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Protein conformational changes and myelin solubilization by anion-detergent solutions

Jaime Monreal[a], Pedro Carmona[b], Pilar Regueiro[a] and Ricardo S. Diaz[a]

[a]*Instituto Cajal, CSIC, Avenida del Doctor Arce 37, E 28002 Madrid, Spain* and [b]*Instituto de Optica, CSIC, Serrano 121, E 28006 Madrid, Spain*

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The addition of sodium sulfate to a myelin suspension in sodium phosphate buffer at neutral pH, containing octyl glucoside detergent (OG), increases the membrane solubility more than 5-fold by an unknown structural mechanism. FTIR spectroscopy has been applied to investigate anion effects on the conformational structure of myelin proteins. Sulfate and sulfate-phosphate media, but not phosphate alone, induce a great conformational protein disorder. The addition of the detergent to the anion mixture solution prevents the myelin from protein denaturation. The conformational transitions have also been quantified through the amide I region. Explanations of these changes and their connections with myelin solubility are also included.

Myelin membrane solubilization; Detergent; Sulfate-phosphate anion; FTIR spectroscopy; Protein structure

1. INTRODUCTION

Membrane solubilization is an important prerequisite for the isolation of individual molecules and for studying their membrane-related functions. The peculiar multilamellar structural organization of the myelin sheath, its particular biochemical composition and the strong protein–protein and lipid–protein interactions which take place among myelin components significantly contribute to the great stability and resistance of this membrane to being disordered [1–3]. These properties result in a more difficult solubilization of myelin by detergents in comparison to other biomembranes. Whereas the solubilization of myelin components by detergents has long been a matter of concern and there exists biochemical information covering some aspects of this important problem, such information is still fragmentary [4–9]. Our group have recently carried out a comparative study of brain myelin solubilization in several aqueous detergent solutions normally used in membrane biochemistry [10]. At comparable molar concentrations, two nonionic detergents, i.e. octyl glucoside (OG) and Lubrol PX, proved relatively better myelin solubilizers than the detergents related to the bile salts, i.e. cholate and CHAPS. The two former detergents solubilized more protein than lipid and the latter two more lipid than protein from myelin membranes. The detergent concentrations required for myelin solubilization were reduced substantially when the salt concentration of the media was increased. Each of the detergents studied, including the above four, sodium dodecyl sulfate (SDS), Triton X-100 and Zwittergent 3-14 had its own advantages and drawbacks as myelin protein extractors.

OG was preferred in this work, given its versatility as a solubilizer and its high critical micellar concentration (CMC). To solubilize 70% proteins in a buffer suspension of myelin it is necessary to reach a concentration of at least 200 mM octyl glucoside in the medium. This high detergent concentration is denaturating (unpublished results) and expensive. However, the same amount of myelin was almost completely solubilized when suspended in 0.1 M sodium phosphate buffer pH 6.7, containing 0.1 M sodium sulfate and 30 mM OG. Therefore, the addition of moderate concentrations of sodium sulfate increases the membrane solubility by more than 5-fold [10] by an unknown structural mechanism and with a great saving of this expensive detergent. Moreover, sodium sulfate is used, added to the phosphate buffer mobile phase, in side-exclusion high-performance liquid chromatography (SE-HPLC) of proteins to minimize adhesion to the gel matrix of the columns and to improve their separation [11].

Given that OG does not interfere in the infrared amide I region [12,13], we have used FTIR spectroscopy as a nondestructive technique to investigate the effect of phosphate buffer, sulfate solution, sulfate-phosphate buffer, and the presence of OG in the last anion solution on the conformational structure of myelin proteins.

2. MATERIALS AND METHODS

2.1. *Myelin purification*

Myelin was purified from calf brain white matter, according to

Correspondence address: J. Monreal, Instituto Cajal, CSIC, Avenida del Doctor Arce 37, E 28002 Madrid, Spain. Fax: (34) (1) 5854154.

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Aveldaño et al. [10], and was maintained at −80°C. No spectroscopic differences, due to storage conditions, were observed.

