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OPTICAL PROPERTIES OF AN OUTER MEMBRANE LIPOPROTEIN FROM *ESCHERICHIA COLI*

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Summary

The infrared spectrum of a structural lipoprotein from the *Escherichia coli* outer membrane indicated the lipoprotein had and α-helical conformation but no sign for the existence of β-structures. From circular dichroism spectra of the lipoprotein, the α-helical content of the protein was found to be as high as 88% in 0.01—0.03% sodium dodecyl sulfate in the presence of 10^{-5} M Mg^{2+} at pH 7.1 and 23°C. When sodium dodecyl sulfate concentration increased higher than 0.1%, the α-helical content of the lipoprotein decreased to about 57%. Divalent cations, such as Mg^{2+} and Mn^{2+}, were found to increase the helical content of the lipoprotein. The high α-helical content of the lipoprotein was observed in a wide range of temperatures (23 to 55°C). The significance of the high α-helical content of the lipoprotein is discussed in light of the three-dimensional molecular models of the lipoprotein proposed previously.

Recently we have purified and paracrystallized a structural lipoprotein of molecular weight 7200 from the outer membrane of *Escherichia coli* [1]. This membrane protein has been extensively investigated from various points of view. Its entire chemical structure has been determined [2,3], its biosynthesis has been studied [4—10], and its cell-free synthesis directed by the purified messenger RNA has been shown [11,12]. Furthermore, the structure of its messenger RNA [13] and ultrastructures of its paracrystals [14] have been investigated. The lipoprotein has been shown to have a very high α-helical content [1,15,16] and three-dimensional molecular assembly models have been proposed by Inouye [17] and Braun [16].

In the present paper we have performed a further detailed analysis of the optical properties of the purified lipoprotein under various conditions.

Materials and Methods

Purification of the lipoprotein

The lipoprotein was purified from *E. coli* B (late exponential phase; Grain Processing Corp., Muscatine, Iowa) according to the method of Inouye et al. [1].

Infrared spectrum

Infrared spectra were measured by a Perkin-Elmer 567 Grating Infrared Spectrophotometer. A standard polystyrene film was used to calibrate absorption peak frequencies. 3 mg of the lipoprotein were used to prepare KBr solid films. The ^2H_2O treated lipoprotein was prepared as described by Nakamura et al. [18].

Circular dichroism measurements

Circular dichroism spectra were measured with a JASCO J-20A Automic Recording Spectropolarimeter. For measurements of circular dichroism spectra at different temperatures, a Cary Model 60 Spectropolarimeter equipped with a 6001 Circular Dichroism Accessory was used. The instruments were standardized with an aqueous solution of (+)-10-camphorsulfonic acid. Mean residue ellipticity, $[\theta]$, in (degree · cm^2)/dmol was calculated from the following equation:

$$[\theta]_{208 \text{ or } 222 \text{ nm}} = (\theta_{208 \text{ or } 222 \text{ nm}}/10)\,(M_{\text{mrw}}/L \cdot c)$$

where θ, M_{mrw}, L and c represent the observed ellipticity, the mean molecular weight of the amino acid residues, the light path length of the cell, and the concentration of the protein in g/ml, respectively. Spectra were traced at 2 cm per minute and the scale was 10 mdegree/cm.

For each circular dichroism spectrum α-helical content was estimated from the following two independent equations:

$$\% \text{ helix} = ([\theta]_{208\text{nm}} - 4000)/(29\,000) \times 100\% \quad (1)$$

$$\% \text{ helix} = ([\theta]_{222\text{nm}} - 2340)/(30\,300) \times 100\% \quad (2)$$

Eqn. 1 was derived by Greenfield and Fasman [19] on the basis of data from polylysine. Eqn. 2 was obtained from data of five globular proteins by Chen and Yang [20].

Other measurements

The concentrations of the lipoprotein were determined by amino acid analysis with a JEOL 6-AH analyzer. The lipoprotein was hydrolyzed in 6 M HCl for 16 h at 110° C in a vacuum sealed tube before analysis.

Magnesium content was determined spectrophotometrically at 285.2 nm with a Perkin-Elmer 503 Atomic Absorption Spectrometer.

