Acta Chemica Scandinavica B 35 (1981) 29 – 32

Purification and Characterization of Maltose Binding Protein of *Saccharomyces cerevisiae*

MAJ-RITA SIRO, KATRIINA VIITANEN and TIMO LÖVGREN

Department of Biochemistry and Pharmacy, Åbo Akademi, SF-20500 Åbo 50, Finland

A maltose binding protein was purified from a membrane fraction of *Saccharomyces cerevisiae* by affinity chromatography on epoxy-activated Sepharose 6 B to which maltose was coupled. The product was checked for purity by disc gel electrophoresis in the presence of urea. Two separate bands were obtained.

The binding of maltose to the purified binding protein was studied at pH 4.8 by equilibrium dialysis using varying concentrations of ligand. The data were plotted according to Scatchard and a biphasic plot was obtained. The different slopes correspond to the apparent dissociation constants of 0.47×10^{-5} and 1.1×10^{-4} M, respectively. The heterogeneous binding was considered to depend on the presence of molecules with differing affinities for the ligand.

Glucose was shown to inhibit the binding of maltose to the purified protein. Only the apparent low affinity dissociation constant of the maltose-protein complex was affected. A change of the constant from 1.1×10^{-4} M in the absence of glucose to $1{,}5 \times 10^{-4}$ M in the presence of glucose was obtained.

Binding proteins taking part in the transport of compounds across plasma membranes are many but only some attempts to isolate these proteins from eucaryotic cells have been reported. The existence of a maltose binding protein in *Escherichia coli* was shown by Hagelbauer and Adler.[1] It has been isolated by Kellermann and Szmelcman [2] and Ferenci and Klotz.[3] Several studies performed suggested that the maltose binding protein could exist in two conformations differing in their affinity towards maltose.[2,4-5] Similar observations have been reported for the galactose binding protein [6,7] and for mitochondrial Ca(II) binding protein.[8]

The utilization of maltose by the yeast *Saccharomyces cerevisiae* is controlled by an inducible mechanism.[9-15] The uptake of maltose from the medium is mediated by α-glucoside permease and the intracellular hydrolysis is catalyzed by α-glucosidase (α-D-glucoside glucohydrolase, EC. 3.2.1.20). This communication describes the purification of a maltose binding protein by affinity chromatography from a membrane fraction prepared from the yeast *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Preparation of the maltose binding protein. The maltose binding protein was isolated from commercial baker's yeast propagated on molasses. The yeast was obtained from Oy Alko Ab (Rajamäki, Finland). Fresh yeast cells (50 g wet wt) were suspended in cold buffer solution (100 ml) containing 400 mM KCl, 20 mM triethanolamine and 1 mM $MgCl_2$.[16] The pH of the solution was adjusted to 4.0 with HCl. The suspension was homogenized with Ballotini glass beads No. 12 (2 parts of dry beads to 1 part of suspension) for eight minutes at 4 °C in a homogenizer (Waring Blender Model 1042). The glass beads were removed by decanting and washed several times with buffer. The combined homogenate and washings were fractionated at 4 °C in a Sorvall superspeed RC 2-B Automatic refrigerated centrifuge as follows: 10 min at $6000 \times g$, 35 min at $14000 \times g$ and 60 min at $40000 \times g$. The individual sediments were washed with the buffer solution until no α-glucosidase activity was found and their binding affinity for maltose was tested by equilibrium dialysis. The α-glucosidase activity was determined using 2.2 mM *p*-nitrophenyl α-D-glucopyranoside in 0.05 M sodium potassium phosphate buffer pH 6.5 by following the release of *p*-nitrophenol at 400 nm.[17] The membrane fraction sedimenting at $40000 \times g$ contained the highest

0302-4369/81/010029-04$02.50
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specific maltose binding activity and this fraction was used for the affinity chromatographic isolation step.

Affinity chromatography. Epoxy-activated Sepharose 6B (4 g) was prepared according to the instructions given by Pharmacia. The coupling solution contained 300 mg maltose dissolved in 12 ml 0.1 M NaOH. Coupling was allowed to proceed on a shaker overnight. The wet gel (12 ml) was packed in a Pharmacia column (K 16/20) and equilibrated with 0.02 M citrate buffer pH 4.8. The membrane fraction sedimenting at 40000 × *g* was dissolved in the buffer mentioned and a total of 7.6 mg protein was applied on the column at room temperature. Excess material was washed out of the column with the citrate buffer, pH 4.8. The elution of bound protein was performed at a flow of 10 ml/h with 0.05 M phosphate-citrate buffer pH 8.0 in opposite direction to application. Fractions of 2.9 ml were collected. The UV-absorbing material eluted between 23.3 and 49.3 ml was pooled and concentrated at 4 °C in a Diaflo pressure filtration unit using filter UM 10.

