A MAJOR OUTER MEMBRANE PROTEIN (O-8) OF *ESCHERICHIA COLI* K-12 EXISTS AS A TRIMER IN SODIUM DODECYL SULFATE SOLUTION

Fujio YU, Shigeyuki ICHIHARA and Shoji MIZUSHIMA
Laboratory of Microbiology, Faculty of Agriculture, Nagoya University, Chikusa-ku, Nagoya 464, Japan

Received 4 December 1978
Revised version received 24 January 1979

1. Introduction

Outer membrane protein O-8 (identical with Ib[1], 1b[2] and c[3]) of *Escherichia coli* is a matrix protein specific for K-12 [4]. This protein exhibits a strong interaction with the peptidoglycan layer even in the presence of sodium dodecyl sulfate (SDS) [5–7], and is thought to play a central role in the assembly of the outer membrane on the peptidoglycan layer [8]. This protein was purified in SDS solution [9]. The purified O-8 is functionally active as a component of the receptor for phage T4 [10].

We have studied the physico-chemical properties of O-8 in SDS solution and determined its molecular weight. The protein exists as a trimer in SDS solution unless it is heated. Furthermore, the trimer is stable in a SDS solution containing 8 M urea. Anomalous migration behavior of O-8 on urea–SDS polyacrylamide gel electrophoresis was also studied.

2. Materials and methods

2.1. *Outer membrane protein O-8*

Protein O-8 was purified to homogeneity from *E. coli* YA21 (K-12, *met leu* F^- λ^-) as in [7,9]. The amount of O-8 was determined by dry weight measurement after removal of SDS by acetone extraction [11] and dialysis. Heat-treatment of the protein was carried out in 1% SDS at 100°C for 5 min.

2.2. *Sedimentation analysis*

Sedimentation equilibrium and velocity experiments were carried out at 0.4 mg protein/ml with a Hitachi UCA-1 ultracentrifuge equipped with an ultraviolet absorption scanning system. Either buffer A or buffer B was used. Buffer A contained 0.1 M sodium phosphate (pH 7.2), 0.04 M NaCl, 0.1% SDS and 0.02% sodium azide. Buffer B was the same as buffer A except that it also contained 8 M urea.

2.3. *Binding of SDS to protein*

The amount of SDS bound to O-8 (1.5–5 mg) was determined by a gel filtration technique based on the method in [12]. A Sephacryl S-200 column (1 × 43 cm) equilibrated with buffer A or B was used. Protein was determined by the Lowry method [13], using pure O-8 as standard. The amount of SDS was determined as in [14].

2.4. *Crosslinking*

Protein O-8 was crosslinked in SDS solution with dimethylsuberimidate as in [15]. Crosslinking was also carried out after addition of 8 M urea. Crosslinked samples were desalted by dialysis against 1% SDS and analyzed by SDS–polyacrylamide gel electrophoresis in the presence of 8 M urea as in [16] with 4% polyacrylamide.

3. Results and discussion

3.1. *Purified O-8 exists as a trimer in SDS solution*

Physico-chemical properties of O-8 were studied in buffer A or B at SDS concentration above the critical micellar concentration (0.1%) at 25°C. They are summarized in table1.

Table 1
Summary of physico-chemical properties of O-8 before and after heat-treatment in SDS solution

	SDS bound/g protein (δ_D) g	$M(1-\phi'\rho)$	Molecular weight (M)	Sedimentation coefficient (s) × 10^{-13} s	Stokes radius (R_s) Å
Nontreated	0.63 (0.2)	4.3×10^4	121 000	7.4	53
Heat-treated	1.30 (0.72)	1.7×10^4	38 000	3.0	51.5

All determinations were carried out in buffer A, except those in parentheses that were in buffer B

