Note

Production of Functional Lectin in Pichia pastoris Directed by Cloned cDNA from Aleuria aurantia

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A plasmid bearing a nucleotide sequence of fucose-specific lectin of Aleuria aurantia was constructed and expressed in a methylotrophic yeast, Pichia pastoris. The product showed almost the same hemagglutinating activity as the lectin produced in Escherichia coli, the properties of which were quite similar to the native one. Because of glycosylation of the product, the molecular mass was larger than that of the native one, and it acquired higher thermostability.

Key words: Aleuria aurantia lectin; heterogenous gene expression; glycosylation of lectin; improvement of thermostability

Lectins are sugar-binding proteins, some of which have great value as specific probes for investigating the structure and function of carbohydrate chains on a cell surface.1) The lectin (AAL) from fruit bodies of Aleuria aurantia, an ascomycete mushroom, is a fucose-specific lectin, and has unique sugar-recognition properties.2,3) α-L-Fucopyranosyl residues are widely distributed in glycoproteins and glycolipids, and in many cases, the residues constitute parts of important antigens such as the blood group antigen H4) and the stage-specific embryonic antigens.5) Therefore AAL has been widely used as a tool for the study on fucose-containing saccharides, and thus it is commercially available.

We have investigated the structure of AAL by cloning cDNA6,7) and genomic DNA,8) and by crystallization.9) In this report we describe production of a large amount of functional AAL in Pichia pastoris, a methylotrophic yeast, and found that AAL produced in this yeast was glycosylated, and acquired higher thermostability.

P. pastoris GS115 (His4-) and plasmid pPIC9K were purchased from Invitrogen (Carlsbad, USA). An expression plasmid, pPIC9K-AAL, was constructed as follows: Because of the existence of two XhoI-recognition-sites in the plasmid pPIC9K, a BamHI-EcoRI fragment (288 bp) containing one XhoI site was introduced into pUC19, and the cloned AAL gene9) was inserted into the XhoI-EcoRI site of the plasmid. The resultant BamHI-EcoRI fragment was cleaved from the plasmid, and then inserted into pPIC9K. Plasmid, pPIC9K-AAL, was linearized at the his4 gene by SalI, and introduced into P. pastoris GS115 (His4-) by electroporation using a Gene Pulser (Bio-Rad). By this procedure, AAL, having the same N-terminal amino acid as that of the native AAL, could be produced in the culture fluid.

Among the His+ transformants chosen, G418-resistant colonies were selected on plates containing 1% yeast extract, 2% peptone, 2% dextrose, and 6 mg/ml G418 (Sigma). P. pastoris bearing pPIC9K-AAL was cultured in a 3-liter table-top glass fermentor (Model TS-M, Takasugi Seisakusho, Tokyo) using basal salts medium with addition of methanol as a carbon source and an inducer for the gene expression according to the manufacturer’s instruction entitled Pichia Fermentation Process Guidelines. Cultivation was ceased at 72 hr after methanol addition, and a portion of the culture fluid was put through sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1A).

AAL was purified using a fucose-starch column, and the hemagglutinating activity was assayed as reported.9) As shown in Table 1, purified AAL produced in P. pastoris showed almost the same specific activity of hemagglutination as that produced in E. coli9) which was purchased from Nichirei Co., Ltd. (Tokyo). Properties of AAL produced in E. coli were reported to be quite similar to the native AAL.9) The culture fluid was estimated to contain 67 mg of AAL per liter.

The molecular mass of AAL produced in P. pastoris was about 36 kDa on SDS-PAGE, which was larger than that produced in E. coli (Fig. 1B). The band cross-reacted with anti-AAL serum, indicating that this band is AAL (Fig. 1C). The product
Fig. 1. SDS-PAGE, Immunoblotting, and PAS-Staining.
Culture fluid (10 µl) of the transformant bearing pPIC9K-AAL was resolved by 12.5% SDS-PAGE, and stained with Coomassie Brilliant Blue (A). Purified AAL produced in P. pastoris (lane 1) and that produced in E. coli (lane 2) were put through SDS-PAGE, and stained with Coomassie Brilliant Blue (B). The protein bands were transferred to a membrane, immunoblotted with anti-AAL serum, and made visible with alkaline phosphatase (C). The protein bands were stained with PAS reagent (D).

Table 1. Purification of Recombinant AAL Expressed in P. pastoris

<table>
<thead>
<tr>
<th>Step</th>
<th>Total titer</th>
<th>Total protein* µg</th>
<th>Specific activity Titer/µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture fluid</td>
<td>3,200</td>
<td>1032</td>
<td>3.1</td>
</tr>
<tr>
<td>Fucose column</td>
<td>800</td>
<td>16.8</td>
<td>48</td>
</tr>
</tbody>
</table>

* Protein was measured by the method of Bradford.10)

was glycosylated, since it was positively stained by periodic acid-Schiff (PAS) reagent11) as shown in Fig. 1D. It should be noted that two glycosylation recognition sequences (138NGS and 269NSS) are found in the amino acid sequence of AAL.7) Native AAL was reported to have no carbohydrate,7) as was AAL produced in E. coli, thus the carbohydrate moiety is not essential to the activity of AAL. As shown in Fig. 2, AAL produced in P. pastoris showed higher thermostability than that in E. coli. It has been reported that glycosylation of a protein resulted in acquisition of higher thermostability.12)

Results presented in this paper indicate that AAL produced in P. pastoris was functional, had almost the same specific activity of hemagglutination as the native one, and acquired higher thermostability because of glycosylation. Moreover, purification of recombinant AAL in a large amount can be easily done by affinity chromatography using a fucose-starch column. Therefore the AAL preparation produced in P. pastoris can be used as a useful tool for investigations of fucose-containing saccharides in the field of molecular cell biology and clinical diagnosis.13)

Fig. 2. Thermostability of AAL.
AAL (0.25 µg) produced in P. pastoris (●) and that in E. coli (○) were dissolved in phosphate-buffered saline, pH 7.4, and treated for 10 min at the temperature indicated. After cooling on ice, hemagglutinating activity was measured.

References
10) Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein

