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# ASSOCIATIVE PROPERTIES OF THE ESCHERICHIA COLI GALACTOSE BINDING PROTEIN AND MALTOSE BINDING PROTEIN

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SUMMARY The periplasmic galactose binding protein and maltose binding protein of Escherichia coli are recovered mostly in dimeric form when purified, from osmotically-shocked bacteria, in the presence of protease inhibitors and 2-mercaptoethanol without dialysis and concentration of the shock fluid. The specific ligands, galactose (but not glucose) for galactose binding protein, and maltose for maltose binding protein, provoque the monomerisation of the dimeric native forms. These results are discussed in relation to the function of both binding proteins in transport and chemotaxis.

## INTRODUCTION

Most periplasmic binding proteins of Gram negative bacteria have been described as monomers with molecular weights ranging from 22,000 to 42,000 (1) ; however, aggregating properties have been reported for the leucine-isoleucine-valine binding protein (2) and dimers of the galactose binding protein have been described, when the protein was dissolved in buffer, after storage as an ammonium sulfate precipitate (3). In this study, we present evidence that the galactose binding protein (GBP), and the maltose binding protein (MBP) of Escherichia coli, which have been purified by others, as monomers of molecular weight 35,000 (GBP) and 40,000 (MBP) (4,5) are recovered in multimeric form (mostly dimeric), when purified rapidly in the presence of protease inhibitors and 2-mercaptoethanol. Furthermore, the specific ligands, galactose (but not glucose) for GBP, and maltose for MBP, provoque the monomerisation of the dimeric native forms.

## MATERIALS AND METHODS

Materials D-glucose, D-galactose and D-maltose were from Merck. [$^{14}$C] sugars were from C.E.A., France. $^{35}$S sulfate 25 Ci/mg was from Amersham. Phenylmethylsulfonyl fluoride was from Sigma. Protein markers were from Calbiochem.

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Purification of galactose binding protein and maltose binding protein
A single procedure was developed for purification of both proteins. E. coli K12, strain pop3325, maltose constitutive, (from Dr. M. Schwartz, Institut Pasteur, Paris), was grown, at 35°C, in minimal medium, containing 10 mCi/l of [$^{35}$S] sulfate at a final concentration 0.2 mM, and $10^{-3}$ M fucose. The cells were harvested at the end of the exponential phase ; they were osmotically shocked at 0°C in distilled water containing $5x10^{-4}$ M $MgCl_2$ as described by Nossal and Heppel (6) ; immediatly after shock, the supernatant shock fluid was adjusted to pH 7.3 with buffer containing 0.01 M sodium phosphate pH 7.3, 2 mM 2-mercaptoethanol, $10^{-4}$ M phenylmethylsulfonyl fluoride (PMSF). The shock fluid was immediatly loaded on a hydroxylapatite (from Dr. G. Bernardi, Paris) column, equilibrated in the same buffer ; elution was made with a linear gradient of 0.01 M to 0.5 M sodium phosphate buffer containing 2 mM 2-mercaptoethanol, $10^{-4}$ M PMSF. Both proteins were eluted in the same fractions ; the pooled fractions were dialysed (4 hours) against $10^{-2}$ M Tris pH 8.1, 2mM mercaptoethanol, $10^{-4}$ M PMSF and loaded on a QAE-Sephadex column, equilibrated in the same buffer ; elution was made with a linear gradient of 0 to 0.5 M KCl in the same buffer ; galactose binding protein and maltose binding protein containing fractions were pooled, dialysed against $10^{-2}$ M Tris pH 8.1, 2 mM 2-mercaptoethanol, $10^{-4}$ M PMSF and loaded on a DEAE-cellulose (DE 52 Whatman) column equilibrated in the same buffer ; elution was made with a linear gradient of 0 to 0.3 M NaCl in the same buffer. The galactose binding protein was eluted at 0.07 NaCl and the maltose binding protein at 0.15 M NaCl. The proteins were dialysed against $10^{-2}$ M Tris pH 8.1, 2 mM 2-mercaptoethanol, and stored at -70°C. Detection of [$^{35}$S] GBP and [$^{35}$S] MBP in the first steps of the purification was made with specific antibodies prepared as described in (5). Galactose and maltose binding activities were measured by the filtration procedure described previously (7) : twenty volumes of a saturated ammonium sulfate solution were added to one volume of reaction mixture (binding protein + [$^{14}$C] sugar) ; the samples were filtered on cellulose esters filters and rinsed three times with saturated ammonium sulfate at 0°C. The filters were counted in a dioxane-based scintillation mixture ; a blank was made without the [$^{14}$C] sugar. Both binding proteins were fully active in binding their specific ligands.

