CRYPTICITY OF PERIPLASMIC ENZYMES

Involvement of protein b in the permeability of the outer membrane of *Escherichia coli*

E. YAGIL, I. R. BEACHAM*, Ahuva NISSIM and G. PRICE*

Department of Biochemistry, George S. Wise Centre for Life Sciences, Tel-Aviv University, Tel-Aviv, Israel and **Department of Botany and Microbiology, School of Biological Sciences, University College of Wales, Aberystwyth, Wales*

Received 4 November 1977

1. Introduction

The cell envelope of gram-negative bacteria includes an outer membrane structure (OM) which acts as a barrier to certain small molecules, most notably some detergents and antibiotics [1—3]. The permeability of the OM has been studied in detail [4—7]; uncharged molecules mol. wt $\lesssim$ 800 pass through, but higher molecular weight solutes tend to be excluded. The OM of gram-negative bacteria contains 2—4 major proteins (mostly associated with peptidoglycan) with some variability between species, strains and method of protein separation. In *Escherichia coli* K12 there are four major proteins: a, b, c, d (notation [8,9]). Three of the OM proteins of *Salmonella typhimurium* are responsible for conferring permeability on the OM [5,6]. Termed 'porins', they were suggested to form hydrophilic pores or channels through the membrane [8,10].

Between the outer and cytoplasmic membranes are located a group of mostly degradative enzymes [11]. The substrates of these 'periplasmic' enzymes must readily pass through the outer membrane. However, even though the OM is readily permeable to such substrates, these enzymes show slightly reduced expression in intact cells relative to extracts [12—14], indicating that the OM is, nevertheless, a partial barrier.

We have reported [13,14] mutants, termed 'cryptic', in which the expression of some periplasmic enzymes is reduced in intact cells. It was shown that crypticity is due to a much reduced permeability of the OM to the substrates of these enzymes, resulting from a lack of one or two of the major proteins (proteins b and/or c). The electrophoretic resolution in this study did not permit us to determine which of these proteins were involved, but provided in vivo evidence for one or both of these proteins functioning as porins.

Our study was mainly concerned with crypticity of periplasmic nucleotidases. Crypticity towards periplasmic β-lactamase in mutants of *Salmonella typhimurium* lacking 'porins' has been reported [15]. These findings provided further in vivo evidence for the role of the OM proteins in determining permeability of the OM.

We now present evidence that mutants isolated in various laboratories and by different methods but all lacking OM protein b are impaired in the penetration of 5′-AMP through the OM. The permeability towards adenosine is also affected, though to a lesser degree.

2. Materials and methods

2.1. *Organisms*

E. coli strain AB1157-1 (parental), 5A-1 (*ush*$^-$) and 3—4 ('cryptic') have been described [13,14]. Strain 3—4 lacks protein b but retains c (Van Alphen et al., submitted). The selection of this strain was based on its inability to dephosphorylate exogenous 3′-AMP [13].

Strains JC7620 (parental), PC2040 and PC2041 are

Direct correspondence to I. R. Beacham; present address: School of Science, Griffith University, Nathan, Brisbane, Qld 4111, Australia

from Lugtenberg's laboratory (supplied by Miss H. S. Felix, Phabagen Collection); the latter two mutants lack protein b; they were isolated as resistant mutants to phages T3, T4 and T7 [16].

Strains W1485 (parental), W1485ParI and W1485ParII come from Schnaitman's laboratory. The two mutants lack protein c; they were isolated by their resistance to phage PA-2 [17].

Strains P400 (parental) and P530 are from Reeve's laboratory (supplied by Henning); P530 lacks both proteins b and c (Lugtenberg, personal communication; [18]). P530 was selected as a colicin resistant mutant [18].

2.2. *Media*

M9 minimal medium was used [13,20].

2.3. *Uptake of labelled substrates*

Uptake of labelled substrates was measured as described [14,20]. The concentration of substrates was 1 μM in fig.1 and 0.5 μM in figs 2 and 3.

3. Results and discussion

The reduced breakdown of 5′-AMP by intact cells was originally used to study 'cryptic' strains such as 3–4 [13]. However, the uptake of 5′-AMP by intact cells was later found to be more convenient [14]; the uptake of the adenosine moiety is solely dependent

