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Cloning and expression of a functional fucose-specific lectin from an orange peel mushroom, *Aleuria aurantia*

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Aleuria aurantia lectin (AAL) shows sugar-binding specificity for L-fucose. A λgt11 expression library was constructed from *A. aurantia* poly(A) RNA and screened with a polyclonal antiserum directed against AAL. An immunopositive clone carrying 1.3-kb *Eco*RI fragment was obtained. The fragment encoded AAL, but lacked a nucleotide sequence corresponding to the two amino-terminal amino acids. The 5'-terminal part of the fragment was replaced with a chemically synthesized DNA fragment and inserted into an expression vector to yield a plasmid pKA-1. *Escherichia coli* carrying pKA-1 expressed functional AAL and the recombinant AAL showed the same immunological properties as those of natural AAL.

Lectin; Cloning; (*Aleuria aurantia*)

1. INTRODUCTION

Lectins are sugar-binding proteins and of great value as specific probes for investigating the structure and function of carbohydrate chains on the surface of animal cells [1]. The widespread occurrence of lectins has been reported among higher plants, vertebrates as well as microorganisms including fungi [2,3].

One of the lectins in fungi has been purified from fruiting bodies of orange peel mushroom, *Aleuria aurantia*, and characterized. This lectin (AAL) has a molecular mass of 72 kDa and is composed of two identical subunits [4]. AAL exhibits the uncommon specificity for L-fucose and has been used as a valuable tool for probing fucose-containing carbohydrates [5]. Because of a limited supply of AAL from natural sources, we attempted to isolate a cDNA clone encoding AAL and to express functional AAL in *Escherichia coli*.

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2. MATERIALS AND METHODS

2.1. *Determination of amino-terminal amino acid sequence of AAL*

Amino-terminal amino acid sequencing of AAL was performed with use of 5 μg of the purified lectin [4] by a protein sequencer 470A (Applied Biosystems) equipped with PTH analyser 120A.

2.2. *General cloning procedures*

Preparation of poly(A) RNA from *A. aurantia* fruiting bodies was performed by the method of Okayama et al. [6]. The RNA was converted to cDNA using a cDNA synthesis kit (Boehringer Mannheim). cDNA library was constructed with a λgt11 cloning kit (Amersham), and screened immunologically with an AAL-antiserum directed against purified AAL. Immunopositive plaques were detected chromogenically using an immunoscreening system (Amersham). Recombination of DNA [7,8], and DNA sequencing [9] were performed as described in the references.

2.3. *Hemagglutination and inhibition assay*

The titration of lectin was performed by serially diluting the sample with phosphate-buffered saline (PBS), and then mixing

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with an equal volume (25 μl) of 1% human type O erythrocytes in PBS. In inhibition assays, AAL (titer 4) was incubated at room temperature for 1 h with L-fucose which had been serially diluted with PBS and assayed for the residual hemagglutination activity. The minimum concentration of fucose that inhibited the hemagglutination reaction completely was determined.

3. RESULTS AND DISCUSSION

3.1. *Cloning of AAL cDNA*

Among 10^5 plaques, one positive clone (λAAL-1) containing a 1.3-kb *Eco*RI fragment was isolated from the library. The fragment was subcloned into plasmid pUC18 [10] and the resulting plasmid was designated as pAL-1 (fig.1). The nucleotide sequence of the 5′-terminal part of the *Eco*RI insert and its derived amino acid sequence are shown in fig.2. The amino acid sequence of the amino-terminal part of AAL was also presented in fig.2. Comparing these two sequences, it was revealed that pAL-1 lacked a nucleotide sequence corresponding to the two amino-terminal amino acid residues.

3.2. *Expression of AAL in* Escherichia coli

Since the plasmid pAL-1 was found to lack a nucleotide sequence corresponding to two amino-terminal residues of the AAL polypeptide, addition of the nucleotides that correspond to initiation methionine and the wanted amino acids to the cloned cDNA was attempted for the expression of recombinant protein. As summarized in fig.3, plasmid pKK-AAL and an expression vector pKA-1 have been constructed. *E. coli* JM109 [11] carrying pKA-1 (abbreviated as *E. coli* JM109[pKA-1]), *E. coli* JM109[pKK-AAL] and *E. coli* JM109[pKK223-3] were cultured in LB medium at 37°C for 16 h after induction with 1 mM isopropyl-β-D-thiogalactoside. After crushing the cells using a sonic oscillator, supernatant and debris fractions were separated by centrifugation and the protein products were analyzed on a SDS-15% polyacrylamide gel electrophoresis