Results

Infrared spectra

Fig. 1 shows the amide I and II regions of the infrared spectrum of a dried

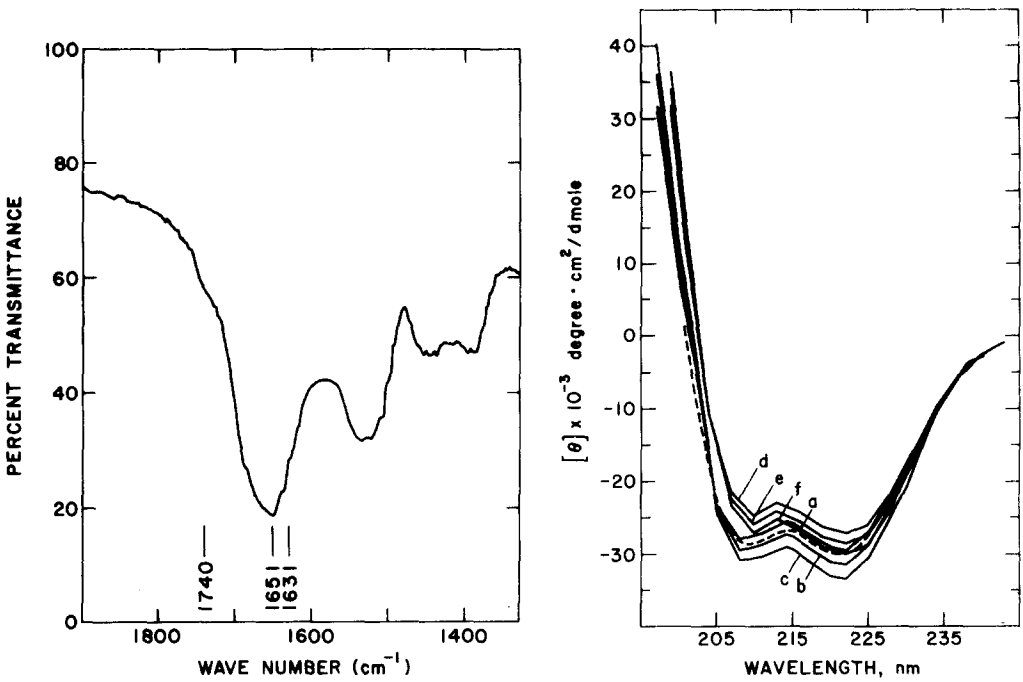

Fig. 1. Infrared spectrum of a KBr film of the lipoprotein treated with ^2H_2O.

Fig. 2. Comparison of an experimental circular dichroism spectrum of the lipoprotein in 0.015% sodium dodecyl sulfate containing 10^{-5} M Mg^{2+} at pH 7.2 and 23°C with computed circular dichroism spectra. ------, experimental and ———, computed curves. Curves a, b and c were computed from the data of polylysine obtained by Greenfield and Fasman [19] (Eqn. 1) assuming 85% helix and 15% random coil, 90% helix and 10% random coil, and 95% helix and 5% random coil, respectively. Curves d, e, and f were computed from the data of five globular proteins reported by Chen et al. [24]. (Eqn. 2) assuming 85% helix and 15% random coil, 90% helix and 10% random coil, and 95% helix and 5% random coil, respectively.

film of the lipoprotein treated with ^2H_2O. The amide I band has a peak at 1651 cm^{-1} and no shoulder at 1631 cm^{-1} indicating the lipoprotein contains α-helical and/or random coil but little β-structure [18,21]. There is no significant shift of the amide I band as compared to the untreated lipoprotein (data not shown) suggesting that the helical structure is dominant since random coil is the only conformation which readily changes the position of the amide I band upon ^2H_2O treatment [22]. It has been shown that there are two fatty acids linked to the N-terminal glycerylcysteine of the lipoprotein through ester bonds [3]. Thus the shoulder at 1740 cm^{-1} could be due to the stretching of the C = 0 of those ester groups as observed in other fatty acid esters [23].

Circular dichroism spectra

In order to determine the helical content of the lipoprotein we have attempted to measure the circular dichroism spectra of the lipoprotein. Since the lipoprotein was not soluble in water, the circular dichroism spectra of the lipoprotein were measured in the presence of sodium dodecyl sulfate. Fig. 2 shows the circular dichroism spectrum of the lipoprotein in 0.015% sodium

dodecyl sulfate containing 10^{-5} M Mg^{2+} at pH 7.2 and 23°C. The spectrum was compared with computed theoretical curves based on data from polylysine [19] (curves a, b, and c) and five globular proteins [20,24] (curves d, e, and f). Curves a and d were computed assuming that the lipoprotein has 85% helix and 15% random coil; b and e were obtained for 90% helix and 10% random coil; c and f were for 95% helix and 5% random coil. As shown in Fig. 2, the experimental spectrum is just between the theoretical curves a and b, indicating the lipoprotein contains 88% helix and 12% random coil under the stated conditions. Combinations of α-helix and β-structure instead of random coil did not result in better fitting than a and b in Fig. 2. It can thus be concluded that the lipoprotein had very little or no β-structure under these coniditions as suggested by its infrared spectrum (Fig. 1).

