ARRANGEMENT OF THE MALTOSE-INDUCIBLE MAJOR OUTER MEMBRANE PROTEINS, THE BACTERIOPHAGE λ RECEPTOR IN *ESCHERICHIA COLI* AND THE 44 K PROTEIN IN *SALMONELLA TYPHIMURIUM*

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1. Introduction

Permeation of small hydrophilic molecules through the outer membrane in Gram-negative bacteria is by passive diffusion through waterfilled pores [1,2]. These pores are formed by major outer membrane proteins, porins, proteins Ia and Ib [3] in *Escherichia coli* [4–6] and the 36 k and 34 k proteins [7] in *Salmonella typhimurium* [8,9]. We have studied the arrangement of these porins mainly by crosslinking techniques and presented evidence that protein I in *E. coli* is a trimer [10]. A similar arrangement was also found in *S. typhimurium* for the 36 k and 34 k porins (E.T.P., in preparation).

λ receptor protein [11] in *E. coli* seems to function as a pore for maltose and maltooligosaccharides [12,13] as well as for some other sugars [14]. Recently we identified an analogous maltose-inducible major outer membrane protein in *S. typhimurium* [15], which also functions as a pore (E.T.P., in preparation). Here we have studied the arrangement of these maltose-inducible porins and present evidence suggesting that these porins are also trimers.

2. Materials and methods

The *E. coli* K12 strain TK24, lacking proteins Ia, Ib and II* [16] has been described [15]. The *S. typhimurium* strain SH6896 lacking both 36 k and 34 k proteins was kindly donated by P. H. Mäkelä.

Bacteria were grown in Luria broth [17] with maltose (0.4%) to induce λ receptor and 44 k protein, or in M9 minimal salts medium [17] with maltose (0.4%) as the carbon source and appropriate amino acids (1 mM). To obtain radioactively-labeled proteins [^{35}S]methionine was added (10 μCi/ml medium) to cells growing in minimal medium when they reached 10^8 cells/ml, and growth continued to 5×10^8 cells/ml before harvesting.

λ receptor and 44 k protein were isolated as peptidoglycan complexes [18] using isolated cell envelopes [15] as starting material. In the mutants lacking proteins Ia and Ib or 36 k and 34 k, λ receptor or 44 k protein is the major peptidoglycan-associated protein [15,19]. The proteins were released from peptidoglycan by salt treatment [20] and further purified using SDS–sucrose gradient centrifugation [10].

Crosslinking was done at room temperature as in [10,21] using the cleavable reagents tartryl-di(glycyl-azide) (TDGA; 1.3 nm bridge length) [22] and bis-methyl-4,9-diaza-5,8-dioxo-6,7-dihydroxy-dodecane-bisimidate (DOBE; 1.7 nm) [21] or the not truly cleavable dimethylsuberimidate (DMS; 1.1 nm) at concentrations indicated. The reaction was terminated by adding an excess of Tris–HCl (pH 7.4). The synthesis of DOBE will be described elsewhere (P. W., in preparation).

Crosslinked complexes were analyzed by SDS–polyacrylamide gel electrophoresis in slab gels using a continous buffer system [23] or a discontinous buffer system [10,21]. The latter was used in symmetrical two-dimensional electrophoresis [22].

3. Results and discussion

Isolated protein–peptidoglycan complexes of λ receptor or 44 k protein were incubated for 30 min with the crosslinking reagents at various concentrations (0.5–40 mM), and analyzed by SDS–gel electrophoresis. Maximal yields of crosslinked complexes were obtained using the reagent at 10–20 mM. DOBE proved to be most effective of these reagents. TDGA was as good for λ receptor but not as effective for 44 k crosslinking, DMS was inferior in both cases. Both of these proteins were not as easily crosslinked as protein I in *E. coli*. The maximal yield of crosslinked complexes obtained with λ receptor and 44 k protein was 20–30%. Figure 1A shows the results obtained with DOBE, analyzed using 10% discontinous gels. One multimer was obtained from λ receptor (fig.1A, slot 2) and two different ones from 44 k protein (fig.1A, slot 4). To prove that these complexes were derived from λ receptor and 44 k protein, respectively, the crosslinked proteins were analyzed by two-dimensional electrophoresis. The crosslinks in the protein complexes were cleaved between the two electrophoretic steps [22,21]. The results (fig.1B,C) demonstrate that the complexes seen in one-dimensional gels are multimers of λ receptor (fig.1B) and 44 k (fig.1C), respectively.