Equilibrium dialysis. Maltose binding activity was measured by equilibrium dialysis in 0.1 M citrate buffer pH 4.8 in highly polished Plexite cells (1 ml). Half-cells containing the protein sample (6 μg) and labeled ^{14}C maltose (310 MBq/mol, Amersham) at one side and the buffer at the other were separated by dialysis tubing Visking size 3 – 20/32 (Med. Cell International) presoaked in sodium bicarbonate. The liquid volume at each side of the tubing was adjusted to 250 μl with the buffer above. Each half-cell contained a small glass bead for sufficient stirring. Dialysis was performed at room temperature with gentle shaking and equilibrium was attained after four hours. Samples of 100 μl were removed in two aliquots from each of the half-cells and counted in 5 ml scintillation liquid (0.6 % w/v 2,5-diphenyloxazole (PPO) and 0.08 % 1,4-bis-(4-methyl-5-phenyloxazolyl)benzene (POPOP)). The radioactivity was measured with an LKB-Wallac 8100 liquid scintillation counter. The increment of radioactivity in the compartment containing protein was taken as a measure of binding. When the effect of glucose on the binding of maltose to the protein was studied nonlabeled glucose was added together with labeled maltose to the half-cell containing the protein.

Disc-gel electrophoresis. Disc-gel electrophoresis was carried out in 10 % polyacrylamide gels at pH 8.9 in the presence of 10 M urea and in 7.5 % acid polyacrylamide gels containing 5 M urea.[18] A total of 15 – 50 μg protein was applied on a gel. A 0.25 % solution of Coomassie Brilliant Blue in methanol – acetic acid – water (5:1:5 v/v/v) was used for staining.

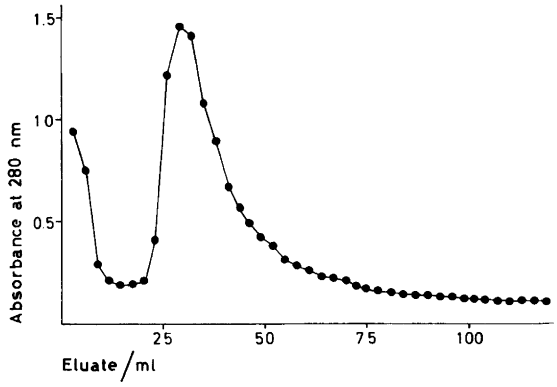

Fig. 1. Elution profile of the maltose binding protein.

Determination of protein. Protein was determined by a modification of the Lowry method[19] using bovine serum albumin as a standard.

RESULTS

A typical elution profile of the binding protein is shown in Fig. 1. The bound protein is eluted in one major peak. The UV-absorbing material obtained in the first fractions, when the column is eluted in the opposite direction to application, consists of insoluble material that does not penetrate the gel when the sample is applied. By the affinity chromatography step the maltose binding protein was purified 6 times relative to the membrane fraction.

The final product was checked four purity by gel electrophoresis in different conditions in the presence of urea. Two separate bands were observed. The one that moved faster contained most of the protein. Attemps to check the maltose binding activity of the bands were not successful probably because of the denaturating conditions.

Equilibrium dialysis experiments were performed with the purified protein using varying concentrations of maltose. The binding of maltose was measured at 24 μg protein per ml. The binding activity as a function of maltose concentration is shown in Fig. 2. At low maltose concentrations (the inset) the curve does not follow the classical Michaelis-Menten hyperbola. The shape is sigmoid instead. The heterogeneous behaviour is even more pronounced when the data are plotted according to Scatchard[20] (Fig. 3). The

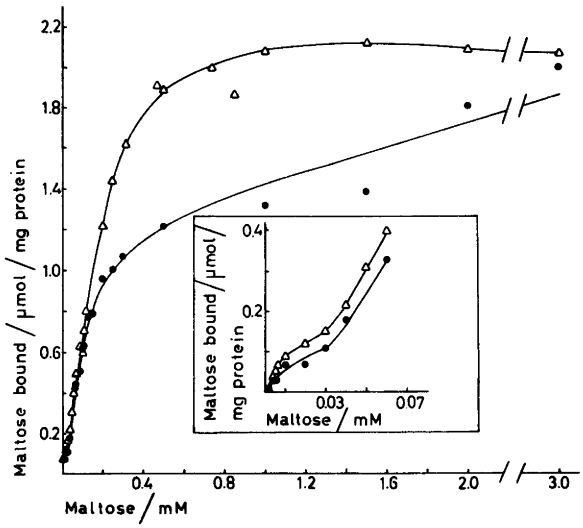

Fig. 2. Maltose binding activity measured by equilibrium dialysis (△), the binding activity in the presence of 5 mM glucose (●).