When sodium dodecyl sulfate concentration increased higher than 0.03% the ellipticity at 208 nm is noticeably lower than that at 222 nm. Previous studies have shown that the presence of sodium dodecyl sulfate seemed to enhance the ellipticity at 208 nm [25—27]. The reason for this effect of sodium dodecyl sulfate is not known. Fig. 3 shows a circular dichroism spectrum of the lipoprotein in 0.1% sodium dodecyl sulfate. As discussed above the ellipticity at 208 nm is much lower than at 222 nm. None of the theoretical circular dichroism spectra computed from possible combinations of α-helix, random coil, and β-structures fitted the experimental spectrum in Fig. 3. Therefore knowing that ellipticities at the 208 nm region were modified in higher sodium dodecyl sulfate concentrations we have attempted to fit the spectrum with theoretical ones only in regions above 218 nm. In Fig. 3, curves a, and b were computed on the basis of the data from polylysine [19], assuming that the lipoprotein contains 66% α-helix and 34% random coil, and 66% α-helix and 34% β-structure, respectively. Curves c and d were calculated on the basis of the data from five globular proteins [20,24], assuming that the lipoprotein contains 66% α-helix and 34% random coil, and 66% α-helix and 34% β-structure, respectively. The best matchings above 218 nm are observed in curves a and c both computed assuming 66% helix and 34% random coil structure.

Effect of sodium dodecyl sulfate and Mg^{2+} on the helical content

Fig. 4 shows the helical content of the lipoprotein with and without exogenous Mg^{2+} in various concentrations of sodium dodecyl sulfate at pH 7.2. When sodium dodecyl sulfate concentrations were higher than 0.05% the helical content was estimated only by equation (2) since equation (1) is based on the molar ellipticity at 208 nm, which became anomalously high at high sodium dodecyl sulfate concentrations as discussed above. In lower than 0.05% sodium dodecyl sulfate, both Eqns. 1 and 2 were used independently to estimate the helical content. Fig. 4 shows that at low sodium dodecyl sulfate concentrations, the lipoprotein had a higher helical content in the presence of Mg^{2+} than in the absence of the ion. At these low sodium dodecyl sulfate concentrations (<0.03%), the helical contents estimated by Eqn. 1 were found to be more appropriate than those estimated by Eqn. 2 from the comparison of experimental spectra with computed spectra as shown in Fig. 2. As sodium dodecyl sulfate concentration increased higher than about 0.03% the helical content of the lipoprotein decreased. The solution with exogenous Mg^{2+} has a faster rate

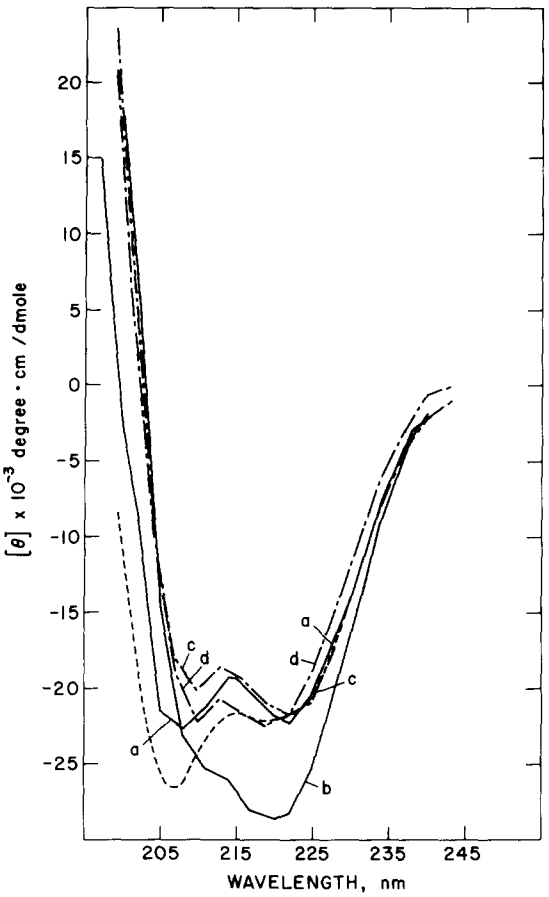

Fig. 3. Comparison of an experimental circular dichroism spectrum of the lipoprotein in 0.1% sodium dodecyl sulfate at pH 7.2 and 23°C with computed circular dichroism spectra. - - - - - -, experimental; and ——— and — · — · — computed curves. Curves a, and b were computed from the data of polylysine obtained by Greenfield and Fasman [19] assuming 66% α-helix and 34% random coil, and 66% α-helix and 34% β-structure, respectively. Curves c and d were obtained from the data of five globular proteins reported by Chen et al. [24], assuming 66% α-helix and 34% random coil, and 66% α-helix and 34% β-structure, respectively.