Since the molecular weights of the multimers seen in 10% gels were not exact multiples of the molecular weights of the monomers, and since we have observed anomalous behaviour of crosslinked complexes on SDS gels [10], the crosslinked λ receptor and 44 k were analyzed on continuous gels of several acrylamide concentrations (3–9%). The Ferguson plots [24,25] derived from this analysis (fig.2) show that monomers

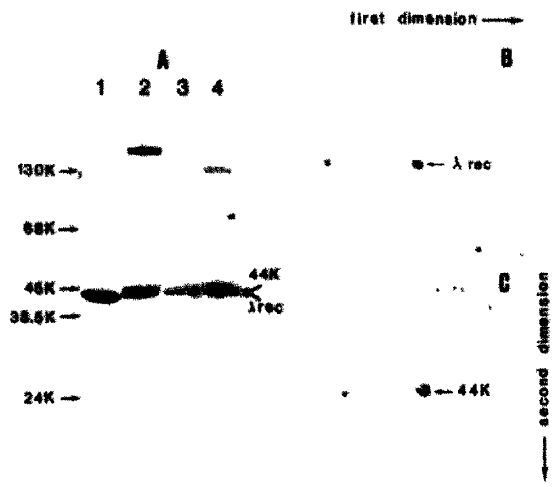

Fig.1. Crosslinked λ receptor and 44 k protein analyzed using discontinous SDS–acrylamide (10%) gels. Isolated protein–peptidoglycan complexes were crosslinked with 20 mM DOBE for 30 min before electrophoresis. (A) Slot 1, λ receptor control; slot 2, λ receptor crosslinked with DOBE; slot 3, 44 k protein control; slot 4, 44 k protein crosslinked with DOBE. The arrows at left indicate the migration of standard proteins: β-galactosidase (mol. wt 130 k) bovine serum albumin (68 k) ovalbumin (45 k) *E. coli* maltose binding protein (38.5 k), and trypsinogen (24 k). (B) Crosslinked λ receptor analyzed using symmetrical two-dimensional SDS–gel electrophoresis. (C) Crosslinked 44 k protein analyzed using two-dimensional electrophoresis. The cleavage of the crosslinks between dimensions in B and C was as in [10,21].

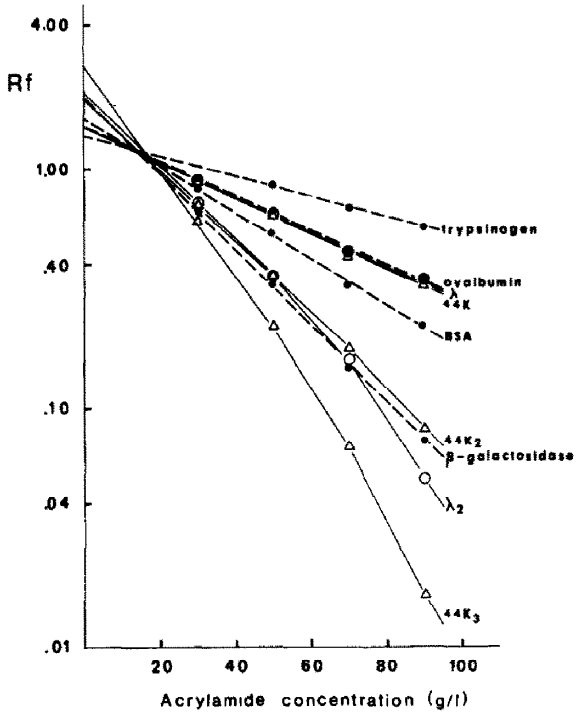

Fig.2. Ferguson plots [24,25] of crosslinked complexes. Protein–peptidoglycan complexes of λ receptor and 44 k protein were crosslinked with 10 mM DOBE for 30 min and subjected to electrophoresis in continuous SDS–polyacrylamide gels. The logarithm of mobility of the protein band relative to that of the tracking dye (R_F) was plotted against the total acrylamide concentration. Symbols: (●) standard proteins; (○) λ receptor (λ) and crosslinked complex of λ receptor (λ_2); (△) the 44 k protein (44 k) and crosslinked complexes of 44 k protein (44 k_2, 44 k_3).

