Biochimica et Biophysica Acta, 1104 (1992) 201–206

BBAMEM 75527

Reconstitution of the binding protein-dependent galactose transport of *Salmonella typhimurium* in proteoliposomes

G. Richarme, A. El Yaagoubi and M. Kohiyama

Biochimie Génétique, Institut Jacques Monod, Université Paris 7, Paris (France)

(Received 5 August 1991)

Key words: Reconstitution; Galactose transport; Binding protein dependence; Proteoliposome; (*S. typhimurium*)

Binding protein-dependent transport systems mediate the accumulation of diverse substrates in bacteria. The binding protein-dependent galactose transport of *Salmonella typhimurium* has been reconstituted in proteoliposomes. The proteoliposomes were made with proteins solubilized and renatured from inclusion bodies produced by a bacterial strain containing a plasmid with the *mgl* (methylgalactose permease) operon of *Salmonella typhimurium*. Galactose transport is dependent both on the addition of the purified galactose binding protein to the transport assay, and on ATP. The interaction between the liganded galactose binding protein and proteoliposomes displays Michaelis type kinetics with a K_m of around 15 μM. Galactose transport is coupled to ATP hydrolysis with a stoichiometry (ATP / galactose) of 2.5 : 1. Galactose transport in proteoliposomes is not significantly inhibited by the uncoupler carbonylcyanide *m*-chlorophenylhydrazone, but is inhibited by 0.5 mM vanadate. The present reconstitution of galactose transport in proteoliposomes suggests that the MglA, MglC and MglE proteins have been solubilized and renatured in an active form from the inclusion bodies of the *mgl* hyperproducing strain.

Introduction

The binding protein-dependent transport systems of Gram-negative bacteria form a class of at least thirty permeases implicated in the active transport of ions, sugars, amino acids and oligopeptides (reviewed in Refs. 1 and 2), and belong to a superfamily of membrane proteins which extends to eukaryotic cells and includes the multidrug resistance P-glycoprotein [3] and the cystic fibrosis transmembrane conductance regulator [4]. Each of these binding protein-dependent transport systems consists of a periplasmic substrate-binding protein which serves as the primary recognition site for transport, and three or four additional inner membrane associated proteins implicated in substrate translocation and energy coupling. The binding protein-dependent galactose transport system of *Salmonella typhimurium* (formerly named the methyl-galactose permease) consists of a periplasmic galactose-binding protein (MglB protein) and three inner-membrane proteins (the MglA, MglC, MglE gene products) [5]. Some results based on a correlation between low ATP levels and reduced binding protein-dependent transports in vivo [6] led to the suggestion that these transports were energized by ATP hydrolysis. However, other studies implicated the proton-motive force [7], acetyl phosphate [8], lipoic acid [9] and succinate [10] in binding protein-dependent transports. A central role for ATP has been recently supported by the discovery of a consensus ATP binding site in the sequence of the inner membrane components OppD, HisP and MalK of several binding protein-dependent transport systems [11], by an ATP requirement for binding protein-dependent transport in membrane vesicles [12,13] and by a demonstration that ATP hydrolysis occurs in vivo concomitantly with binding protein-dependent transport [14–16]. In a recent study (manuscript submitted for publication), we report the purification of the MglA gene product as a galactose-dependent ATPase. In the present study, we describe the reconstitution of binding protein-dependent galactose transport in proteoliposomes; the proteoliposomes have been made with proteins solubilized and renatured from inclusion bodies obtained from a bacterial strain hyperproducing the mgl operon of *Salmonella typhimurium*. Galactose transport in proteoliposomes is dependent on the addition of the galactose binding

Correspondence: G. Richarme, Biochimie Génétique, Institut Jacques Monod, Université Paris 7, 2 place Jussieu, 750005 Paris, France.

protein in the transport assay, and of ATP internally trapped during the formation of the proteoliposomes.