The molecular weight (M) was determined from the following equation [17]:

$$M = \frac{2RT}{\omega^2(1-\phi'\rho)} \quad \frac{\mathrm{d}\ln c}{\mathrm{d}r^2} \qquad (1)$$

where ω is the radial velocity of rotation, ρ is the density of the solvent, and ϕ' is the effective partial specific volume of the protein. The factor $1-\phi'\rho$ of the equation can be replaced by $1-\bar{\nu}_p\rho + \delta_D(1-\bar{\nu}_D\rho)$ according to [17], where $\bar{\nu}_p$ is the true partial specific volume of the protein, $\bar{\nu}_D$ the partial specific volume of SDS, and δ_D the amount of SDS bound to the protein. The values of $M(1-\phi'\rho)$ of O-8 before and after heating in SDS solution were determined by sedimentation equilibrium to be 4.3×10^4 and 1.7×10^4, respectively. The amount of SDS bound to native O-8 was 0.63 g/g protein. This amount was considerably smaller than that observed with a wide variety of proteins (1.2–1.5 g/g protein [18]). Upon heating in SDS solution, the amount was increased to 1.30 g. The density of the solvent (buffer A) was determined to be 1.01 g/cm^3. The true partial specific volume of O-8 was calculated to be 0.712 cm^3/g from the amino acid composition [11] as in [19]. The partial specific volume of SDS of 0.87 cm^3/g was employed [17].

From these data, the molecular weights of O-8 before and after heating in SDS solution were calculated to be 121 000 and 38 000, respectively. The former is about 3 times the latter. Providing that O-8 after heating in SDS solution is a monomer, we concluded that purified O-8 is a trimer. This conclusion is consistent with the finding in [15], where a crosslinking study suggested that the matrix protein of *E. coli* B and protein I of *E. coli* K-12 (a mixture of O-8 and O-9) exist in the outer membrane as trimers. Recently, it was also shown by sedimentation analyses that the matrix proteins of *E. coli* B and *Salmonella typhimurium* exist as trimers (Nakae, T. personal communication). A molecular weight of 38 000 has been estimated for O-8 after heating in SDS solution, from the migration position on polyacrylamide gel [9].

As shown in table 1, Stokes radii (R_s) of the O-8–SDS complexes before and after heating in SDS solution were calculated from sedimentation equilibrium and velocity measurements to be 53 Å and 51.5 Å, respectively, according to the following equation [17]:

$$s = \frac{M(1-\phi'\rho)}{6\pi\eta N R_s}$$

where N is Avogadro's number and η is the viscosity of the solvent. It is assumed that R_s is generally a linear function of the inverse error function, erf^{-1}, of $1-K_D$ [20]. Partition coefficients (K_D) of O-8 and standard proteins complexed with SDS were determined by the gel filtration technique, and Stokes radii of standard protein–SDS complexes were taken from [17,21]. As shown in fig.1, standard protein obeyed the assumption. On the other hand, protein O-8, particularly before heating, behaved anomalously, being eluted faster for its R_s value. Fibrous proteins were indicated [22] to be anomalously retarded in gel filtration. The end-on insertion of them into the gel pores that contributed to the retardation was discussed. It is assumed that in SDS solution proteins are generally unfolded [23,24]. Therefore, the faster elution of the O-8 trimer–SDS complex for its R_s value is most likely the reflection of a highly folded

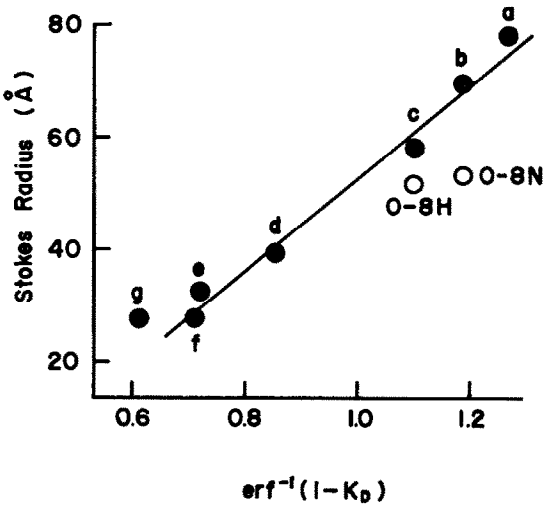

Fig.1. Chromatography of protein O-8 on Sephadex G-200 column (2.5 × 95 cm) in SDS solution (buffer A). The sample was dissolved in 2% SDS–1% 2-mercaptoethanol solution and applied on the column. Stokes radii of protein–SDS complexes were plotted as described in the text. *Abbreviations:* O-8H, heat-treated O-8 monomer; O-8N, non-treated O-8 trimer. Standard proteins: a, bovine serum albumin; b, catalase; c, ovalbumin; d, chymotrypsinogen A; e, β-lactoglobulin; f, hemoglobin; g, lysozyme.

structure of the O-8 trimer in SDS solution. This is consistent with our previous observation that the structure of major outer membrane proteins including O-8 is stable even in SDS solution [9,25].