2.2. *Spectroscopy*

Aliquots of purified myelin were suspended, at 16 mg protein/ml as measured by the method of Aguilar et al. [6], in each of the following solutions: (a) distilled water (myelin control), (b) 0.2 M sodium phosphate buffer, pH 6.7, (c) 0.1 M sodium sulfate solution, (d) 0.1 M sodium phosphate buffer, pH 6.7, containing 0.1 M sodium sulfate, and (e) the last saline solution containing 30 mM OG detergent. The infrared spectra of these samples were recorded using CaF_2 cells with a path length of about 10 μm. A Perkin-Elmer infrared spectrophotometer, model 1725X, assisted by a computer, was used. Sixty-four scans were recorded for each sample and their respective blanks. Difference spectra were obtained by following the criterium of eliminating the absorbance of the band at about 2100 cm^{-1} [14]. Second derivative spectra were performed to more accurately identify the positions of band components in the amide I region by using an Obey program (Perkin-Elmer). The protein secondary structures were quantified from the difference spectra, which were adjusted to a sum of Lorentzian functions by means of a fitting program for the resolution of spectral profiles in the amide I region [15]. Each type of protein secondary structure was estimated as percentage of the areas corresponding to the bands located in the ranges of 1655–1648 cm^{-1} (α-helices), 1645–1615 cm^{-1} (β-sheets) and 1663–1658 cm^{-1} (random coils) [16–19]. The assignment of the random coil amide I vibrational modes to bands around 1660 cm^{-1} is supported by the shifting of these bands towards lower frequencies when going from H_2O to D_2O. In membrane systems, the shifting of the random coil amide I band components is about 10–12 cm^{-1} and those of the ordered structures (helices and β-sheets) are about 2 cm^{-1} [18].

3. RESULTS

The spectrum of myelin control suspended in water (Fig. 1a) shows the absorption maximum of the amide I band at 1654 cm^{-1} (1657 cm^{-1} in the second derivative spectrum of Fig. 2a). This indicates that the average protein conformational structure in the native state is mainly α-helical [16–19]. Small proportions of β-sheet structures are also observed in the 1645–1615 cm^{-1} range (1642–1626 cm^{-1} in the second derivative spectrum, Fig. 2a). The random coil structure appears as a very slight shoulder which is more visible, however, in the second derivative spectrum at about 1663 cm^{-1}.

Fig. 1b corresponds to myelin suspended in phosphate buffer, the spectrum of which is similar to that of the myelin control and therefore its second derivative spectrum is not shown in Fig. 2.

Fig. 1c reflects the effect of sodium sulphate solution. In comparison with myelin control, an evident increase of β-sheets is observed as shown by the band near 1626 cm^{-1} and the bands in the second derivative spectrum at 1680 and 1619 cm^{-1} (Fig. 2b). At the same time, a disordering of the polypeptide backbone occurs as shown by the increase of the halfbandwidth in the amide I band region, in such a way that even this broad amide I band masks to some extent the lipid carbonyl bands located in the 1750–1700 cm^{-1} region.

The spectrum of myelin suspended in sodium phosphate buffer containing sodium sulfate (Fig. 1d) shows an absorption maximum at 1661 cm^{-1}. The proteins