Biogel P-100 chromatography Columns (80 cm x 0.7 cm) of Biogel P-100 (100-200 mesh) were equilibrated at 22°C with the column buffer, $10^{-2}$ M Tris pH 8.1, 2 mM 2-mercaptoethanol (other constituents were added in the column buffer as described in the text) ; 150 µl of the purified protein solution was applied to the column ; the flow rate was 2 ml/h ; fractions of 10 drops were collected. The void volume of the column was determined with dextran blue ; protein markers serum albumin (68,000) and chymotrypsinogen (25,000) were included in each experiment.

Glycerol gradients ultracentrifugation Glycerol gradient ultracentrifugation was performed with a Spinco SW/50 rotor, in a Spinco model l2-65B ; isokinetic glycerol gradients (5 to 20%) of 4.8 ml were prepared in $10^{-2}$ M Tris pH 8.1 buffer containing 2 mM 2-mercaptoethanol, and other constituents as indicated in the text. Samples and standards of 100 µl were applied to the gradients and centrifuged at 4°C for 17 hours at 45,000 rpm. Fractions of 200 µl each were collected. $S_{20,w}$ was calculated on the basis of a linear relationship of the sedimentation coefficient to the distance migrated in the gradient. Bovine serum albumin was used as a standard for these calculations.

RESULTS Fig. 1A shows chromatography of the maltose binding protein on a Bio-Gel P-100 column equilibrated in buffer containing no maltose. The maltose binding protein was eluted as a peak, at an elution volume lying between the void volume of the column and the elution volume of the serum albumin (68,000)

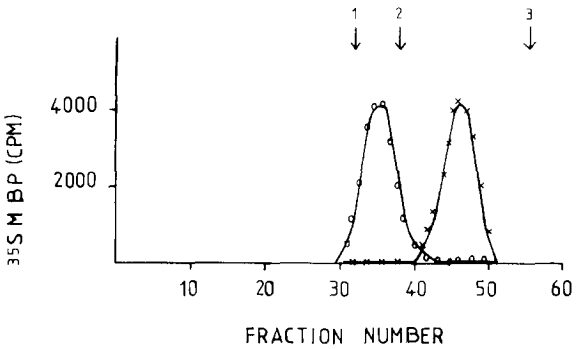


figure 1 Bio-Gel P100 chromatography of maltose binding protein in the absence and in the presence of maltose. 150 µl of a mixture containing MBP (50 µg), serum albumin and chymotrypsinogen (1 mg each) was loaded on a column equilibrated in buffer containing no maltose (o). 150 µl of a mixture containing MBP (50 µg), $10^{-3}$ M maltose, serum albumin and chymotrypsinogen (1 mg each) was loaded on a column equilibrated in buffer containing $10^{-3}$ M maltose (x). 50 µl of each fraction was counted for radioactivity. Arrows indicate 1 : void volume, 2 : serum albumin elution volume, 3 : chymotrypsinogen elution volume.

marker ; this peak corresponds to a dimeric form of the maltose binding protein ; in some experiments a small fraction of the protein was eluted at the void volume of the column, indicating the presence of higher multimeric forms (not shown). Fig. 1B shows chromatography of the same preparation of maltose binding protein on a Bio-Gel P-100 column containing $10^{-3}$ M maltose, a specific ligand of the maltose binding protein. In this experiment, the maltose binding protein was eluted at an elution volume which corresponds to a molecular weight of 40,000, the molecular weight reported for the monomeric maltose binding protein (5). The results from both columns show that the maltose binding protein, purified as described in this study, exists in multimeric forms, mostly dimeric, in equilibrium with the monomeric form, and that the ligand, maltose, provoque the monomerisation of the dimeric native form.

