THE RECEPTOR OF BACTERIOPHAGE λ: EVIDENCE FOR ITS DIMERIC NATURE

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1. Introduction

It has been shown by genetic and physiological studies that lamB-protein, a constituent of the outer membrane of *Escherichia coli* K12, facilitates the transport of maltose, maltodextrins and other sugars [1–5] and is also involved in maltose chemotoxis [2]; in addition, lamB-protein serves as receptor for bacteriophage λ [6,7]. The apparent molecular weight of the lamB-protein, determined by polyacrylamide gel electrophoresis in presence of sodium dodecyl sulfate (SDS), was reported to be 47 000 [4,8]. Furthermore, some protein-chemical data are already available [8], but otherwise little is known about the biochemistry of this protein. By assaying the phage inactivating ability of the lamB-protein, I will demonstrate here that the smallest, biologically-active unit of the λ-receptor is most likely the dimer of the lamB-protein.

2. Materials and methods

2.1. *Strains*

Bacteriophage $\lambda b_2 vh$ [9], which is a host range mutant of the 'grand virulent' strain $\lambda b_2 v$ [10,11], and its host *E. coli* K12 CR63 [12] were kindly provided by Dr M. Schwartz.

2.2. *λ-Receptor and λ-receptor assay*

Purified receptor of phage λ was a generous gift of Dr M. Schwartz; it was isolated from strain *E. coli* Hfr G6 [13], purified as in [6] and stored frozen in 0.01 M Tris·HCl (pH 7.5).

The concentration of active λ-receptor was deduced from the rate by which phage λ gets inactivated [6].

Phage inactivation was followed at 37°C in 0.01 M Tris·HCl (pH 7.5), containing 1 mM $MgSO_4$ and 0.02% bovine serum albumin (TMB buffer). Bovine serum albumin not only protects the phage against inactivation [6], but also prevents the λ-receptor from aggregation and inactivation (data to be published).

2.3. *SDS–polyacrylamide slab gel electrophoresis*

The discontinuous Laemmli system was used [14]. The acrylamide concentration in the lower gel was 10%, in the upper gel 3%. The sample buffer was as in [14] except that it contained in addition 3.3% sodium cholate. Staining and destaining were performed as in [6]. For determination of apparent molecular weights the following markers were used: phosphorylase *b* (mol. wt 100 000; obtained from Dr H. Buc), bovine serum albumin (mol. wt 68 000), sheep γ-globulin (mol. wt 50 000 for the heavy chain and mol. wt 23 500 for the light chain; purchased from Miles Labs) and ovalbumin (mol. wt 43 000). The molecular weights of the marker proteins were taken from [15].

For recovery and localization of λ-receptor activity in the gel slab a 1 cm broad strip covering the sample of interest was cut out from the gel immediately after the end of electrophoresis, frozen with powdered dry ice and then cut into 0.4 mm thick slices with a Mickle gel slicer. Each slice was transferred into

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0,4 ml ice-cold TMB buffer, vigorously shaken, incubated overnight at 4°C, diluted further with ice-cold TMB buffer, if necessary and assayed biologically as in section 2.2. If the presence of bovine serum albumin is not wanted, it may be replaced by detergents.

For determination of the apparent molecular weight of active λ-receptor its position in the gel was compared with the position of Coomassie blue-stained marker proteins applied to the same gel slab, taking into account the expansion of the gel during the staining and destaining procedures.

3. Results

When the λ-receptor used in this study is boiled for 3 min in presence of 2% SDS, 3.3% sodium cholate and 5% mercaptoethanol and then subjected to a discontinuous SDS–gel electrophoresis [14], it becomes apparent that the λ-receptor, purified according to [6], contains besides the lamB-protein (mol. wt 47 000) two other proteins, which are probably identical with the proteins Ia and Ib described in [16]; see fig.1b. However, when the λ-receptor is boiled for 3 min in a sample buffer which lacks mercaptoethanol, an additional band with app. mol.wt ~95 000 appears; simultaneously, the band representing the monomer of lamB-protein is found in decreased quantity, whereas the intensity of the contaminating band remains unchanged (see fig.1c). This finding suggests that the λ-receptor, pretreated with sample buffer for 3 min at 100°C in the absence of mercaptoethanol, exists in two forms: as monomer and as dimer. When this pretreatment is prolonged for 15 min, only the monomer of lamB-protein is found; on the other hand, when the λ-receptor is pretreated with the same sample buffer for 3 min only at 90°C, the lamB-protein migrates exclusively at the position of the dimer (data not shown). When the band migrating with an apparent molecular weight of the dimer of lamB-protein is cut out of the gel after staining with Coomassie blue, washed free of acid and boiled in 2% SDS and 5% mercaptoethanol, the presumptive dimer is indeed converted to the monomer, showing that the 95 000 band is most probably composed only of lamB-protein (data to be published).