Materials and Methods

Bacteria. The strain of *Escherichia coli* K12 LA 5709 (F-*mgl*572 *lac*Y *gal*E *pts*F *arg recA srl*) carrying plasmid pHG 30, a pBR322 derivative containing the *mglA, mglB, mglC, mglE* genes of *Salmonella typhimurium* was from the laboratory of Prof. W. Boos (Fachbereich Biologie, Konstanz University, FRG) [5]. It was grown in LB medium supplemented with 0.4% glucose (in order to repress the *mgl* genes) to an absorbance of 0.5 at 600 nm and then transferred for 2 h in the same medium but without glucose to obtain expression of the *mgl* genes.

Solubilisation and renaturation of the membrane proteins. All procedures were carried out at 4°C (except when indicated). The bacteria (4 g wet weight) were washed with 30 ml of 100 mM Tris hydrochloride (pH 7.4) and lysed at 20°C in 3 ml of 50 mM potassium phosphate (pH 6.8), 2 mM dithiothreitol, 5 mM ATP, 10 mM galactose, 20% sucrose followed by addition of 1 ml of 1 mg/ml lysozyme (freshly prepared), 0.1 g of sonicated phospholipides (asolectin) in 1 ml of water and EDTA (added slowly (2 min)) to a concentration of 5 mM; 2% Triton X-100, 0.1 mg of pancreatic DNase and 5 mM $MgCl_2$ were then added. The whole mixture was incubated for 1 h at 0°C, centrifuged for 10 min at $5000 \times g$ to eliminate the cellular debris; the supernatant was centrifuged for 10 min at $40000 \times g$; the white pellet, containing protein aggregates [17], was washed three times with buffer containing 50 mM potassium phosphate, 0.5 mM EDTA, 5 mM $MgCl_2$, 2 mM dithiothreitol, 0.1 mg/ml asolectin, 2 mM ATP. It was solubilised in 10 ml of 6 M guanidine-HCl, 1% CHAPS, 1 mM dithiothreitol, 50 mM Tris-HCl (pH 7.4) (1 h at 37°C) and renatured by dilution in 100 ml of a solution containing 20 mM Tris-HCl (pH 8), 0.5 mM EDTA, 5 mM $MgCl_2$, 2 mM dithiothreitol, 15% glycerol, 0.5% CHAPS, 0.2 mg/ml asolectin (buffer A) at 20°C; the protein solution was allowed to stand for 2 h at 20°C and it was clarified by centrifugation (10 min at $20000 \times g$); the supernatant was concentrated to 5 ml by ultrafiltration, and was dialyzed overnight against 50 ml of buffer A; the concentrated solution was used for the formation of proteoliposomes.

Proteoliposomes. Proteoliposomes were formed following a dilution procedure: 2 ml of solubilized protein in buffer A (0.5 mg), 1 ml of soybean phospholipids (40 mg/ml) (asolectin from Associated Concentrates) previously resuspended by sonication in buffer B (20 mM Tris-HCl (pH 8), 0.5 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 15% glycerol) and 50 μl of 10% CHAPS were incubated for 1 h at 0°C, sonicated for 3 s, freezed-thawed once and diluted fifty times in 160 ml of 20 mM Tris-HCl, 0.5 mM EDTA, 5 mM $MgCl_2$, 1 mM dithiothreitol, 3 mM ATP at 18°C. Proteoliposomes were collected by centrifugation for 1 h at $200000 \times g$ and resuspended in 500 μl of the preceeding buffer without ATP. Liposomes were prepared in the same way in the absence of added protein. Protein concentration of proteoliposomes was determined as described in Ref. 18.

Transport assay. 20 μl of proteoliposomes (50 μg protein/ml) were added to 20 μl of 180 μM [^{3}H]-galactose and 20 μl of 150 μM galactose binding protein at 20°C; at the indicated times, 8-μl aliquots were poured into 1 ml of buffer B at 20°C; the whole was filtered onto 0.22 μm Millipore filters GSWP and washed with 2×1 ml of buffer B at 0°C.