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1                                   10
Pro-Thr-Glu-Phe-Leu-Tyr-Thr-Ser-Lys-Ile-Ala-Ala-Ile-Ser-Trp-
1                                  30
GAA TTC CTC TAC ACC TCG AAA ATT GCA GCC ATC TCT TGG
Glu Phe Leu Tyr Thr Ser Lys Ile Ala Ala Ile Ser Trp

16                                  25
Ala-Ala-Thr-Gly-Gly-Arg-Gln-Xaa-Arg-Val-Tyr-Phe-Gln-Asp-Leu-
                                   60
GCT GCC ACC GGC GGC CGC CAG CAA CGC GTC TAC TTC CAA GAC CTT
Ala Ala Thr Gly Gly Arg Gln Gln Arg Val Tyr Phe Gln Asp Leu
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Fig.2. The amino-terminal amino acid sequence of AAL and the 5′-terminal nucleotide sequence of the *Eco*RI 1.3-kb fragment. Amino acids are numbered taking amino-terminal proline as 1. Xaa (23rd residue) indicates unidentified amino acid. The nucleotide sequence of the coding strand is given from 5′ ⟶ 3′, and the bold numbering takes the 5′-terminal G as 1. The predicted amino acid sequence is given below the nucleotide sequence.

[12]. *E. coli* JM109[pKA-1] produced a protein which migrated at the same position as AAL. This protein was positively stained by immunoblotting using the anti-AAL serum after transfer to a nitrocellulose filter (not shown).

The extract derived from *E. coli* JM109[pKA-1] showed activity to agglutinate human type O blood cells like natural AAL. However, no activity was observed in the extract of either *E. coli* JM109[pKK-AAL] or *E. coli* JM109[pKK223-3]. The recombinant AAL occurs in a soluble form within *E. coli* cells, thus should be easily recovered and purified (to be published elsewhere). In contrast, in the case of pea lectin, the recombinant lectin formed insoluble aggregates [13]. To determine the sugar specificity, hemagglutination-inhibition experiments were carried out. When natural and recombinant AALs were used at the same hemagglutinating activity (titer 4), the activities were equally inhibited by fucose at a minimum concentration of 0.39 mM. From these observations, it was concluded that recombinant AAL is functional and shows the same agglutination-inhibition properties as natural AAL.

The amount of recombinant AAL produced in *E. coli* was approximately 60 mg per liter culture.

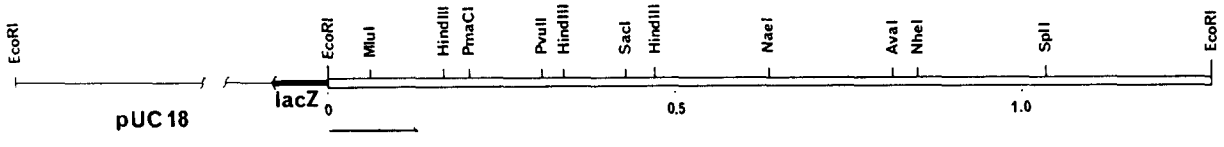

Fig.1. Restriction map of plasmid pAL-1. Open bar represents the insert DNA from *A. aurantia*. Arrow indicates the direction of the AAL translation in vivo. Restriction sites are indicated and numbers represent the distance given in kb.