of both proteins behave normally, giving straight lines. The lower molecular weight crosslinked complex of 44 k seems to have almost normal mobility in these gels. However, the mobilities of the λ receptor multimer and the larger multimer of 44 k as seen in 10% gels are clearly too low. Both of these complexes have aberrantly low mobility in the higher acrylamide concentrations (> 5%). This is probably due to modifications in the proteins resulting from crosslinking. Thus the apparent molecular weights of the multimers were estimated from 3% gels and the values obtained: 105 000 for λ receptor multimer and 110 000 and 165 000 for 44 k multimers, best fit with a dimer of λ receptor and dimer and trimer of 44 k. With prolonged reaction times (2 h) trimers of λ receptor (mol. wt 160 k) were also observed (fig.3). Trimer

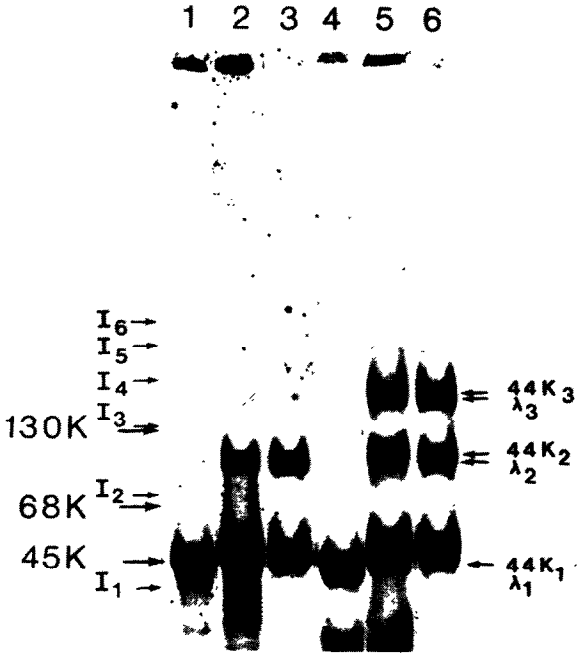

Fig.3. Autoradiograph of crosslinked λ receptor and 44 k protein analyzed on continuous SDS–polyacrylamide (3%) gels. Crosslinking of the protein–peptidoglycan complexes (slots 2, 5) or the salt released and purified proteins (slots 3, 6) was done with 20 mM DOBE for 2 h before electrophoresis. Slots 1–3 λ receptor (slot 1, uncrosslinked control); slots 4–6, 44 k protein (slot 4, uncrosslinked control). The arrows at left indicate the migration of standard proteins as in fig.1. In addition the migration of crosslinked complexes ($I, I_2, I_3, I_4, I_5, I_6$) of protein I are indicated by arrows.

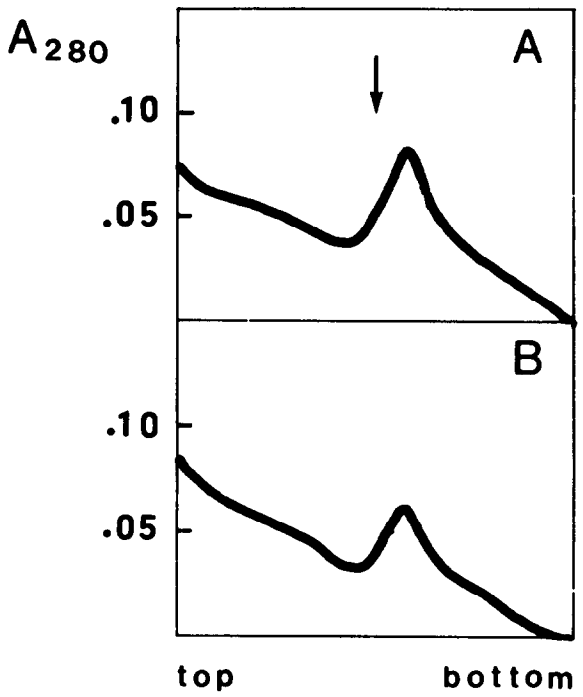

Fig.4. Sedimentation of salt released λ receptor (A) and 44 k protein (B) in a 5–28% (w/w) sucrose gradient in presence of 0.1% SDS [10]. The centrifugation was for 15 h at 130 000 × *g* using an SW60 rotor in a Sorvall OTD-2 ultracentrifuge. The arrow indicates the sedimentation of protein I trimers [10] under identical conditions.