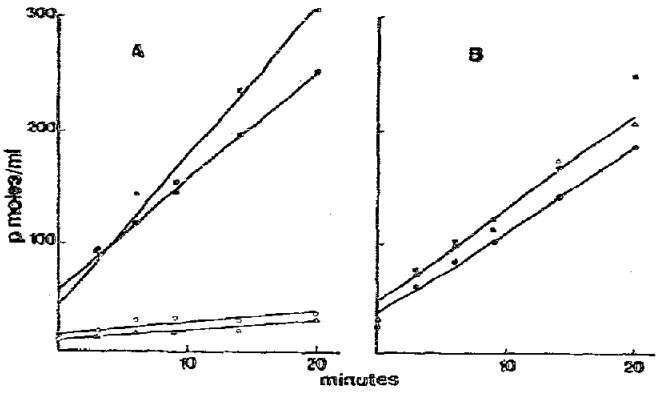

Fig.1. Uptake of 5′-[^{3}H]AMP by mutant and parental *E. coli* strains. These experiments were carried out in supplemented minimal medium containing 0.2% casamino acids. 1A. (×) P400 (parental); (○) P530; (●) AB1157-1 (parental); (△) 3–4. 1B. (●) W1485 (parental); (×) W1485ParI; (△) W1485ParII.

on breakdown of 5′-AMP by 5′-nucleotidase [20]. Thus, one feature of the 'cryptic' phenotype is the dramatic reduction in uptake of 5′-AMP by intact cells despite the 'cryptic' presence of active 5′-nucleotidase. (The mutants were selected for their resistance to AMP plus 5-fluorouracil [13].) Clearly if this is due to a lack of one of the OM proteins, then independently isolated deficient mutants should also be impaired in uptake of 5′-AMP. Such mutants have been isolated as phage or colicin resistant clones

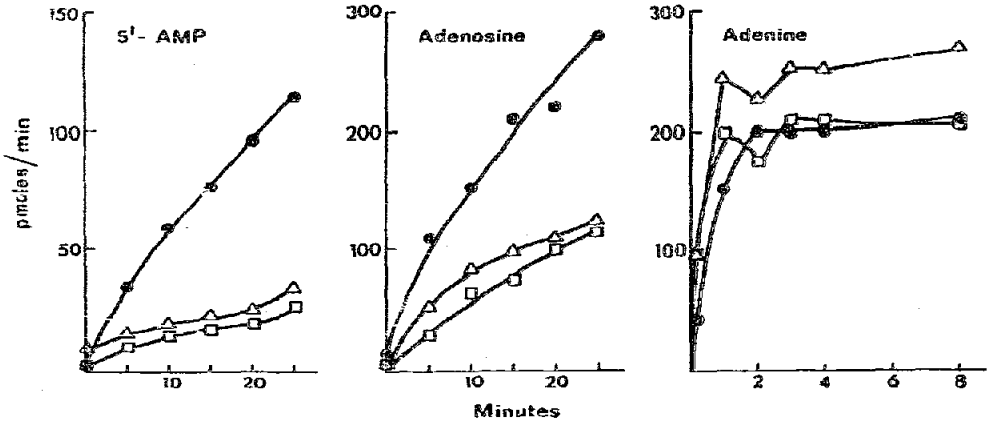

Fig.2. Uptake of [^{3}H]AMP, [^{14}C]adenosine and [^{14}C]adenine in mutant and parental strains. (●) JC7620 (parental); (△) PC2040; (□) PC2041.

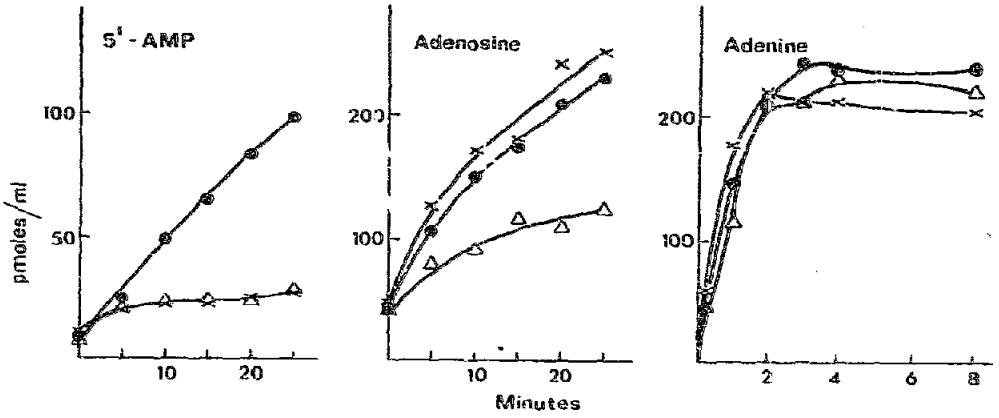

Fig.3. Same as in fig.2. (●) AB1157-I (parental); (△) 3–4; (×) 5A-1 (*ush*)

[16–19,21,22] and by selection for copper resistance [23] (see also section 2.1.). We have tested mutants deficient in proteins b or c or both. All mutants lacking b (P530, PC2040, PC2041 and 3–4) show greatly reduced uptake of 5′-AMP (figs 1A, 2 and 3). Mutants, however, which lack only protein c (W1485ParI and W1485ParII) did not show this trait (fig.1B). These results show that protein b and not c is involved in the formation of porins which enable the passage of 5′-AMP through the OM.

