fluka). Asolectin in powder was dissolved in chloroform and after that evaporated under the stream of nitrogen gas. The formed thin lipid film on flask was dissolved and mixed in a vortex in phosphate buffer (5 mM, pH 7.4). Liposomes were formed by extrusion of suspension by using LiposoFast-Basic™ apparatus (Avestin, Inc.) with 400 nm pore polycarbonate membrane. Solutions of dT₁₅ and CuP were prepared in the same phosphate buffer. For the final DCDR experiment, porphyrin/oligonucleotide complex (mixed solution of 1.6 μM CuP and 45 μM oligonucleotide) was added to the liposome suspension (asolectin concentration of 0.4 mg/ml or 0.52 mM). These concentrations were found as optimal to reach sufficiently thin layer after the drop drying on the DCDR plate that provided well reproducible micro-Raman spectra.

For DCDR experiments, 2 μl drops of the sample (liposome suspension or its mixture with CuP/oligonucleotide complex) were deposited on the DCDR slide (Tienta SpectRIM™) and dried for about one hour at room temperature. Spectra were collected by a confocal Raman microspectrometer LabRam HR800 (Horiba Jobin-Yvon) with a nitrogen cooled CCD detector and a 600 grooves/mm grating spectrograph. Used 100 $\times$ objective provided 1–2 μm laser spot on the sample. Raman spectra were excited by a 514.5 nm line of Ar⁺ ion laser (3 mW power at the sample) and collected with acquisition time of 5 $\times$ 60 s. Supplementary and reference Raman measurements in bulk solutions or suspensions were performed by using a standard Raman spectrometer equipped with a Spex 270M spectrograph (Jobin-Yvon) with a single 1800 grooves/mm grating and a liquid nitrogen cooled CCD detector.

3. Results and discussion

Porphyrin/oligonucleotide complex: Cationic CuP is known to bind strongly to oligonucleotides notably by Coulombic interaction. In the case of oligothymidylates, the porphyrin macrocycle was suggested to be almost perpendicular to the self-stacked thymine planes of dT_n, the complex being additionally stabilized by van der Waals' interactions of other type than stacking [6]. The equilibrium association constant of the CuP/dT₁₅ complex was found to be $\sim 1.2 \times 10^6 \text{ M}^{-1}$ at room temperature. It means that in the prepared mixed solution of CuP and the oligonucleotide, almost all CuP should exist in

complexes with the prevailing stoichiometry of one porphyrin per one oligonucleotide. It was proved by UV-Vis absorption spectrum of the CuP/oligonucleotide mixture that the position of Soret band really corresponds to CuP bound to oligonucleotide.

Raman spectrum of the mixed solution does not contain any resolvable band of the oligonucleotide due to its low concentration. CuP Raman spectrum is, on the other hand, well resolvable because of its resonance enhancement, even though its concentration is significantly lower. Comparison with the spectrum of pure CuP solution has revealed that the CuP/oligonucleotide complex formation causes downshift of the band at 1645 to 1640 cm^{-1} , while positions of other strong porphyrin bands remain unchanged.

Liposomes: Employed sample of asolectin is composed of about equal proportion of lecithin, cephalin and phosphatidylinositol and of other phospholipids and polar lipids in minor amounts. Mixture of different lipids gives rise a liposome membrane, which is not so uniform and rigid as in the case of uniform lipid composition often used in model studies, and resembles thus better the natural biomembrane.

Liposome suspension deposited on the DCDR plate formed a distinct ring after drying. Micro-Raman mapping showed that the ring contains assembled liposomes with minor buffer contribution while the ring interior contains only the buffer, mostly in a microcrystal form (micro-image not shown). We found that Raman spectrum of liposomes on the DCDR plate in the 2800–3000 cm^{-1} region, which reflects arrangement of hydrophobic chains, was within an experimental error identical with that obtained from the original suspension (and corresponded to a liquid crystalline phase). This has proved that liposomes concentrated in the ring without excess of bulk water do not lose their structural arrangement at least in a microscopic scale.

System of liposomes and porphyrin/oligonucleotide complexes: Bright field micro-image of the drop of mixed the porphyrin/oligonucleotide solution with the liposome suspension, dried on the DCDR plate is shown in Fig. 1 (left). The dried drop maintained almost round shape with well developed edge ring and irregular reddish patches in its interior.

Micro-Raman measurements revealed that the internal region is covered by remains of dried CuP/oligonucleotide solution. Figure 1 (right) shows (upper curve) a typical Raman spectrum from this region, consisting of strong CuP Raman bands at 1005, 1367 and 1640 cm^{-1} and bands of the buffer at 392, 534, 890 and 965 cm^{-1} . Position of the 1640 cm^{-1} band confirms that CuP is still complexed with oligonucleotide.

Fig. 1. Left: Bright field micro-image (50 $\times$ objective) of the drop of the CuP/dT₁₅ complex solution mixed with liposome suspension, dried on the DCDR plate (bar = 20 μm). Right: Typical Raman spectra obtained from the central region (upper curve) and the edge ring (middle curve) compared with the reference Raman spectrum from the ring formed by a pure liposome suspension (lower curve).

Raman spectra from the edge ring contain well resolved bands of both porphyrin (385, 1005, 1367, 1640 cm^{-1}) and lipids (875, 1302, 1440, 1656, 2853, 2903, 2935 cm^{-1}). Typical spectrum is shown in Fig. 1 (right-middle curve). Presence of CuP in the ring indicates that it has been bound to a large molecular system and therefore transported to the drop edge during drying. Withal, the 1640 cm^{-1} band implies durability of the CuP/oligonucleotide complex. It means, that part of the CuP/oligonucleotide complexes bind to liposomes and the “coffee ring” drying mechanism makes their robust separation from the CuP/oligonucleotide complexes remaining free in the solvent.

Variations of Raman spectra taken from different locations inside the ring concern mainly the strong bands in the 2800–3000 cm^{-1} region that belong to CH stretching modes of lipid chains. Mutual intensity ratios of 2853, 2903 and 2935 cm^{-1} bands are indicators of the state of the hydrophobic liposome region and respond sensitively to liposome interactions with other molecules [5,8]. Obtained Raman spectra differ more or less from the spectrum obtained from the ring formed by the pure liposome suspension without the CuP/oligonucleotide complex, which is shown in Fig. 1 (right) as the lower curve. We attribute these spectral deviations to reorientation of lipid chains in liposome as a consequence of their interaction with the porphyrin/oligonucleotide complex.

4. Conclusions

DCCR was demonstrated as a promising technique for Raman studies of liposome systems. The main advantage is a perfect accumulation of liposomes in the “coffee ring” without remarkable loss of the lipid structural arrangement and a complete separation of smaller molecules or molecular complexes that are not bound to liposomes. This permits Raman studies of even weak interacting molecular systems present at low percentages in the original liposome suspension.

Application of this approach to our system revealed that complexation of oligonucleotides with cationic porphyrin brought about its possible binding to liposome and that this interaction causes certain reorientation of the lipid chains.

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