This conclusion is further supported by the following calculation. In general, the frictional ratio (f/f_{min}), given by the following equation, of a globular protein is 1.1–1.3 in aqueous solution [21]:

$$\frac{f}{f_{min}} = \frac{(M)^{\frac{2}{3}}(1-\phi'\rho)}{6\eta s(N\pi)^{\frac{2}{3}}[\frac{3}{4}(\bar{v}_p+\delta_D\bar{v}_D)]^{\frac{1}{3}}}$$

The frictional ratios of O-8 before and after heating in SDS solution were estimated to be 1.35 and 1.65, respectively, suggesting that the trimer has a conformation close to globular even in SDS solution, while the O-8 monomer, after heating in SDS solution, has a conformation essentially differing from the globular and most likely corresponding to a protein with a melted structure.

3.2. *Anomalous behavior of O-8 trimer on urea–SDS–polyacrylamide gel electrophoresis*

The migration velocity of O-8 in SDS–polyacrylamide gel was increased upon heating in SDS solution [9]. This can be accounted for by the dissociation of the O-8 trimer to a monomer as discussed above. On the other hand, in SDS–polyacrylamide gel containing 8 M urea, native O-8 migrated even faster than did the heat-dissociated protein (fig.2a,2b and [9]). This was first thought to be due to the dissociation of the O-8 trimer to a monomer in the urea–SDS solution. However the following crosslinking study showed that O-8 still exists as a trimer in the urea–SDS solution. Protein O-8 was crosslinked in SDS solution and analyzed on urea–SDS–gel (fig.2c–f). Consistent

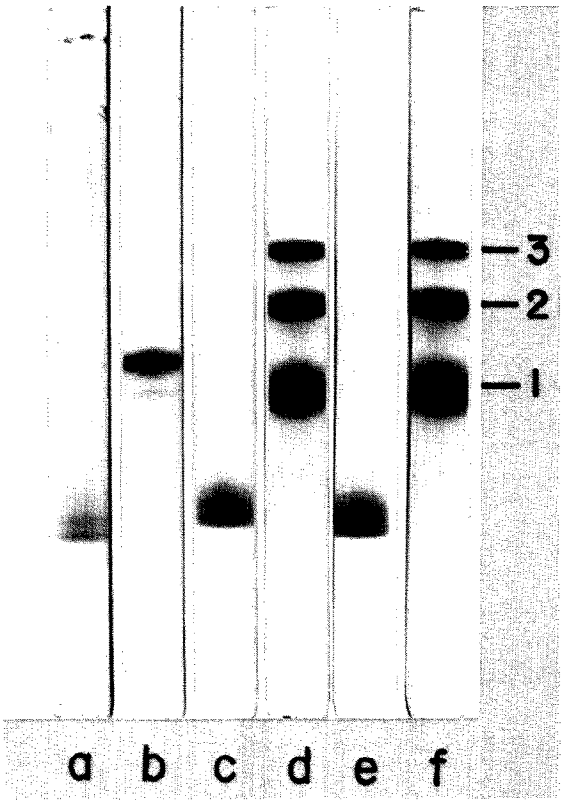

Fig.2. Gel electrophoretic mobility of protein O-8 before and after crosslinking. Samples were analyzed by urea–SDS gel electrophoresis before (a,c,e) and after (b,d,f) heat-treatment. (a,b) Non-crosslinked O-8; (c,d) O-8 crosslinked in 0.1% SDS; (e,f) O-8 crosslinked in 0.1% SDS in the presence of 8 M urea. 1,2 and 3 indicate the position of monomer, dimer and trimer of O-8, respectively.

with the results in [15], after heating in SDS solution, three main bands corresponding to monomer, dimer and trimer of O-8 were observed, indicating that individual O-8 trimers were crosslinked partly or totally (fig.2d). The crosslinking of O-8 took place even in the presence of 8 M urea to the same degree (fig.2f), strongly indicating that O-8 exists as a trimer in the urea–SDS solution.