was the highest multimer obtained for both proteins by crosslinking with DOBE. When purified proteins (see below) were crosslinked similar results were obtained (fig.3). These results suggest that both proteins are arranged as trimers, although the level of trimers seen in the case of λ receptor makes this somewhat ambiguous.

In gradient centrifugation both released, uncrosslinked λ receptor and 44 k protein sedimented as one multimeric species and both have sedimention rates that are faster than that of protein I trimers (fig.4), thus indicating that λ receptor and 44 k protein have a similar multimeric arrangement.

As indicated in fig.3 the peak material from the gradient could be crosslinked in both cases up to trimers.

These results suggest that λ receptor and 44 k protein are present in the outer membrane as trimers.

Thus the trimer arrangement appears to be a general feature for porins in the outer membrane.

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References

[1] Nakae, T. and Nikaido, H. (1975) J. Biol. Chem. 250, 1411–1422.
[2] Decad, G. M. and Nikaido, H. (1976) J. Bacteriol. 128, 325–336.
[3] Schmitges, C. J. and Henning, U. (1976) Eur. J. Biochem. 63, 47–52.
[4] Nakae, T. (1976) Biochem. Biophys. Res. Commun. 71, 844–877.
[5] Bavoil, P., Nikaido, H. and Von Meyenburg, K. (1977) Molec. Gen. Genet. 158, 23–33.
[6] Van Alphen, W., Van Selm, N. and Lugtenberg, B. (1978) Mol. Gen. Genet. 159, 75–83.
[7] Ames, G. F.-L., Spudich, E. N. and Nikaido, H. (1974) J. Bacteriol. 117, 406–416.
[8] Nakae, T. (1976) J. Biol. Chem. 251, 2176–2178.
[9] Nikaido, H., Song, S. A., Shaltiel, L. and Nurminen, M. (1977) Biochem. Biophys. Res. Commun. 76, 324–330.
[10] Palva, E. T. and Randall, L. L. (1978) J. Bacteriol. 133, 279–286.
[11] Randall-Hazelbauer, L. and Schwartz, M. (1973) J. Bacteriol. 116, 1436–1446.
[12] Hazelbauer, G. L. (1975) J. Bacteriol. 124, 119–126.
[13] Szmelcman, S., Schwartz, M., Silhavy, T. J. and Boos, W. (1976) Eur. J. Biochem. 65, 13–19.
[14] Von Meyenburg, K. and Nikaido, H. (1977) Biochem. Biophys. Res. Commun. 78, 1100–1107.
[15] Palva, E. T. (1978) J. Bacteriol. 136, 286–294.
[16] Henning, U., Höhn, B. and Sonntag, I. (1973) Eur. J. Biochem. 39, 27–36.
[17] Miller, J. H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[18] Rosenbusch, J. P. (1974) J. Biol. Chem. 249, 8019–8029.
[19] Endermann, R., Hindennach, I. and Henning, U. (1978) FEBS Lett. 88, 71–74.
[20] Hasegawa, Y., Yamada, H. and Mizushima, S. (1976) J. Biochem. 80, 1401–1409.
[21] Palva, E. T. and Randall, L. L. (1976) J. Bacteriol. 127, 1558–1560.
[22] Lutter, L. C., Ortanderl, F. and Fasold, H. (1974) FEBS Lett. 48, 288–292.
[23] Davies, G. E. and Stark, G. R. (1970) Proc. Natl. Acad. Sci. USA 66, 651–656.
[24] Ferguson, K. A. (1964) Metabolism 13, 985–1002.
[25] Banker, G. A. and Cotman, C. W. (1972) J. Biol. Chem. 247, 5856–5861.