SYNTHESIS IN MINICELLS OF THE MAJOR PROTEINS OF THE *ESCHERICHIA COLI* OUTER CELL ENVELOPE MEMBRANE

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1. Introduction

Recently Levy [1] reported on the very interesting finding that in *E.coli* minicells extraordinarily stable messenger RNA (half life at least 80 min) exists that is translated mainly into a few proteins associated with the outer membrane of the cell envelope. They were separable electrophoretically into bands (apparent mol. wt.) c (35 000), d (32 000), e (29 000), and f (10 000). Levy identified band f as Braun's lipoprotein [2]. The apparent molecular weights of bands c and d are close to those established chemically for two major outer membrane proteins: our protein I has a molecular weight of about 37 000 [3–5] and our protein II* can exhibit a molecular weight of 33 000 (6; for designations for these proteins used by other workers see [6,7]. We show here that Levy's bands c and d indeed are identical with the chemically fairly well characterized proteins I and II*, and we also report on mutants in minicell producing strains missing proteins I, II* or both.

2. Experimental

Minicells producing strains PA678–54 (the original Adler strain, 8) and χ984 (1) were grown aerobically at 37°C in minimal medium containing 0.4% glucose and 0.2% Casamino acids (Difco) to late log phase. Strain χ984 was supplemented with adenosine and pyridoxine (both at 10 μg/ml). Minicells were purified by several cycles of differential centrifugation in 67 mM potassium phosphate (pH 6.8), containing 5% sucrose. Minicells from 4 l cells were suspended in 10 ml minimal salts medium containing penicillin (1000 U/ml), glucose (0.2%) and radioactive amino acids (2 μCi/ml, ^{14}C-labeled protein hydrolyzate with a specific radioactivity >45 mCi per milliatom C; Amersham CFB 25). After incubation at 37°C for 3 h with shaking minicells were washed with water and placed for 5 min in boiling Na-dodecyl-sulfate (2 ml, 2%) containing 5% mercaptoethanol, 10% glycerol, and 62.5 mM Tris–HCl pH 6.8. Upon centrifugation the supernatant was used for electrophoresis according to Ames [9]. Gels were stained (see [9]) and autoradiographed according to Bonner and Laskey [10].

The purity of minicells was tested by the following internal control experiment. As a very convenient accident strain PA678–54 produces protein II* in normal amounts but its minicells do not synthesize this protein ([1]; see also Results). Cells of PA678–54 were incubated with radioactive amino acids under the same conditions as described for minicells. Protein II* became heavily labeled and thus the absence of label in II* from minicells shows that contamination of minicells with whole cells was not a problem.

3. Results

3.1. *Protein synthesis in minicells*

Minicells from strains PA678–54 and χ984 incubated with radioactive amino acids gave results practically identical with those reported by Levy [1]: in

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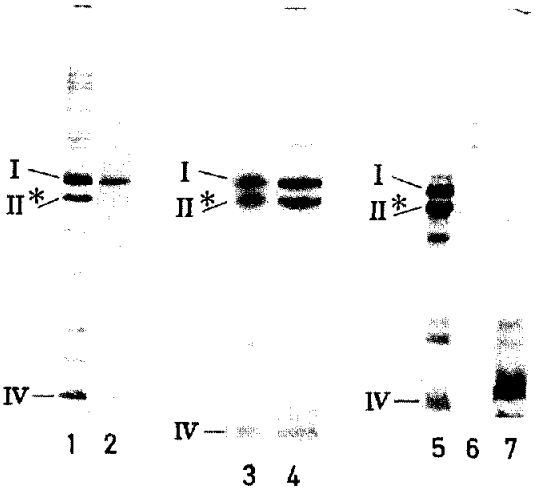

Fig.1. SDS-electrophoreses of minicell proteins. 1,2 strain PA678-54; 1, stained gel and 2, autoradiography. 3,4 strain χ984; 3, stained gel and 4, autoradiography. 5, strain χ984 (stained gel); 6,7 mutant of χ984 missing proteins I and II* (see section 3.3); 6, stained gel and 7, autoradiography. IV, Braun's lipoprotein. The method used for autoradiography [10] renders the gel opaque and also leads to some broadening of the bands, therefore, usually many minor bands are not clearly visible on the photographs of stained gels. The position of proteins I, II*, and IV corresponds to Levy's bands c, d, and f, respectively.