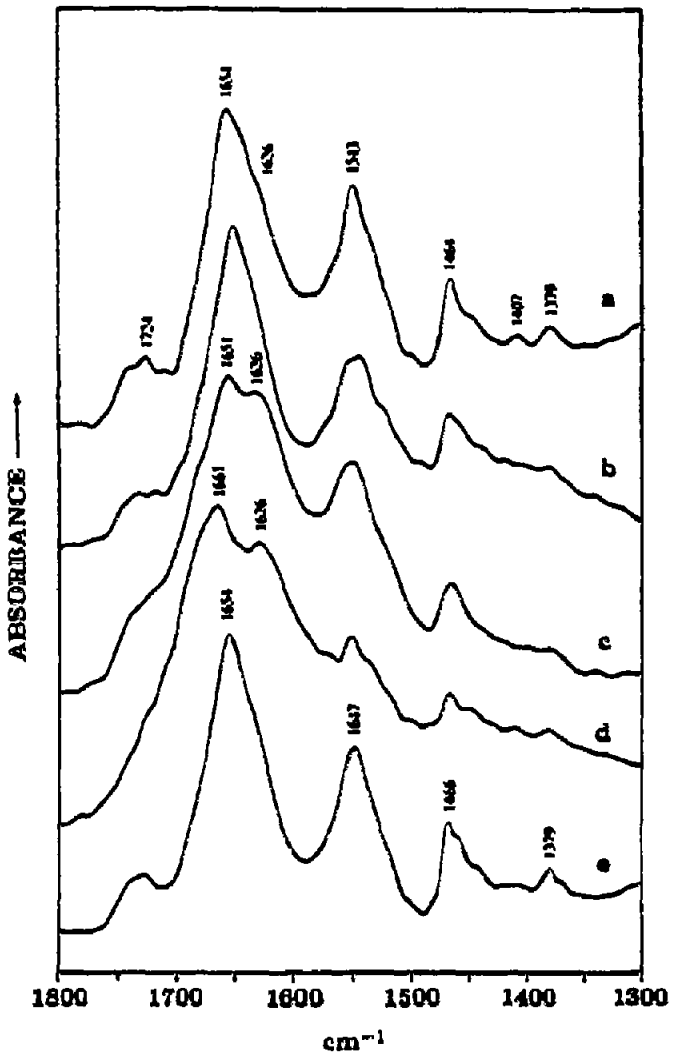

Fig. 1. Infrared spectra of myelin suspensions (16 mg protein/ml) in the following media: a, distilled water; b, 0.2 M phosphate buffer; c, 0.1 M sodium sulfate; d, 0.1 M sodium phosphate buffer containing 0.1 M sulfate; e, 0.1 M sodium phosphate buffer containing 0.1 M sodium sulfate and 0.03 M octyl glucoside detergent.

adopt the predominant random coil conformational structure as well as turns and β-sheets. However, the α-helical bands located around 1650 cm^{-1} are masked by the aforementioned strong bands at 1661 and 1626 cm^{-1}. According to this, its respective second derivative spectrum (Fig. 2c) includes the strongest band at 1660 cm^{-1}, which indicates that the disordering effects are increased by a combination of sulfate plus phosphate anions. In comparison with the spectrum of myelin control and with that of the sodium sulfate solution, a greater polypeptide backbone disorder is also observed as indicated by the halfbandwidth.

When OG is added to the latter anion mixture solution (Fig. 1e and its second derivative spectrum in Fig. 2d), a spectrum similar to that of the myelin control is observed, where the predominant secondary structure,