Purification of the galactose binding protein. This purification was made from the *Escherichia coli* strain LA 5709 containing plasmid pHG 30 by an osmotic shock procedure, followed by two column chromatographies on hydroxylapatite and DEAE cellulose as described in [19]. Galactose binding protein was deprived of bound ligand as described previously [19].

ATP determination. For these experiments, proteoliposomes were prepared in the presence of 0.3 mM ATP and washed twice at 0°C in buffer B. Aliquots of proteoliposomes were taken in parallel during transport assays and ATP was assayed by a luciferin-luciferase assay as described [9,20].

ATPase. ATPase activity was measured at 20°C by adding protein in 2 μl to 3 μl of 100 μM [^{3}H]ATP (1.5 Ci/mmol)/1 mM $MgCl_2$ and 10 mM galactose when indicated. ATP and ADP were separated by chromatography on polyethyleneimin cellulose as described in Ref. 21.

Gel electrophoresis. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (12.5%) was performed according to the method of Laemmli [22]. Protein samples were incubated 1 h at 37°C in sample buffer (instead of boiled) before loading the gel. Coloration of protein bands was made by silver staining [23].

Materials. ATP (disodium salt) was obtained from Sigma. [^{3}H]ATP and [^{3}H]galactose were obtained from Amersham and were used at 1.5 Ci/mmol. Triton X-100 was from Boehringer and asolectin (soybean phospholipids) was from Associated Concentrates, Woodside, Long Island, NY 11377 USA. All other chemicals were obtained from Sigma and were reagent grade.

Results

Extraction, solubilisation and reconstitution of membrane proteins in proteoliposomes

The insoluble protein aggregates from the *E. coli* strain LA 5709 carrying plasmid pHG 30 were solubilized as described in Methods in a buffer containing 6

M guanidine-hydrochloride, 1% CHAPS and 2 mM dithiothreitol, and renatured by dilution in a CHAPS containing buffer. The solubilized protein fraction possesses a galactose-dependent ATPase activity (4 nmol/min per mg of protein in the presence of galactose, 0.6 nmol/min per mg of protein in the absence of galactose). It shows several bands on a polyacrylamide gel (Fig. 1), including bands at 51 000, 29 000 and 21 000 daltons which correspond to the molecular weights of the MglA, MglC and MglE gene products determined by molecular cloning experiments [5]. Enrichment of the polypeptide migrating at 51 000 daltons during the purification of the MglA protein (manuscript submitted) and of the polypeptides migrating at 29 000 and 21 000 daltons on a MglB protein-Sepharose affinity column (manuscript in preparation), suggest that these polypeptides represent the MglA, MglC and MglE proteins.

Reconstitution of galactose transport

Proteoliposomes containing ATP accumulate galactose when the purified galactose binding protein is added to the transport assay (Fig. 2). Galactose accumulation occurs at an initial rate of 7 nmol/min per mg of protein and 36 nmol per mg of protein are accumulated in less than 10 min. Omission of the galactose binding protein during the transport assay or

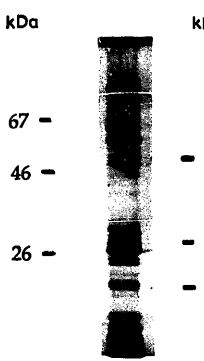

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel of the solubilized proteins. Migration was from top to bottom; the concentration of acrylamide was 12.5%; the gel was stained with silver nitrate. kDa, kilodaltons. The left column shows the position of molecular weight markers and the right column shows the migration of the 51 kDa, 29 kDa and 21 kDa polypeptides.

