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Solubilization of sarcoplasmic reticulum membranes by sodium dodecylsulphate

A Fourier-transform infrared spectroscopic study

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In order to improve our understanding of membrane protein solubilization by sodium dodecylsulphate, sarcoplasmic reticulum vesicles have been treated with this surfactant at different detergent: protein mole ratios. Effects on Ca^{2+}-ATPase activity, membrane protein solubilization, and protein conformation have been independently monitored, and correlations among the various parameters have been observed. The thermal denaturation of sarcoplasmic reticulum proteins in the presence of sodium dodecylsulphate has also been characterized spectroscopically.

Membrane solubilization; Protein denaturation; Sarcoplasmic reticulum Ca^{2+}-ATPase; Surfactant; Fourier-transform infrared spectroscopy

1. INTRODUCTION

The study of intrinsic membrane proteins often requires their previous solubilization; this is usually carried out in the presence of surfactants [1,2]. In some instances, e.g. membrane reconstitution, 'mild', non-denaturing detergents, usually nonionic amphiphiles, are recommended [1]. In addition, membrane proteins are often solubilized for gel electrophoretic analysis. The most widely used surfactant in this context is, by far, sodium dodecylsulphate (SDS) [3,4].

Sarcoplasmic reticulum (SR) has been commonly used in membrane research [5]. Previous studies from this laboratory have characterized the solubilization of SR membranes by the nonionic surfactant Triton X-100 [6,7]. The present work is intended to describe the solubilization process induced by SDS, whose empirical effects are much better known than the molecular details of its interaction with membrane components. The effects of SDS on SR membranes have been examined from the point of view of membrane solubilization, changes in Ca^{2+}-ATPase activity, and modifications of protein structure. The latter have been studied by Fourier-transform infrared (FT-IR) spectroscopy, a method that has been successfully applied to the conformational analysis of soluble and membrane proteins [8–11].

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Abbreviations: D/P, detergent:protein mole ratio; FT-IR, Fourier-transform infrared; SDS, sodium dodecylsulphate; SR, sarcoplasmic reticulum

2. MATERIALS AND METHODS

Isolation of SR from rabbit back and leg muscle has been described previously [5,6]. For activity and turbidimetric studies aliquots of the sarcoplasmic vesicle suspension (0.9 ml) were treated with 0.1 ml of the appropriate SDS solutions in order to obtain final surfactant concentrations ranging from 10^{-5} to 10^{-2} M. Final protein concentration was 1 mg/ml unless otherwise stated. Equilibration was allowed to take place at room temperature and the turbidity measured as adsorbance at 550 nm in a Uvikon 860 Kontron spectrophotometer. Proteins were determined by the method of Lowry et al. [12]; determinations in the presence of SDS were made following the modification of Wang and Smith [13]. Ca^{2+}-dependent ATPase activity was assayed as described previously with an ATP-regenerating system [6]. For calculations of detergent:protein mole ratios (D/P) it was assumed that all the protein in our SR preparations was Ca^{2+}-ATPase ($M_r = 115000$). In addition, detergent:protein mole ratios are only an approximation because they are calculated on the total rather than the effective (membrane-bound) detergent; however, the high affinity of SDS for membranes ensures that our approximation is valid for comparative purposes within the limits of this work.

For FT-IR spectroscopy samples containing 2% (w:w) protein in 0.1 M KCl, 10 mM Hepes pD 7.4 (D_2O medium) or 3% (w:w) in 0.1 M KCl, 10 mM Hepes pH 7.4 (H_2O medium) were introduced in a Harrick cell (Harrick, Ossining, NY) with CaF_2 windows and 25 μm (D_2O medium) or 6 μm (H_2O medium) spacers. Buffer contribution was subtracted by keeping a straight line between 1900–1300 cm^{-1} using the highest possible subtraction factor for the buffer. 256 (D_2O medium) or 512 (H_2O medium) scans were collected in a Nicolet 10 DX spectrometer, apodized with a Happ-Gentzel function and Fourier transformed to give a nominal resolution of better than 2 cm^{-1}. The spectra were transferred to an IBM personal computer where the resolution enhancement calculations were performed by standard methods [8–11, 14].