Since it thus seems that the outer membrane is less permeable to 5′-AMP in protein b-deficient strains, the question arises as to whether permeability to adenosine or adenine is affected. Figures 2 and 3 show that a number of the strains having greatly reduced uptake of 5′-AMP also show reduced uptake of adenosine, but to a lesser degree; uptake of adenine is not affected. As well as the parental strains, a 5′-nucleotidase-deficient mutant strain (*ush*$^-$) [13,20] is also included as a control. This strain predictably shows no uptake of the adenosine moiety of 5′-AMP (fig.3) [20], but is unimpaired in adenosine uptake. It is notable in this connection that some *tsx*$^-$ mutants, which also lack an OM protein, show impaired uptake of adenosine and other nucleosides [24]. Thus the uptake of adenosine can seemingly proceed via at least two proteins in the OM.

In summary, a variety of mutants lacking protein b are impaired in the uptake of 5′-AMP and adenosine. In conjunction with other studies ([4–7,15,22,23,24]; Van Alphen et al., submitted), we interpret this as further in vivo evidence that this protein is involved in promoting the permeability through the outer membrane.

Acknowledgements

We thank Ben Lugtenberg and C. A. Schnaitman for communicating experimental results prior to publication. We also thank them, Miss H. S. Felix and Ulf Henning, for sending us bacterial strains. Work performed in the laboratory of E.Y. was supported by a grant from the US-Israel Binational Science Foundation.

References

[1] Wilkinson, R. G., Gemski, P. and Stocker, B. A. D. (1972) J. Gen. Microbiol. 70, 527–554.
[2] Tamaki, S., Sato, T. and Matsuhashi, M. (1971) J. Bacteriol. 105, 968–975.
[3] Leive, L. (1974) Ann. NY Acad. Sci. 235, 109–127.
[4] Nakae T. and Nikaido, H. (1975) J. Biol. Chem. 250, 7359–7365.
[5] Nakae, T. (1976) J. Biol. Chem. 251, 2176–2179.
[6] Nakae, T. (1976) Biochim. Biophys. Res. Commun. 71, 877–884.
[7] Nikaido, H. (1976) Biochim. Biophys. Acta. 433, 118–132.

[8] Lugtenberg, B., Peters, R., Bernheimer, H. and Berendsen, W. (1976) Mol. Gen. Genet. 251–262.
[9] Lugtenberg, B., Meijers, J., Peters, R., Van der Koek, P. and Van Alphen, L. (1975) FEBS Lett. 59, 254–258.
[10] Lugtenberg, B., Bronstein, H., can Selin, N. and Peters, R. (1977) Biochim. Biophys. Acta 465, 571–578.
[11] Heppel, L. A. (1971) in: Structure and Function of Biological Membranes (Rothfield, L. I. ed) Academic Press, New York.
[12] Brockman, R. M. and Heppel, L. A. (1968) Biochemistry 7, 2554–2562.
[13] Beacham, I. R., Kahana, R., Levy, L. and Yagil, E. (1973) J. Bacteriol. 116, 957–964.
[14] Beacham, I. R., Hass, D. and Yagil, E. (1977) J. Bacteriol. 129, 1034–1044.
[15] Nikaido, H., Song, S. A., Shaltiel, L. and Nirminen, M. (1977) Biochem. Biophys. Res. Commun. 76, 324–330.
[16] Havekes, L. M., Lugtenberg, B. J. J. and Heckstra, W. P. M. (1976) Mol. Gen. Genet. 146, 43–50.
[17] Bassford, P. J., Diedrich, D. C., Schnaitman, C. L. and Reeves, P. (1977) J. Bacteriol. 131, 608–622.
[18] Henning, U. and Haller, I. (1975) FEBS Lett. 55, 161–164.
[19] Davies, J. K. and Reeves, P. (1975) J. Bacteriol. 123, 102–117.
[20] Yagil, E. and Beacham, I. R. (1975) J. Bacteriol. 121, 401–405.
[21] Schmitges, C. J. and Henning, U. (1976) Eur. J. Biochem. 63, 47–52.
[22] Verhoef, C., de Graff, P. J. and Lugtenberg, B. J. (1977) Mol. Gen. Genet. 150, 103–105.
[23] Lutkenhaus, J. F. (1977) J. Bacteriol. 131, 631–637.
[24] Hantke, K. (1976) FEBS Lett. 70, 109–112.