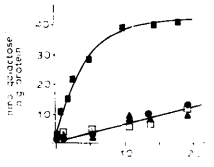

Fig. 2. Reconstitution of galactose transport in proteoliposomes. Uptake was assayed in the presence of 3 mM internal ATP, 50 μM external MglB protein, and 60 μM [^{3}H]galactose (●); ATP, no MglB (○); MglB, no ATP (□); MglB, ATP, liposomes instead of proteoliposomes: an amount of liposomes identical to the amount of proteoliposomes was added as judged from the absorbance at 530 nm (▲).

omission of ATP during the formation of the proteoliposomes result in a drop of galactose uptake to a level corresponding to that obtained with liposomes prepared in the absence of the solubilized proteins. The initial rate of this slow galactose uptake is 10-times lower than the rate of the rapid galactose accumulation. This slow uptake is likely to represent a non specific permeability of the liposomes for galactose. The galactose accumulation in proteoliposomes cannot be attributed to a simple binding reaction since it does not occur in ATP-loaded liposomes even in the presence of galactose binding protein; moreover the accumulation of galactose is dependent on the internal volume of the proteoliposomes: The presence of 0.6 M sucrose in the transport assay, which does not affect significantly the initial velocity of uptake reduces the accumumation by a factor of three (not shown). The binding protein-dependent galactose transport is linearly dependent on the concentration of the proteins used for the formation of the proteoliposomes, in the range of 0–0.5 mg/ml studied (Fig. 3).

Source of energy

The requirement for ATP, internally trapped in the proteoliposomes, suggests that ATP is the source of energy for transport. These results are consistent with early suggestions [6] and with recent evidence indicating ATP as an energy source for other binding protein-dependent transport systems [12,13,15,16]. The uncoupler carbonylcyanide *m*-chlorophenylhydrazone does not significantly inhibit the ATP-dependent galactose transport in proteoliposomes (Fig. 4). This suggests that the energisation of galactose transport by ATP is not due to a protonmotive force, which would have been created by ATP hydrolysis. Galactose transport in proteoliposomes is inhibited by vanadate; a 60% inhibition of the initial rate of galactose transport is obtained at a concentration of 0.5 mM vanadate

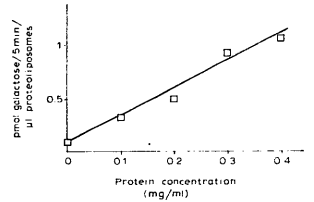

Fig. 3. Galactose transport by proteoliposomes made with different amounts of protein. Proteoliposomes were made as described in Methods with a definite amount of lipids (1 ml at 40 mg/ml) and different amounts of solubilized protein (1 ml at the concentration indicated in abscissa).

(Fig. 4). This inhibition is reminiscent of the vanadate inhibition of the ATPase activity of the purified MglA protein (manuscript submitted for publication) and of the vanadate inhibition of histidine transport in membrane vesicles [13].

Dependence of galactose transport on galactose binding protein and galactose concentrations

Increasing galactose concentrations from 0 to 100 μM at a constant galactose binding protein concentration of 30 μM leads to an increase of transport velocity up to 30 μM galactose followed by a plateau from 30 μM to 100 μM galactose (Fig. 5, curve A). This curve is consistent with a dependence of galactose transport on the liganded galactose binding protein: In fact, due to the high affinity of the galactose binding protein for galactose (K_d = 0.4 μM) [24], the concentration of the liganded binding protein is close to the total galactose concentration up to 30 μM galactose and remains

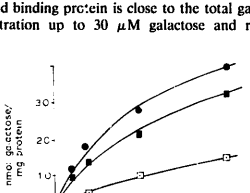

Fig. 4. Effect of inhibitors on transport. Transport was assayed in the presence of 3 mM internal ATP, 50 μM MglB and 60 μM [^{3}H]galactose. No addition (●); addition of 10 μM carbonylcyanide *m*-chlorophenylhydrazone in the transport assay (■); addition of 0.5 mM vanadate (added during the formation of the proteoliposomes) (□). The binding protein-independent galactose transport was subtracted.