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3. RESULTS

Membrane solubilization by detergents may be conveniently monitored by means of turbidity measurements [6]. Changes in turbidity have been expressed as '% turbidity', i.e. percent decrease in turbidity with respect to the untreated sarcoplasmic reticulum suspension. The effect of SDS upon sarcoplasmic reticulum is not linearly related to the amount of detergent (Fig. 1). It is apparent that the surfactant produces little change in absorbance at 550 nm up to 7×10^{-4} M (D/P≈ 80:1); an increase in turbidity at 3×10^{-4} M is probably due to vesicle aggregation and/or fusion [15]. Above 7×10^{-4} M, SDS produces a sudden decrease in A_{550}. Surfactant concentrations higher than 2×10^{-3} M do not cause any further decrease in turbidity indicating that solubilization is complete. This decrease in absorbance at 550 nm is preceded by an increase in ATPase activity at SDS concentrations above 10^{-4} M. The increase is maximum around $6\text{–}8\times10^{-4}$ M and then the activity decreases until complete inactivation, concomitant with complete solubilization (Fig. 1).

The conformation of SR in the presence of SDS at D/P ≈ 80:1, when surfactant-induced ATPase activation is maximal, and 1200:1, when the membranes are fully solubilized and the enzyme activity is lost, has been studied by FT-IR. Spectral measurements require higher protein concentrations than turbidity or enzyme assays. However, it has been shown that similar detergent:protein ratios produce the same effects over a large range of protein concentrations [6]. Previous studies have also demonstrated that, in order to study protein conformational changes, both D_2O and H_2O media should be used [9,10,16]. The 1600–1700 cm^{-1} region of the infrared spectrum of SR shows, as its main feature, the so-called Amide I band, with a maximum at 1648 cm^{-1} (original spectrum not shown). However, if Fourier derivative is performed (Fig. 2A), peaks corresponding to protein structure are seen in native (SR D_2O medium) at 1625, 1635, 1647, 1656, 1668, 1682 and 1698 cm^{-1}. α-Helix appears as the major protein structure, with contributions of β-turns and unordered structure as shown previously [16]. In the presence of SDS at D/P 1150:1 (Fig.2E) there are protein bands at 1631, 1643, 1656, 1668, 1680 and 1690 cm^{-1}. The derivative intensity ratio is not a good measurement of the integrated intensity of the different bands, but in combination with the original spectrum can give a qualitative idea of the events taking place in the protein; an increase of the 1643 cm^{-1} band, attributed to random coil, together with a decrease in the contribution of the band around 1630–1635 cm^{-1} (β-sheet) can be expected from these derivative spectra. This behaviour is compatible with the band shift and the bandwidth changes in the original spectrum. This analysis is confirmed by the Fourier derivative spectra of the Amide I band in

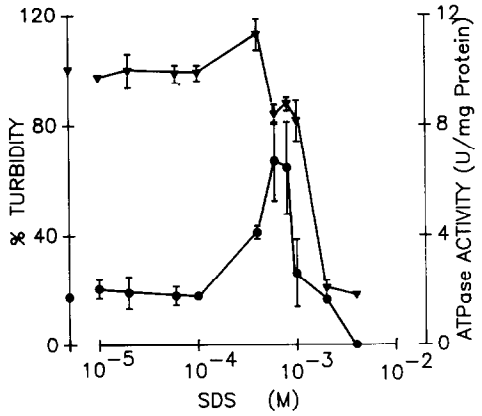

Fig. 1. The effect of SDS on sarcoplasmic reticulum suspensions. (▼) Changes in turbidity; (●) changes in Ca^{2+}-ATPase activity. Data correspond to average values ± SE (n = 4).

