MAJOR PROTEINS OF THE *ESCHERICHIA COLI* OUTER CELL ENVELOPE MEMBRANE

Preliminary characterization of the phage λ receptor protein

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1. Introduction

The outer cell envelope membrane of Gram-negative bacteria harbors a set of a few major or abundant proteins. For some of those from *Salmonella typhimurium* and *Escherichia coli* it has been shown that they function as non-specific hydrophilic pores for various low molecular weight hydrophilic solutes [1–4], and such proteins have therefore been called porins [1]. In *E. coli* K-12, these porins are represented by the group of proteins I, polypeptides Ia and Ib ([5,6]; identical with proteins 1a/1b [7,8]; b/c [9]; 0-9/0-8 [10,11]; A_1/A_2 [12]). Such a porin of *E. coli* BE is the matrix protein [13], probably corresponding to protein Ia in K-12 strains. In agreement with porin function it has been shown that these polypeptides are trans-membrane proteins [13–15]. In maltose-induced cells the receptor for phage λ (*lam*B protein) also constitutes a major outer membrane protein [16]. It is known to much increase the outer membrane's permeability for maltose and maltotriose, and is thought to form a pore for these sugars [17] and, to a certain degree, also for glucose and lactose [18].

There are striking similarities between *E. coli*'s proteins I and *lam*B. Both are tightly but non-covalently bound to the murein layer of the cell envelope [13] (for *lam*B: Schnaitman, personal communication). In the murein–protein complexes obtained as in [13] both are completely resistant to proteolytic degradation ([13,19] and see below). Similarity concerning functions and the behavior just mentioned may allow to expect a similar structure of the two proteins, perhaps arisen by a common origin. Since, in addition, protein–chemical data are not yet available for the interesting *lam*B protein we have isolated it and performed a preliminary characterization. Also, some knowledge on the final *lam*B gene product should be of importance to a characterization of possible biosynthetic precursors [20,21].

2. Experimental

Cells (Hfr G6 [22]; a kind gift from Dr W. Boos) were obtained commercially from E. Merck, Darmstadt, where they had been grown aerobically on a minimal medium [23] with maltose (2%) as sole carbon source. Envelopes were prepared as in [23] for the isolation of other major outer membrane proteins. Lyophilized envelopes from 200 g cell paste were suspended (5 mg/ml) in 20 mM Tris–HCl, pH 7.5 and pronase was added at 200 μg/ml. Upon incubation for 4 h at 37°C the material was washed once with the same buffer, once with water, and lyophilized (yield: 8 g dry wt). There was no loss of specific receptor activity after the pronase treatment. The substance was homogenized (10 mg/ml) at room temperature in 10 mM Tris–HCl, pH 7.5, containing 3% SDS, 5 mM EDTA, and 0.1% mercaptoethanol. After centrifugation for 1 h at 30 000 × *g* the pellet was again extracted in the same way with 0.5 vol. same solution. Combined supernatants were lyophilized, dissolved with about 100 ml water and acetone was added to final conc. 90%. The precipitate was washed 4 times with 90% acetone and lyophilized

from water (yield: 3.5 g dry wt). Chromatography on Biogel P200 was essentially as in [23] for other major proteins; protein was dissolved (20 mg/ml) in the extraction buffer, kept at 100°C for 5 min and centrifuged for 1 h at 39 000 × *g*. The supernatant of 2 g material was applied to one column (10 × 100 cm). The receptor protein eluted shortly after the void volume and was separated to about 50% from protein I. The yield of pure *lam*B protein from one column was 5–10 mg, the purification is partially documented in fig.1. Considering the cellular concentration of the protein [16] and the about 8–10 fold higher yields reached in isolation of protein I [23] which is present at about the same concentration [13,19], the *lam*B protein yield is rather poor.

Alkylation of protein with iodo [1-^{14}C]acetamide (Amersham CFA. 326) was as in [24]. 34 nmol *lam*B protein or 35 nmol protein I were reacted with 10 μCi amide. Protein was recovered by chromatography on a 1 × 30 cm Sephadex G-50 superfine column in 50% acetic acid.

SDS–polyacrylamide gel electrophoreses and preparation of tryptic fingerprints were as in [5,6]. N-Terminal amino acid sequences were determined with the manual Edman procedure, and phenylthiohydantoins were identified by thin-layer chromatography [25]. Proteins I and III were from *E. coli* B/r [23].

3. Results

3.1. λ-*Receptor protein*

In complete agreement with the data of [16] we observed in electrophoretic profiles of cell envelopes from maltose grown cells a major protein with app. mol. wt 47 000, and this protein was missing in a strain resistant to phage λ. Murein–protein complexes were prepared as in [13] from maltose-induced cells and from a *lam*B deletion mutant (MB 17 [22]). Their corresponding electrophoretic profiles are shown in fig.1 and, expectedly, the former inactivated while the latter did not inactivate the phage. In fact, as little as 0.1 μg total protein/ml (about half of which is protein I, fig.1) sufficed to inactivate 90% of 1.3 × 10^3 phages (assay [26]: 30 min at 37°C). These data, with [16], leave no doubt that the protein represents the *lam*B gene product.