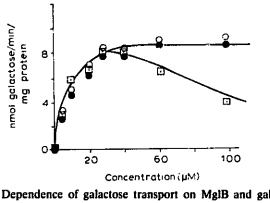

Fig. 5. Dependence of galactose transport on MglB and galactose concentration. The abscissa indicates the concentration of either total galactose (curve A), total MglB (curve B), or the calculated concentration of the MglB-galactose complex (Curve C). In A (●), The MglB concentration was 30 μM while galactose concentration was varied; in B (□), total galactose concentration was 30 μm, while MglB was varied; in C (○), the concentrations of MglB and galactose were varied simultaneously while maintaining them in equimolar ratio. Galactose up take was assayed during 2 minutes.

constant from 30 to 100 μM galactose. Thus transport velocity increases up to 30 μM galactose but becomes constant thereafter because only the concentration of bound galactose and not the free form counts for transport.

Increasing galactose binding protein concentration from 0 to 100 μM at a constant galactose concentration of 30 μM leads to an increase of transport until a 30 μ galactose binding protein concentration is reached (Fig. 5, curve B); this is followed by an inhibition of transport as the binding protein concentration exceeds the galactose concentration. This result is consistent with the liganded binding protein being the substrate for transport, and the inhibition observed for binding protein concentrations higher than galactose concentration can be explained if the unliganded binding protein competes for the membrane components of the transport machinery.

When galactose and galactose binding protein are added in equimolar amounts in increasing concentrations from 0 to 100 μM, the concentration of the galactose-galactose binding protein complex is similar to that of the added components, since the affinity of the MglB protein for galactose is high (0.4 μM K_d). In these conditions, the initial rate of galactose uptake shows saturation kinetics, with a half-maximal velocity of around 15 μM, which represents the affinity of the liganded binding protein for the proteoliposomes (Fig. 5, curve C).

ATP hydrolysis during transport

The hydrolysis of ATP internally trapped in the proteoliposomes is negligible unless galactose and the purified galactose binding protein are added to the assay medium (Fig. 6); (there is no detectable ATP hydrolysis if galactose or the galactose binding protein

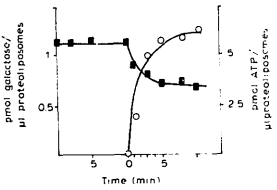

Fig. 6. Galactose uptake and concomitant ATP hydrolysis. Proteoliposomes were prepared in the presence of 0.3 mM ATP. They were incubated in the presence of 40 µM [^{3}H]galactose; at time 0, the purified galactose binding protein was added to a final concentration of 40 µM. ATP (■) and binding protein-dependent galactose transport (○) were assayed in parallel as described in Methods. The low galactose transport in the absence of the mglB protein was subtracted.

alone is added to the proteoliposomes (not shown)). In the presence of the galactose binding protein, galactose is accumulated at a rate of 8 nmol/min per mg of protein and ATP is hydrolyzed at a rate of 20 nmol/min per mg of protein. This represents a stoichiometry of 2.5 ATP hydrolyzed per galactose transported. ATP hydrolysis occurs concomitantly with galactose transport and ceases after the plateau of galactose accumulation has been attained (though ATP has not been entirely consumed during the accumulation of galactose). The tight coupling between galactose transport and ATP hydrolysis is probably related to a perfect coupling between galactose transport and the MglA ATPase in the proteoliposomes, and to the absence of other ATPase activities in the protein fraction used for the formation of the proteoliposomes (unpublished results).

Discussion

This paper describes the reconstitution of the binding protein-dependent galactose transport in proteoliposomes. Galactose transport is dependent: (i) on the addition of the MglB protein during the transport assay, (ii) on the presence of ATP in the interior of the proteoliposomes, (iii) on the presence of the solubilized proteins from the *mgl* operon hyperproducing strain during the formation of the proteoliposomes. Omission of any one of these three components results in a reduced galactose transport activity, which represents a non specific permeability of the liposomes for galactose.