H_2O medium (Fig. 2B) where maxima are seen at 1658 and 1653 cm^{-1}, indicating the presence of random coil and α-helix structures with additional bands at 1645 and 1635 cm^{-1}, attributed to β-sheet [17].

When SR is in the presence of SDS (D/P ≈ 80:1) and the enzyme activity of the Ca^{2+}-ATPase shows a maximum, the spectral changes are somewhat intermediate between the two situations described above. In D_2O medium (Fig. 2C), there is an increase in the 1645 cm^{-1} band, now at 1643 cm^{-1}, but not as large as with the high SDS concentration. The 2 cm^{-1} shift suggests an increased proportion of the random coil component. The 1670–1700 cm^{-1} region, where the bands arising from β-turns appear, gives rise to a single band at 1682

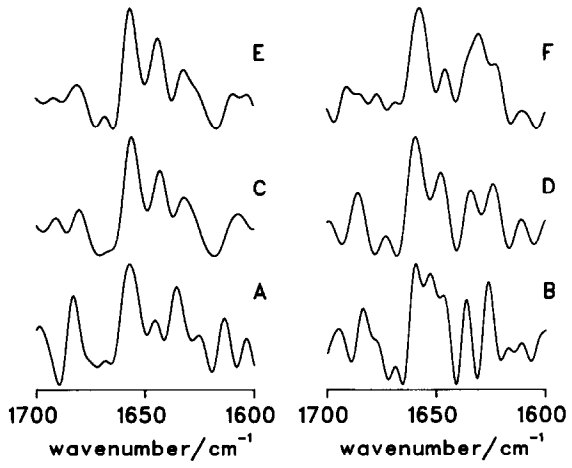

Fig. 2. Fourier derivative spectra of native and detergent-treated sarcoplasmic reticulum. The infrared spectrum of SR prepared according to Nakamura et al. [5] is indistinguishable from the preparation in the E_1 state as described by Arrondo et al. [16]. Derivatives were obtained with a power of 3 and a breakpoint of 0.3. The original spectra were taken in either D_2O (A,C,E) or H_2O (B,D,F) buffer. (A,B) Native sarcoplasmic reticulum; (C,D) sarcoplasmic reticulum in the presence of SDS at a ≈80:1 detergent:protein mole ratio; (E,F) id. id. at a ≈1150:1 detergent:protein mole ratio.

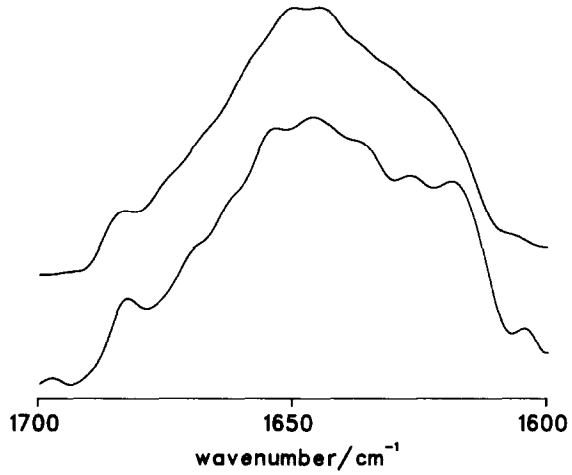

Fig. 3. Thermal denaturation of sarcoplasmic reticulum. The curves correspond to Fourier self-deconvolved spectra using a full-width at half-height of 14 and a resolution enhancement factor of 2. The measurements were made in D_2O buffer, in the absence (lower curve) or presence (upper curve) of SDS at ≈1150:1 detergent:protein mole ratio.

cm^{-1}. The signal appearing at 1635 cm^{-1} in native SR is now at 1633 cm^{-1} having also decreased in intensity, whereas at the high SDS concentration it shifts up to 1631 cm^{-1}. The Fourier-derivative spectra in H_2O (Fig. 2B,D,F) confirm the D_2O results with the merging of the 1653 (α-helix) and 1659 cm^{-1} (random) bands.