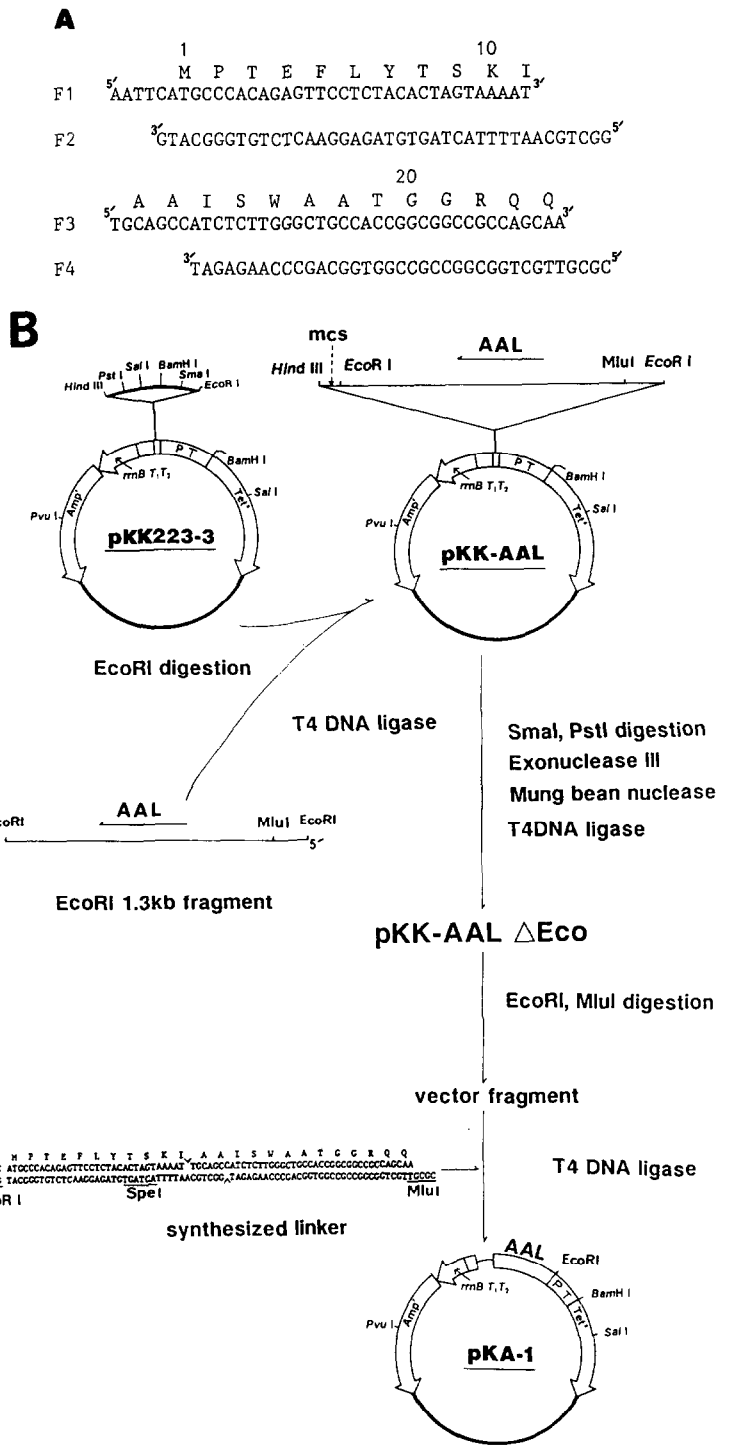

Fig.3. Strategy for the construction of the expression vector pKA-1. (A) Nucleotide sequences of the chemically synthesized DNA fragments. Expected amino acids are indicated above the coding strand with numbering of initiation methionine as 1. (B) Scheme for the construction of pKA-1. mcs, multi-cloning site; PT, *tac* promoter; rrnB T1T2, ribosomal RNA transcription terminators T1 and T2.

Thus we are able to obtain a large amount of functional AAL and use it as a tool for probing fucose-containing oligosaccharides and glycoproteins.

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REFERENCES

[1] Lis, H. and Sharon, N. (1986) Annu. Rev. Biochem. 55, 35–67.
[2] Liener, I.E., Sharon, N. and Goldstein, I.J. (1986) The Lectins, Academic Press.
[3] Gold, E.R. and Balding, P. (1975) The Receptor-Specific Proteins, Plants and Animal Lectins, Elsevier.
[4] Kochibe, N. and Furukawa, K. (1980) Biochemistry 19, 2841–2846.
[5] Yamashita, K., Kochibe, N., Ohkura, T., Ueda, I. and Kobata, A. (1985) J. Biol. Chem. 260, 4688–4693.
[6] Okayama, H., Kawaichi, M., Brownstein, M., Lee, F., Yokota, T. and Arai, K. (1987) Methods Enzymol. 154, 3–28.
[7] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[8] Huynh, T.V., Young, R.A. and Davis, R.W. (1985) in: DNA Cloning, vol.1. A Practical Approach (Glover, D.M. ed.) pp.49–78, IRL Press, Oxford.
[9] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[10] Norrander, J., Kempe, T. and Messing, J. (1983) Gene 26, 101–106.
[11] Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) Gene 28, 351–359.
[12] Laemmli, U.K. (1970) Nature 227, 680–684.
[13] Stubbs, M.E., Carver, J.P. and Dunn, R.J. (1986) J. Biol. Chem. 261, 6141–6144.