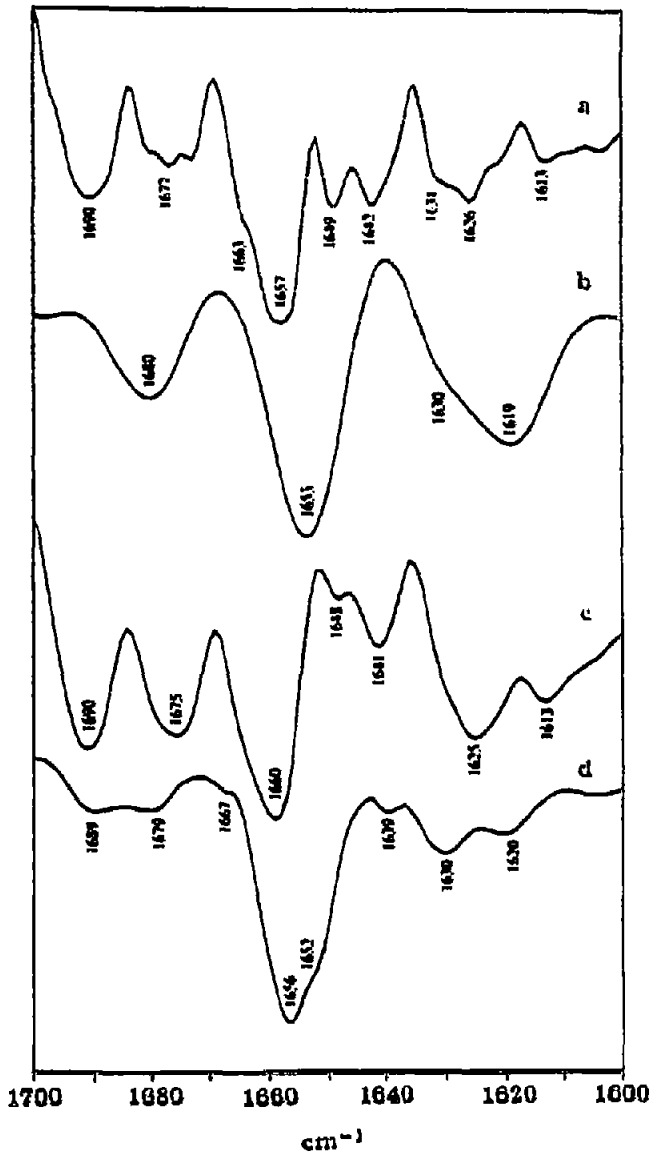

Fig. 2. Second derivative spectra of myelin suspensions (16 mg protein/ml) in the following media: a, distilled water; b, 0.1 M sodium sulfate; c, 0.1 M sodium phosphate buffer containing 0.1 M sodium sulfate; d, 0.1 M sodium phosphate buffer containing 0.1 M sulfate and 0.03 M octyl glucoside detergent.

on the basis of the above, is α-helical, and small amounts of β-sheet and random coil structures are also present.

All these qualitative results are consistent with the quantitative ones included in Table I, where myelin control shows a great proportion of α-helices (51%) and smaller amounts of β-sheet as well as unordered structures (29% and 20%, respectively). When myelin was suspended in phosphate buffer, no significant percentages of conformational changes were observed. However, in sodium sulfate solution, an 11% decrease for α-helices takes place, and at the same time β-sheet and unordered structures increase (6% and 5%, respectively)

Table I

Estimates of protein secondary structures in myelin samples suspended in different media[a]

Media[b]	α-Helices	β-Sheets	Unordered
Water	51	29	20
Sodium phosphate buffer	50	30	20
Sodium sulfate in water	40	35	25
Sodium sulfate in sodium phosphate buffer	20	36	44
Sodium sulfate in sodium phosphate buffer and octyl glucoside detergent	50	31	19

[a]Percentages of areas.
[b]Conditions used as described in section 2.

at the expense of α-helices. When the membrane is suspended in phosphate buffer containing sodium sulphate, a great decrease in α-helices is observed (31%) as a result of the moderate increase of β-sheets (7%) and the strong rising of unordered structures (24%). However, these conformational changes do not take place when OG is present in the latter anion medium, which is reflected by the maintaining of the protein structure of the membrane as shown by the percentages of areas obtained (50% α-helices, 31% β-sheets and 19% unordered) which are very similar to those obtained for myelin suspended in water.

4. DISCUSSION

A comparative structural study of myelin suspended in different media has been carried out by infrared spectroscopy, mainly through the infrared amide I band region. Some significant results emerge from this study as shown in Figs. 1 and 2, and in Table I. Firstly, the great protein conformational changes produced by sodium sulfate solution and preferentially by sodium-phosphate buffer containing sodium sulfate. Secondly, the avoiding of these protein conformational changes when OG is present in the latter anion mixture solution. Thirdly, these results are in contrast with those obtained for sodium phosphate buffer, in which the anion practically does not alter the protein conformational structure of myelin. This means that the said conformational changes are caused by the sulfate anion, either alone in water solution or preferentially in the presence of the phosphate anion.

The protein conformational changes could be explained on the basis of electrostatic repulsions between the lipids with negative charges and sulfate anions (SO_4^{2-}). According to this interpretation, the repulsion would detach lipids from the myelin proteins, particularly from proteolipid and basic proteins which are the main protein components of this membrane. In this way, water would be allowed to reach protein domains to form water clusters [20]. Consequently, water would

cause increases of β-sheet and unordered structures at the expense of α-helices through breaking intrachain α-helical hydrogen bonds [12,13].