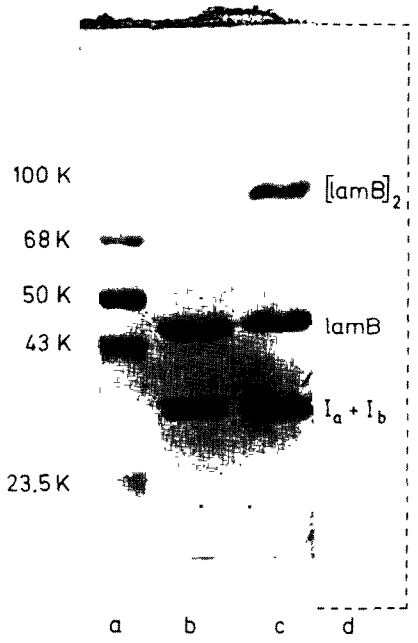

Fig.1. SDS–polyacrylamide gel electrophoresis. (a) Molecular weight standards as in section 2.3. The numbers indicate the molecular weights in kilodaltons according to [15]; (b) λ-receptor, boiled for 3 min in 2% SDS, 3.3% sodium cholate and 5% mercaptoethanol; (c) λ-receptor, boiled for 3 min in 2% SDS and 3.3% sodium cholate; lamB and $[lamB]_2$ stand for the monomer and dimer of lamB-protein Ia and Ib stand for proteins Ia and Ib [16]. (d) The same sample as in (c) was applied, but before staining this part of the gel was cut out and assayed for λ-receptor activity as in section 2.3. The result is seen in fig.2.

In order to find out which band of the lamB-protein carries biological, i.e., phage inactivating activity, another aliquot of the sample used for slot c in the gel of fig.1 was applied to the same gel. After the end of electrophoresis a strip covering this sample (see fig.1d) was cut out of the gel, sliced and assayed for receptor activity as in section 2.3. The pattern of biological activity obtained in shown in fig.2. About 95% of total receptor activity is found exactly at the dimer position of lamB-protein, whereas the monomer of lamB-protein is completely inactive ($<10^{-4}$ of total activity recovered). The contaminant band, probably composed of proteins Ia and Ib [16], is also completely inactive. A few % of total receptor activity migrate slower than the dimer of lamB-protein; they

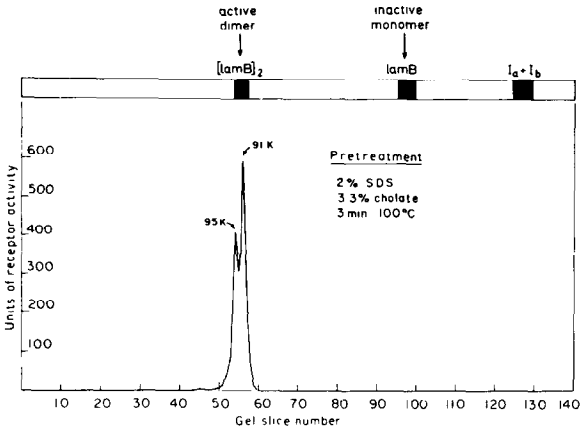

Fig.2. Recovery of λ-receptor activity after SDS gel electrophoresis. Part d of the gel slab in fig.1 was fractionated and assayed for λ-receptor activity as in section 2.3. Receptor activity is given in arbitrary units. In the upper part the electropherogram of sample c of fig.1 is depicted. 91 K and 95 K are the apparent molecular weights (kilodaltons) of active λ-receptor, determined as in section 2.3.

probably represent higher oligomers of the lamB-protein, which are present in a concentration too low to be detected by Coomassie blue staining. The major peak of λ-receptor activity is reproducibly resolved into two peaks whose apparent molecular weights differ by a value of 4000–5000. In other gels (not shown here) this doublet was also detected by Coomassie blue staining. The relatedness of these two types of lamB-dimers is presently under investigation.

4. Discussion

During the last years many proteins of the outer membrane of *E. coli* were shown to serve as phage receptors (see, for instance, [17] and references therein); in no case, however, the subunit structure which is necessary for the protein to act as phage receptor was identified. In this study I have provided evidence that the smallest unit of active λ-receptor is the dimer of lamB-protein. One may, however, object that the observed inactiveness of the monomer of lamB-protein is due to inappropriate treatment and one should therefore try to find conditions to isolate and keep the monomer of lamB-protein in its 'native' state. Obviously, the data presented cannot rule out the possibility that under suitable conditions also the monomeric lamB-protein can bind and inactivate phage λ. On the other hand, one could imagine that even under physiological conditions the lamB-polypeptide chain gets rapidly denatured unless it assembles to a dimer, which apparently is extremely stable.

It was shown for the lamB-protein [18] and other outer membrane proteins [19,20] that they are synthesized as larger precursors. It remains to be investigated how the two processes, proteolytic cleavage of the precursors and formation of functional oligomers, are temporally and topologically coordinated.

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