The migration behavior of the crosslinked O-8 before heating in SDS solution should be noted (fig.2a,c,e). Regardless of whether the protein had been crosslinked or not, it migrated even faster than the heat-treated monomer O-8. Since these preparations were composed of the trimer of O-8, the result surprisingly indicates that the O-8 trimer migrated faster than the heated monomer. As shown in table 1, 8 M urea appreciably reduced the amount of SDS bound to the O-8 trimer. In addition to the highly folded structure of the O-8 trimer, this reduction may also have contributed to the anomaly, since the smaller the amount of SDS bound, the greater the effect of electric charge of protein itself on the mobility.

Acknowledgements

We thank Dr Taiji Nakae for informing us of his results prior to publication and Amano Pharm. Co. for large scale cultivation of bacteria. This work was supported by a grant from the Ministry of Education, Science and Culture of Japan.

References

[1] Schmitges, C. J. and Henning, U. (1976) Eur. J. Biochem. 63, 47–52.
[2] Diedrich, D. L., Summers, A. O. and Schnaitman, C. A. (1977) J. Bacteriol. 131, 598–607.
[3] Lugtenberg, B., Meijers, J., Peters, R., Van der Hoek, P. and Van Alphen, L. (1975) FEBS Lett. 58, 254–258.
[4] Ichihara, S. and Mizushima, S. (1977) J. Biochem. 81, 1525–1530.
[5] Rosenbusch, J. (1974) J. Biol. Chem. 249, 8019–8029.
[6] Hasegawa, Y., Yamada, H. and Mizushima, S. (1976) J. Biochem. 80, 1401–1409.
[7] Yu, F. and Mizushima, S. (1977) Biochem. Biophys. Res. Commun. 74, 1397–1402.
[8] Yamada, H. and Mizushima, S. (1978) J. Bacteriol. 135, 1024–1031.
[9] Nakamura, K. and Mizushima, S. (1976) J. Biochem. 80, 1411–1422.
[10] Mutoh, M., Frukawa, H. and Mizushima, S. (1978) J. Bacteriol. 136, 693–699.
[11] Ichihara, S. and Mizushima, S. (1978) J. Biochem. 83, 1095–1100.
[12] Helenius, A. and Simons, K. (1972) J. Biol. Chem. 247, 3656–3661.
[13] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–257.
[14] Hayashi, K. (1975) Anal. Biochem. 67, 503–506.
[15] Palva, E. T. and Randall, L. L. (1978) J. Bacteriol. 133, 279–286.
[16] Mizushima, S. and Yamada, H. (1975) Biochim. Biophys. Acta 375, 44–53.
[17] Tanford, C., Nozaki, Y., Reynolds, J. A. and Makino, S. (1974) Biochemistry 13, 2369–2376.
[18] Takagi, T., Tsuji, K. and Shirahama, K. (1975) J. Biochem. 77, 939–947.
[19] Cohn, E. J. and Edsall, J. T. (1943) in: Proteins amino acids and peptides, pp. 157–161, 370–375, Reinhold, New York.
[20] Ackers, G. K. (1967) J. Biol. Chem. 242, 3237–3238.
[21] Fish, W. W. (1975) in: Methods in Membrane Biology (Korn, E. D. et al. eds) vol. 4, pp. 189–276, Plenum Press, London, New York.
[22] Nozaki, Y., Schechter, N. M., Reynolds, J. A. and Tanford, C. (1976) Biochemistry 15, 3884–3890.
[23] Reynolds, J. A. and Tanford, C. (1970) J. Biol. Chem. 245, 5161–5165.
[24] Shirahama, K., Tsujii, K. and Takagi, T. (1974) J. Biochem. 75, 309–319.
[25] Mizushima, S. (1974) Biochem. Biophys. Res. Commun. 61, 1221–1226.