The conformational changes strongly increase when sulfate is added to phosphate buffer (Figs. 1d, 2c and Table I). In fact, in this case the absorption maximum appears at 1661 cm^{-1}, which is attributable to random coil structures, and the amide I band masks to a large extent the carbonyl bands of lipids. These results are not attributable exclusively either to phosphate or to sulphate anions alone, but to sulfate-phosphate hydrogen bonded dianions. Although the phosphate anion in the lyotropic series of Hofmeister is a more effective agent on protein structure than sulfate, in practice phosphate does not produce significant protein conformational changes (Fig. 1b, Table I) because phosphate consists mainly of a mixture of HPO_4^{2-} and $H_2PO_4^-$ anions, at neutral pH, rather than of the more effective PO_4^{3-} [21]. However, the addition of sulfate to phosphate buffer may cause the formation of the hydrogen-bonded dianions $^{-2}O_3POH\cdots O_4S^{2-}$ and $^{-}O_3HPOH\cdots O_4S^{2-}$[22],which would lead to more electrically charged anionic species. Consequently, the resulting dianions would cause stronger electrostatic lipid–dianion repulsions (Figs. 1d and 2c) than the lipid–sulfate (Figs. 1c and 2b) or lipid–phosphate (Fig. 1b) ones. The said electrostatic repulsions between the hydrogen-bonded dianions and myelin lipids would allow greater amounts of water to reach the protein domains and would largely cause the breaking of intrachain and interchain α-helical and β-sheet hydrogen bonds. This leads to a great increase in unordered structure, as revealed by one of the main quantitative results reflected in Table I relative to the 24% increase in unordered structure.

The reported structural disordering effect of sulfate-phosphate is avoided when OG detergent is present in this anion aqueous medium (Fig. 1e, Table I). In this case the membrane protein structures are practically unaltered, as shown by the 50% of α-helices, 31% β-sheets and 19% unordered structures, which are very similar percentages to those of the myelin control. This can be attributed to the anion–lipid electrostatic repulsions facilitating the separation of lipids from the membrane, which results in an easier binding of OG to myelin proteins to avoid their denaturation [23,24].

These interpretations are also consistent with the nature of lipid–protein interactions. Extrinsic proteins, like myelin basic proteins, are associated with membranes through electrostatic interactions as well as minor hydrophobic interactions [25]. Reversely, intrinsic proteins, like myelin proteolipid proteins, interact hydrophobically with lipids by crossing the bilayer several times [26–29]. Moreover, both proteins possess net positive charges which are responsible for a strong preferential binding to acidic lipids with negative charges [25–28]. On the other hand, the aforementioned different suspension media supply various capacities for anion–lipid headgroups electrostatic interactions as occurs in phosphate, sulfate and phosphate-buffer media. A combination of both electrostatic and hydrophobic types of interactions is present in the suspension containing sulfate-phosphate-OG detergent. In the phosphate buffer medium, the negative charges of the anions are not high enough to cause strong electrostatic interactions leading to the removal of lipids from proteins. Therefore, no visible myelin protein conformational changes are observed (Fig. 1b, Table I). Regarding sulfate medium, the anion–lipid electrostatic interactions are greater as a consequence of the two negative charges in sulfate anions. Consequently, a greater separation of lipids from myelin proteins occurs and subsequent protein conformational changes appear (Fig. 1c, Table I). The lipid headgroup-anions electrostatic repulsions are considerably enhanced in sulfate-phosphate suspensions [22]. Comparatively, a higher proportion of lipids is removed from myelin proteins and therefore much greater conformational changes are observed (Fig. 1d, Table I). Finally, when OG is added to the latter suspension medium, the potential protein conformational changes which would be produced by the sulfate-phosphate dianions are avoided by the hydrophobic interactions between the detergent and the myelin proteins [23,24]. Therefore, the protein conformational structures appear to be similar to those of the myelin control (Fig. 1e, Table I).

The interactions leading to the removal of lipids from proteins could be used to explain myelin solubilization in this medium. The removed lipids, which are soluble in sulfate-phosphate solution (unpublished results), facilitate the binding of the nonionic amphiphile detergent to the membrane proteins. The maintaining of the protein structure is produced by the hydrophobic moiety of OG, and the polar fragment of this molecule allows the solubilization of the myelin membrane. This mechanism could explain why sulfate-phosphate-detergent solutions cause enhancement of the myelin solubility and the saving of OG without denaturation of membrane proteins.

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