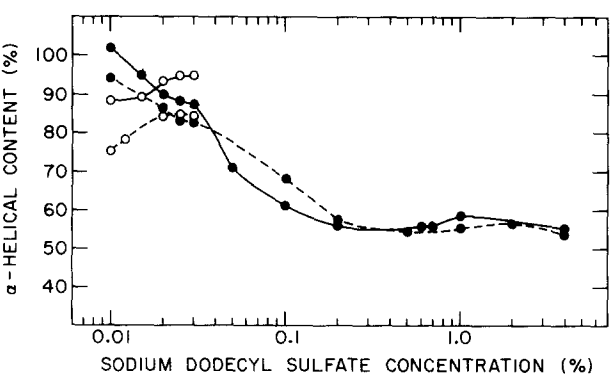

Fig. 4. Effects of sodium dodecyl sulfate and magnesium ion on the helical contents of the lipoprotein. ———, in the presence of 10^{-5} M Mg^{2+}; - - - - - -, no Mg^{2+} was added. ○, α-helical contents estimated by Eqn. 1; ●, by Eqn. 2.

of decrease. With sodium dodecyl sulfate concentrations higher than about 0.15% the helical content is essentially constant (≈57%).

The amino acid composition of the lipoprotein shows that there are more acidic than basic residues. Thus at pH 7.2 the lipoprotein should carry negative charges. Mg^{2+} in low sodium dodecyl sulfate concentration (<0.05%) may bind to those negative groups and hence increase the aggregation of molecules as well as the apparent helical content of the lipoprotein as shown in polypeptides [19,28,29]. At pH 7.2 the ionic strength of 0.01 M sodium phosphate buffer is 0.02. According to Reynolds and Tanford [30] the critical micelle concentration of sodium dodecyl sulfate at this ionic strength is 4 mM which is equivalent to 0.12% sodium dodecyl sulfate. They also showed that only the monomer form of sodium dodecyl sulfate can bind to proteins. Thus beyond the critical micelle concentration (0.12%) there was no further binding of sodium dodecyl sulfate to the lipoprotein resulting in no further change in the helical content. The same effects were also observed with manganous ion indicating that these divalent ions can somehow increase the helical content of the lipoprotein molecular at low sodium dodecyl sulfate concentration. It has been shown that Mg^{2+} was required to paracrystallize the lipoprotein suggesting that Mg^{2+} has an important role in controlling the conformation of the lipoprotein molecule [14].

Effect of temperature on the circular dichroism spectra

The effect of temperature on the circular dichroism spectra of the lipoprotein in 0.1% sodium dodecyl sulfate, pH 7.2, has been studied (data not shown). Between 23 and 55°C the lipoprotein appeared to maintain a stable conformation under the stated conditions. When the temperature rose to 60°C, however, the minimum at 208 nm disappeared and the trough at 222 nm became broader. It is interesting to note that the lipoprotein became less soluble at 60°C and sometimes formed insoluble fibrous structures which showed different ultrastructures from those of the paracrystals. Detailed investigations of the ultrastructures of these fibers are now in progress.

Discussion

Two different three-dimensional molecular assembly models of the lipoprotein have been proposed by Inouye [17] and Braun [16]. In the former model the helical content is predicted to be 91% whereas in the latter model the lipoprotein consists of 66% helical, 31% β-structures, and 3% random structures as predicted from the amino acid sequence using the empirical rules developed by Chou and Fasman [31]. The present studies show that the lipoprotein contains very little, if any, β-structures as revealed by its infrared spectrum (Fig. 1). Furthermore, the circular dichroism data indicate that α-helix is the predominant conformation in the lipoprotein under our experimental conditions. The lipoprotein exhibits about 88% helical structure in the presence of 0.015% sodium dodecyl sulfate which is close to the prediction of the former model [17]. At higher sodium dodecyl sulfate concentration, the apparent helical content decreased to about 75%. In this case the remaining structure appears to be predominantly random coil rather than β-structure from the comparative anal-

ysis of experimental and theoretical spectra.

In addition, the amphipathic helix-phospholipid complex model as proposed by Segrest et al. [32,33] can also be a possible model for the lipoprotein in the membrane. This model also predicts high α-helical content but with different arrangement of phospholipid molecules as proposed by Inouye [17]. However, these conformations of the purified lipoprotein in sodium dodecyl sulfate solution, described in the present study, do not necessarily reflect the actual conformation of the lipoprotein in the membrane.

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