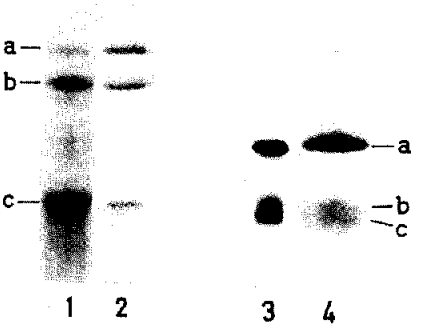

Fig.2. SDS-electrophoreses of cyanogen bromide fragments. 1,2 fragments a–c of protein I; 1, stained gel and 2, autoradiography. 3,4 fragments a–c of protein II*; 3, stained gel and 4, autoradiography. The stained bands correspond to fragments from proteins isolated from whole cells and the radioactivity comes from these proteins synthesized in minicells. These patterns differ from those published for the two proteins [5,6] in that in both cases a few more fragments of lower molecular weights than fragments c exist. They stain only weakly and are difficult to detect if small quantities of fragments are applied to a gel.

minicells from PA678–54 essentially only radioactive bands c and f and from χ984 only radioactive bands c, d and f were found (fig.1). For unknown reasons in both strains we could not always unambiguously identify band e.

3.2. *Proteins I, II* and Levy's bands c,d*

Radioactive band c from minicells (strain PA678–54) labeled with a mixture of ^{14}C-amino acids was eluted from the gel, non-radioactive isolated [11] protein I from the same strain was added, and the mixture was subjected to cyanogen bromide cleavage. Electrophoretic separation of the fragments showed identity of the stained and radioactive patterns (fig.2). Thus, Levy's band is identical with protein I.

Exactly the same procedure was followed for band d (strain χ984) and protein II*. Again, stained and radioactive patterns of the corresponding cyanogen bromide fragments were identical (fig.2), and band d is therefore identical with protein II*.

3.3. *Mutants missing proteins I and II**

Selection for resistance to phage TuII* very frequently and specifically leads to loss of protein II* which acts as a receptor for the phage [12]. Five mutants of strain χ984 resistant to phage TuII* were isolated. All of them still produced minicells and were found to miss protein II*. Minicells from one of these mutants still synthesized protein I but the radioactive band II* was missing.

We have reported that selection for resistance to another phage (TuI, the receptor for this phage is not yet known) can result in mutants missing protein I [13]. Several such mutants were produced in χ984 and one strain of the desired character, i.e., lacking protein I, was found. This murant was in addition made resistant to phage TuII*. Several isolates tested now missed both proteins I and II* and all of them produced minicells. Minicells from one such TuI plus TuII* resistant mutant did not synthesize the proteins corresponding to Levy's bands c and d, i.e., proteins I and II* (fig.1).

4. Discussion

The data reported show that two of the Levy proteins which are synthesized in minicells from very stable mRNA are identical with the major outer membrane proteins I and II* isolated from whole cells. We had shown before [7] that mutants missing these proteins can grow and divide normally. This fact, however, does not yet exclude that they may play a role in cellular division as was suggested by Levy [1]. These proteins normally are present at very high concentrations (about 10^5 copies per protein per cell) and their presence in amounts below about 5% of this concentration would probably no longer be detectable with the methods used. In addition, Levy considered the possibility that two fractions of the relevant mRNAs might exist, and only the minicell species might have the very long half life. Therefore, in mutants 'missing' these proteins they still could have been synthesized at the site of division. The mutants described here show that this is not so. In summary, we conclude that proteins I and II* produced in minicells are not specific for the outer membrane at the cell poles and that their synthesis from stable mRNA is not required for cellular division.

The question remains as to why so very stable mRNA molecules exist for the proteins discussed. In view of the high cellular concentration of these proteins the reason could be an economical one.

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