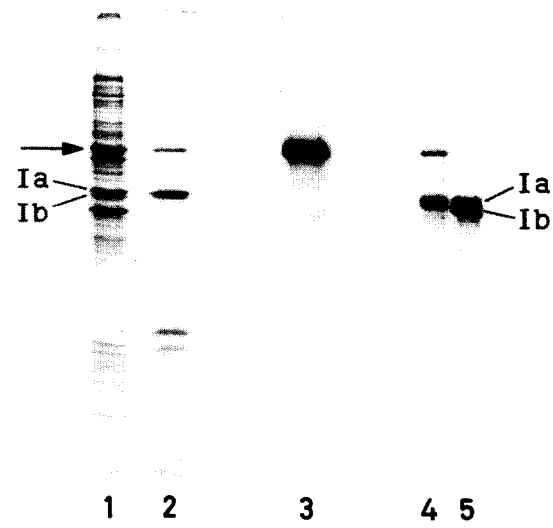

Fig.1. SDS–polyacrylamide gel electrophoreses. (1–3) Purification of the *lam*B protein (arrow): (1) total cell envelope fraction; (2) same preparation after digestion with pronase; (3) *lam*B protein after chromatography on Biogel P 200. (4,5) Murein–protein complexes according to [13]: (4) from Hfr G6 grown on minimal maltose medium; (5) from the *lam*B deletion mutant MB 17 [22] grown on a complete medium (Antibiotic No. 3, Difco).

3.2. *The* λ *receptor protein is a single polypeptide chain*

The amino acid composition of the protein was determined with two different protein preparations prior and after performic acid oxidation. Hydrolysis with 6 N HCl was for 24 h and 48 h with one and for 24 h, 48 h, and 72 h for the other preparation; the results are shown in table 1. Apparently the protein in both cases was contaminated with murein: substances eluting in the positions of diaminopimelate and hexosamine were present in about equimolar amounts. Based on methionine or histidine the minimum chemical molecular weight is about 8000.

On tryptic fingerprints of the protein 38–40 ninhydrin-positive peptides were clearly discernible; the pattern did not show any similarity with that obtained from protein I. Apparent electrophoretic molecular weight, amino acid composition, and number of tryptic peptides then agree well with the existence of a single polypeptide chain with mol. wt 47 000–50 000. Also, a unique N-terminal sequence was found and it is compared with those of other major

Table 1
Amino acid compositions of proteins I and *lam*B

Component	*lam*B protein		Protein I [28]	
	Residues polypeptide (mol/47 kd)	Moles % (mol %)	Residues polypeptide (mol/37 kd)	Moles % (mol %)
Lysine	25	5.4	17	5.2
Histidine	6–7	1.3	2–3	0.6
Arginine	15	3.2	11	3.4
Aspartic acid	70	15	49	15.3
Threonine	24	5.2	20	6.1
Serine	23	4.9	16	4.9
Glutamic acid	43 (50)[a]	9.2	26	7.9
Proline	11	2.4	7	2.1
Glycine	65	13.9	43	13.1
Alanine	36 (50)[a]	7.7	30	9.2
Cysteine	–		1	0.3
Valine	36	7.7	22	6.7
Methionine	5–6	1.3	4	1.2
Isoleucine	24	5.2	13	3.9
Leucine	29	6.2	23	7.1
Tyrosine	13	2.8	20	6.1
Phenylalanine	23	4.9	17	5.2
Tryptophan[b]	16	3.4	5	1.5
Diaminopimelic acid	6–7		–	
Hexosamine	6–7		1	

[a] Number in brackets: values actually found which then were corrected for the apparent contamination with murein (see diaminopimelate–hexosamine values)
[b] Determined as in [31]

Differences between values from different samples (*lam*B protein) and different analyses were 10–15% for most residues; such differences amounted to 20–25% for serine and glycine. Systematic losses of threonine or serine exceeding such differences did not occur during all hydrolysis times, the values are therefore not corrected

outer membrane proteins in table 2.

Analyses of performic acid oxidized protein did not reveal cysteic acid. Since it has been reported that the smallest unit of the protein active as phage receptor is a dimer linked by disulfide bonds [27], the protein was also reacted with iodo-[^{14}C]acetamide (see section 2). Radioactivity was not recovered together with the protein while this was clearly the case with protein I, most likely containing one cysteine residue per polypeptide chain [13,28]. The *lam*B protein we have isolated does not, therefore, appear to contain cysteine.

Table 2
N-Terminal sequences of major outer membrane proteins

Protein (*E. coli* strain)		Sequence	Ref.
*lam*B	(Hfr G6, K-12)	Val – Asp – Phe – x – Gly – Tyr – Ala	
I	(B/r)	Ala – Glu – Ile – Tyr – Asn – Lys – Asp	[29]
II*	(P400, K-12)	Ala – Pro – Lys – Asp – Asn – Thr – Trp	[30]
III	(B/r)	Ala – Thr – Lys – Thr – Val	

At x an identifiable phenylhydantoin was not recovered. For the outer membrane proteins II* and III see [5]

4. Discussion

At the level of analyses performed striking structural similarities between the pore-forming proteins *lam*B and I are not apparent. The overall similarity in amino acid composition (table 1) is not unique for the two proteins since the same is true for the major outer membrane proteins I, II* and III [5]. The N-terminal sequences listed do not (with the possible exception of the first three residues of proteins I and *lam*B) suggest functional importance of these regions concerning protein translocation into the outer membrane.

We here have confirmed Schnaitman's observation (see section 1) that (at least part of) the *lam*B protein is bound to the murein layer in a way similar to protein I [13]. Since the protein can act as phage receptor [26], and in agreement with porin function, it certainly should also represent a trans-membrane protein. Binding of maltose to the protein has not been detected [17], and the large variety of solutes which can pass the outer membrane via protein I certainly do so by simple diffusion [2,3]. Why does the *lam*B pore then appear to be much more substrate specific than protein I [18]? One possibility consists in the presence of the *mal*E gene product, a periplasmic protein which binds maltose with high specificity [17]. If a physical association between *lam*B and binding proteins would exist the latter could inhibit penetration of other solutes that cannot become bound. It would be of interest to examine the porin function of the *lam*B protein in mutants missing protein I and the maltose binding protein.

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