Temperature is known to influence enzyme activity as well as protein conformation. Also, membrane proteins are often heated in the presence of SDS in order to facilitate solubilization [3]. The Fourier self-deconvolved spectrum at 60°C of the protein in the absence of surfactant (Fig. 3) shows bands at 1616 and 1683 cm^{-1}; they have been attributed to extended chains interacting with aminoacid side chains, the so-called β-edge structure [18], also with an increase in the bands corresponding to random coil and α-helix (1645 and 1654 cm^{-1} respectively). In the presence of SDS at high concentration, the infrared spectrum at 60°C is quite different; the band is centered around the random coil structure, and only small shoulders at 1619 and 1685 cm^{-1} are present. The picture for SR at 60°C in the presence of 80:1 SDS (not shown) is again intermediate between the above two.

4. DISCUSSION

The solubilization process of SR by the anionic surfactant SDS is not linearly related to detergent concentration, but occurs at a critical detergent/membrane ratio of about 1.5 μmol SDS/mg protein (D/P ≈ 170:1) (Fig. 1). A similar behaviour had been observed previously for the same membrane with Triton X-100 [6]; the solubilization process took place at about 2 μmol surfactant/mg protein. When surfactant effect upon enzyme activity is compared, SDS also acts in a fashion similar to Triton X-100: low SDS concentrations increase enzyme activity, while higher concentrations have the opposite effect [6]. A typical feature of SDS is that 10^{-2} M surfactant produces complete enzyme inactivation, which was not the case with Triton X-100 [6].

FT-IR reveals that SDS produces, in a concentration-dependent way, a decrease in β-sheet structure, a change in β-turns and an increase in unordered structure. The increase in enzyme activity induced by SDS at D/P ≈ 80:1 is concomitant with a reduction in the β-sheet structure and a rearrangement of β-turns, the effect being similar to that observed with Triton X-100 [7]. The structure of SR consists of an intramembranous, primarily helical domain, and several cytoplasmic domains composed of α-helix, β-sheet, β-turn and unordered elements [19]. The infrared spectrum suggests therefore an influence of the surfactant molecules on the cytoplasmic, rather than the intramembranous domains of Ca^{2+}-ATPase at surfactant concentrations that enhance enzyme activity. However, it has been shown [20] that, at these D/P ratios, the ability of SR vesicles to accumulate Ca^{2+} is impaired, probably because of an increased bilayer permeability. Thus SDS could act as an uncoupler for the Ca^{2+}-ATPase, and the increase in enzyme activity could be due to either or both surfactant effects, on the protein and/or on the bilayer. The detergent action on the protein might consist of a 'loosening' of the channel gate, or of a barrier composed of β-sheet structure in front of the channel gate, thus facilitating the conformational change induced by ATP binding [21] and stimulating Ca^{2+} pumping. At high SDS concentration the picture is different: an increase of the unordered structure is quite obvious, even if there is still some α-helix present. The loss of enzyme activity can be due to at least two factors: one, as pointed above, that the overall protein conformation in the micelle is greatly modified, and the other that, even if the channel structure is not disturbed, it is kept inside the micelle thus hindering the substrate from reaching the enzyme active site.

Temperature is known to produce a conformational change in SR with a midpoint at 42°C. This thermal denaturation 'opens' the polypeptide chain rearranging it and modifying the pattern of intra and intermolecular interactions within the protein and with the solvent [16]. In Fig. 3 this rearrangement is shown by the new bands at 1616 and 1683 cm^{-1} in the thermally denatured SR. However, after thermal denaturation in the presence of SDS, the picture is quite different with almost all the protein in an unordered conformation, suggesting that after polypeptide chain opening there is an interaction with the surfactant molecules that stabilizes an unfolded structure.

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