Galactose transport energized by ATP is not inhibited by the uncoupler carbonylcyanide *m*-chlorophenylhydrazone. This excludes an indirect role of ATP through the formation of a protonmotive force. ATP is hydrolyzed concomitantly with galactose transport, and shows a tight coupling with transport. A stoichiometry of 2.5 ATP hydrolyzed per galactose transported was obtained. Stoichiometries of 5 and 1.7 have been obtained by others for binding protein-dependent transport in proteoliposomes or in bacteria [14,15]. Though a stoichiometry of 2 can be explained by the presence of homodimers of the ATP-binding component, or by the presence of two ATP-binding sites in these ATP-binding components [15], it cannot be excluded that galactose uptake has been underestimated, due to some leakage, or due to a nonquantitative retention of the proteoliposomes on the filters during transport measurements.

Our results indicate that the MglB-galactose complex rather than free galactose is the substrate for galactose transport in proteoliposomes; the apparent K_d of the liganded binding protein for the proteoliposomes is around 15 µM. This value is consistent with the high concentration of the mglB protein in the periplasmic space (which has been estimated to be around 0.5 mM [1]); this affinity is slightly higher than the affinity for the membrane of the HisJ protein implicated in histidine transport (K_d 65 µM [13]), and of the MalE protein implicated in maltose transport (K_d 90 µM [25]).

The binding protein-dependent galactose transport in proteoliposomes reaches a plateau in less than ten minutes. The internal galactose concentration at this plateau can be estimated from the results shown in Fig. 6: The internal volume corresponding to 1 µl of proteoliposomes can be calculated to be 0.018 µl by dividing the amount of ATP determined in the luciferase assay (5.4 pmol per µl of proteoliposomes) by the concentration of ATP used during the formation of the proteoliposomes (0.3 mM). If we assume that the whole population of proteoliposomes can accumulate galactose, the internal galactose concentration can be estimated to be 75 µM by dividing the amount of galactose accumulated (1.3 pmol) by the volume determined above (0.018 µl). At the MglB protein and galactose concentrations used in this experiment (40 µM each), it can be calculated that the free galactose concentration in the assay medium is close to 3 µM, thus yielding a concentration factor of 25. However, it is possible that a significant fraction of the proteoliposomes does not display active transport, which would suggest that galactose is accumulated in a smaller volume, and consequently at a higher concentration than that calculated above. The existence of two populations of liposomes which would be active or inactive for binding protein-dependent galactose transport could explain the relatively high amount of galactose transported in the absence of the binding protein (Fig. 2) (in the two populations of liposomes) as compared

to the amount of galactose transported in a binding protein-dependent manner (in the population of liposomes active for binding protein-dependent transport). The maximal rate of galactose transport approaches 8 nmol/min per mg of protein, and the rate of ATP hydrolysis is 20 nmol/min per mg protein. This rate is somewhat lower than the maximal velocity of the purified MglA protein (200 nmol/min per mg of protein). This discrepancy is not surprising since the mglA protein represents only a fraction of the proteins incorporated in the proteoliposomes. The rate of galactose transport in proteoliposomes is slightly higher than the rate of galactose transport in vivo (3 nmol/min per mg of cell protein [26]).

The binding protein-dependent galactose transport depends on the presence of solubilized proteins from the *mgl* operon hyperperproducing strain during the formation of proteoliposomes. Galactose transport activity depends linearly on the concentration of the solubilized proteins added during the formation of the proteoliposomes. The solubilized proteins have been prepared by solubilisation and renaturation of inclusion bodies from a bacterial strain hyperproducing the *mgl* operon of *Salmonella typhimurium*. We have already purified the MglA protein from such inclusion bodies, by following its galactose-dependent ATPase activity (submitted for publication). The present reconstitution of galactose transport in proteoliposomes suggests that the MglC and MglE proteins have been solubilized, and renatured in a functional form from these inclusion bodies. Complementation of the MglA protein with the MglC and MglE proteins, for reconstitution of an MglB-dependent galactose transport in proteoliposomes should be a useful test for the purification for these two proteins.