The galactose binding protein was sedimented in three linear glycerol gradients, containing respectively : no galactose no glucose, $10^{-3}$ M glucose, $10^{-3}$ M galactose ; as shown in fig 2, in the first two gradients, the galactose binding protein sedimented faster than in the third gradient in the presence of galactose ; in the absence of galactose and glucose, or in the presence of $10^{-3}$ M glucose, a sedimentation coefficient of 6.3 S was determined ; in the presence of $10^{-3}$ M galactose, a sedimentation coefficient of 3.7 S was

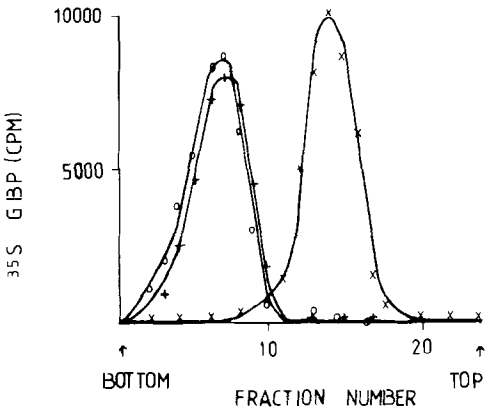


figure 2 Sedimentation of galactose binding protein on glycerol gradients containing : no galactose, no glucose (+), $10^{-3}$ M glucose (o), $10^{-3}$ M galactose (×). 100 µl of GBP (120 µg) was loaded on each gradient. GBP was preequilibrated with the ligand contained in each gradient. 50 µl of each fraction was counted for radioactivity.

calculated which is slightly higher than the value reported for the monomeric galactose binding protein (4). The ratio of the sedimentation coefficients, in the presence and absence of galactose, is compatible with the existence of a dimeric form of the galactose binding protein in the absence of galactose. The same results were obtained using Bio-Gel P-100 chromatography columns. These results show that the galactose binding protein, prepared as described in this study, is recovered in dimeric form, and that galactose, but not glucose, provoque the monomerisation of the dimeric native form.

DISCUSSION Dimeric forms of the maltose binding protein and of the galactose binding protein are described in this study. The specific ligands, maltose (for MBP), and galactose, but not glucose (for GBP), provoque the monomerisation of the dimeric native forms ; furthermore, ligand binding experiments made with these multimeric binding proteins (to be published elsewhere), show a dependence of binding affinity on protein concentration. This reflects a preferential affinity of maltose and galactose for the monomeric form of MBP and GBP.

In the previous report of GBP dimer (3), dimers could only be seen under artificial conditions, after precipitation under saturated ammonium sulfate ; the affinity of galactose for monomer and dimer was identical, and no effect of galactose on the state of aggregation of the protein was described.

The ability of periplasmic binding proteins to form dimers, and the lower affinity of the dimers for the ligands should be important for the transport and chemotactic functions of these proteins. In transport, if the contact of the binding protein-ligand complex with the cytoplasmic membrane (8,9) triggers a dimerisation of the binding protein, possibly induced by its contact with a multimeric pore (10), the consequent decrease of affinity of the dimeric binding protein for its ligand should favor transfer of the ligand to the cytoplasm. The different influence of glucose and galactose on the monomer-dimer equilibrium of GBP might explain the lower efficiency for glucose transport of the galactose binding protein mediated transport (W. Boos, personal communication). In chemotaxis, the maltose binding protein (chemoreceptor for maltose) and the galactose binding protein (chemoreceptor for galactose) interact respectively (9, 12) (13) with two methyl accepting chemotaxis proteins, located in the cytoplasmic membrane, the tar and trg gene products (14); the tar gene product exists as tetrameric protein (15) ; interaction of the tetrameric tar gene product and the maltose binding protein endowed with aggregating properties might promote an aggregation of several tar tetramers that could be important for the chemotactic response. The importance of aggregation phenomena in membrane receptors function has been reported in several systems (16-18).

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