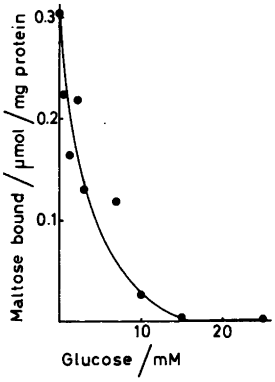

Fig. 4. The effect of glucose on the maltose binding activity of the maltose binding protein measured by equilibrium dialysis.

Scatchard plot is biphasic which indicates that there are two populations of sites, one strong and the other weak. The different slopes correspond to the apparent dissociation constants of 0.47×10^{-5} and 1.1×10^{-4} M, respectively. The extrapolation of the curve to high free maltose concentration indicates that totally 0.22 $\times 10^{-2}$ µmol maltose was bound per µg protein of which only 0.18×10^{-3} µmol was bound with a high affinity.

The effect of glucose on the binding activity was tested by adding varying amounts of glucose to the binding protein in the presence of 50 µM maltose. The results are shown in Fig. 4. It can be seen that glucose efficiently inhibits the binding of maltose. The kinetics of the inhibition was studied in the presence of 5 mM glucose and varying maltose concentrations (Figs. 2 and 3). Glucose seems not to affect the apparent high affinity dissociation constant of the protein-maltose complex (Fig. 3). On the contrary the affinity of the binding protein at the low affinity site decreased in the presence of glucose. A change of the apparent dissociation constant from 1.1×10^{-4} M in the absence of glucose to 1.5×10^{-4} in the presence of glucose was obtained.

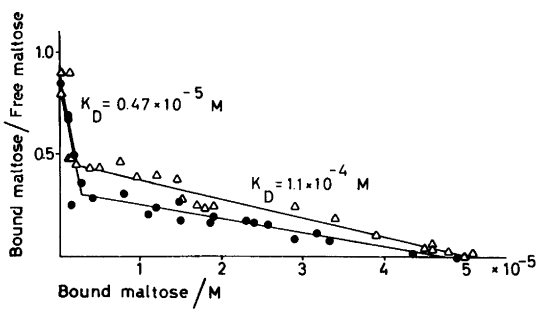

Fig. 3. Scatchard plot of the binding data of Fig. 2 in the absence of glucose (△) and the presence of 5 mM glucose (●).

Discussion. The degree of purification is difficult to determine due to the fact that the maltose binding activity of the yeast homogenate cannot be measured because of its high α-glucosidase activity. The final purification is consequently much greater than the reported 6 times achieved by the affinity chromatographic step.

Sigmoidal ligand binding curves are characteristic of the cooperative binding of ligand to a protein with multiple binding sites. The obtained Scatchard plot (Fig. 3) shows that successive ligand molecules are bound with decreasing affinity. Such a negative cooperativity has been explained by the presence of binding sites of different affinities so that the stronger are occupied first or by the presence of oligomeric proteins composed of identical subunits the affinities of which are changed by binding of ligand. Extrapolation of the part of the Scatchard plot corresponding to the high concentrations of maltose shows that the total amount of maltose bound is 0.22 $\times 10^{-2}$ µmol/µg protein. The amount of ligand bound to the high affinity site (0.18×10^{-3} µmol/µg protein) is

only a small fraction of the total amount. Accordingly, the two kinds of sites seem not to be carried by different forms of one single protein. An inhomogeneous preparation of enzyme containing molecules of differing affinities for the ligand gives a binding curve similar to that of negative cooperativity.

The binding heterogeneity of isolated material could reflect the presence of one constitutive and one inducible maltose binding protein with different affinities for maltose. As is well known the synthesis of α-glucoside permease and α-glucosidase are induced mainly by maltose and (a) common regulatory mechanism(s) are involved.[21] This induction is prevented during catabolite repression.[22] In spite of that, yeast cells grown under repressed conditions show a measurable maltose transporting activity indicating that a maltose transporting system is constitutively present.[23,24] Actually an inducible and a constitutive α-glucosidase has been demonstrated in *Schizosaccharomyces pombe*.[25] Separate mechanisms for the transport of α-methyl thioglucoside (α-TEG) in induced and uninduced yeast cells have been reported.[26] The maltose uptake system and the α-TEG accumulation are related in both their function and induction.[27,28]

The specificity of the maltose binding activity was tested by competition experiments using glucose that affects the maltose fermenting system in yeast cells. In whole yeast cells glucose decreases the initial rate of the uptake of maltose[23] and a rapid increase of K_m from 4 to 50 mM has been found during deadaption of the system in the presence of glucose.[29] The results presented show clearly that glucose inhibits the binding of maltose to the isolated material (Fig. 4). The high and low affinity sites seem to obey different mechanisms of inhibition as only the apparent low affinity dissociation constant of the maltose-protein complex is affected by glucose. Equilibrium binding studies are, however, not enough to distinguish between mechanisms.

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Received July 21, 1980.