Acknowledgments

The authors wish to thank Pr. W. Boos (Konstanz University) for the gift of the *mgl* overproducing strain, Pr. C. Burstein (Université Paris 7) for his help in the determination of ATP pools, and Dr. A. Meier (Institut Paris) for his help for the bacterial fermentation.

References

1 Ames, G.F.L. (1986) Annu. Rev. Biochem. 55, 397–405.
2 Furlong, C.E. (1987) in *Escherichia coli* and *Salmonella typhimurium*, (Neidhardt, F.C., ed.), pp. 768–796, American Society for Microbiology, Washington, DC.
3 Gros, P., Croop, J. and Housman, D.E. (1986) Cell 47, 371–378.
4 Riordan, J.R. and al. (1989) Science, 245, 1066–1073.
5 Müller, N., Heine, H.G. and Boos, W. (1985) J. Bacteriol. 163, 37–45.
6 Berger, E.A. and Heppel, L.A. (1974) J. Biol. Chem. 249, 7747–7755.
7 Plate, C.A. (1979) J. Bacteriol. 137, 221–225.
8 Hong, J.S., A.G. Hunt, P.S. Masters and Lieberman, M.A. (1979) Proc. Natl. Acad. Sci. USA 76, 1213–1217.
9 Richarme, G. (1985) J. Bacteriol. 162, 286–293.
10 Hunt, A.G. and Hong, J.S. (1983) Biochemistry 22, 844–850.
11 Higgins, C.F., Hiles, I.D., Whalley, K. and Jamieson, D.J. (1985) EMBO J. 4, 1033–1040.
12 Dean, D.A., Fikes, J.D., Gehring, K., Bassfor, P.J. and Nikaido, H. (1989) J. Bacteriol. 171, 503–510.
13 Prossnitz, F., Gee, A. and Ames, G.F.L. (1989) J. Biol. Chem. 264, 5006–5014.
14 Bishop, L., Agbanyani, R. Jr., Ambudkar, S.V., Maloney P.C. and Ames, G.F.L. (1989) Proc. Natl. Acad. Sci. USA 86, 6953–6957.
15 Nimmack, M.L., Gallagher, M.P., Peara, S.R., Hyde, S.C., Booth, I.R. and Higgins, C.F. (1989) Proc. Natl. Acad. Sci. USA 86, 8257–8261.
16 Davidson, A.L. and Nikaido, H. (1990) J. Biol. Chem. 265, 4254–4260
17 Marston, F. (1986) Biochem. J. 240, 1–12.
18 Schaffner, W. and Weissmann, C (1973) Anal. Biochem. 56, 502–514.
19 Richarme, G. (1983) Biochim. Biophys. Acta, 748, 99–108.
20 Feingold, D.S. (1970) J. Membr. Biol. 3, 372–386.
21 Shlomai, J. and Kornberg, A. (1980) Proc. Natl. Acad. Sci. USA 77, 799–804.
22 Laemmli, U.K. (1970) Nature 227, 680–685.
23 Wray, W., Boulikas, T. Wray, V.P. and Hancock (1981) Anal. Biochem. 118, 197–203.
24 Miller, D.M., Newcomer, M.E. and Quiocho, F.A. (1979) J. Biol. Chem. 254, 7521–7528.
25 Manson, M.D., Boos, W., Bassford, P.P., Jr. and Radmunsen, B.A. (1985) J. Biol. Chem. 260, 9727–9733.
26 Parnes, J.R. and Boos, W. (1973) J. Biol. Chem. 248, 4436–4440.