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Production and purification of a recombinant human 14 kDa β-galactoside-binding lectin

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The cDNA for a 14 kDa human β-galactoside-binding lectin was inserted into a plasmid carrying a *taq* promoter, and the lectin protein was expressed in *E. coli* cells. The recombinant lectin was extracted from the cells and purified to apparent homogeneity by a single-step chromatography on an asialofetuin-agarose column. Subunit molecular mass (14 kDa), hemagglutinating activity and antigenicity were indistinguishable from those of the human placental lectin. Though the N-terminal of the placental lectin is blocked with an acetyl group, the recombinant lectin was found to have a free amino group. However, the N-terminal amino acid sequences were identical. The recombinant lectin was considered to have the same three-dimensional structure as the placental lectin.

Galactoside binding, β-; Lectin expression; Cell differentiation; (Human lung, *E. coli*)

1. INTRODUCTION

Endogenous β-galactoside-binding proteins (lectins) have been found in various vertebrate tissues and cells [1]. They are extractable with lactose and are specifically inhibited by β-galactoside-containing saccharides. Since they are often found in embryonic tissues and also in tumorigenic cells [2], they are supposed to have important function(s) in some onco-developmental events. However, their physiological functions are not well understood.

Complete primary sequences have been reported for 14K-type lectins of chick skin [3,4], rat lung [5] and human placenta [6], and a nearly complete sequence has also been determined for that of electric eel [7]. The molecules all consist of 134 amino acids and show 50–90% sequence homology. They have free thiol groups instead of disulfide bridges, and as far as examined, their N-terminal amino groups are blocked. We recently isolated a full-length cDNA clone for a human 14K-type lectin from a lung cDNA library [8], and determined its nucleotide sequence; the deduced amino acid sequence was identical with that of the human placental 14K-type lectin [6,9,10]. The result suggested the absence of a signal sequence required for secretion as in the cases of chick [3] and rat [5] lectin cDNAs. Recently, a cDNA for mouse 35K-type lectin was cloned and the deduced amino acid sequence was reported. Its C-terminal half showed a significant homology to the 14K-type lectins, while its N-terminal half has some homology with proteins of the heterogeneous ribonucleoprotein complex [11]. We pointed out that 14K-type lectins have approximately 30% sequence homology to the C-terminal half of one of the IgE-binding proteins isolated from rat basophilic leukemia cells

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Abbreviations: IPTG, isopropyl-β-D-thiogalactopyranoside; EDTA/PMSF/MEPBS, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 4 mM 2-mercaptoethanol, 20 mM sodium phosphate, pH 7.2, 0.15 M sodium chloride; PVDF, polyvinylidenedifluoride; LPS, lipopolysaccharide

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(designated ϵBP in [12]), and also that the mouse 35K-type lectin is a possible counterpart of rat ϵBP in mouse [8]. These results suggest that some β-galactoside-binding lectins are involved in the metabolism of nucleic acids and in the regulation of the immune system. In this context, Levi et al. demonstrated prophylactic and therapeutic effects of an electric eel lectin on experimental autoimmune disease [13]. We also found that human placental lectin could trigger the production by primed macrophages of a cytotoxin capable of destroying malignant cells [14].

These findings emphasize the importance of an extensive study of β-galactoside-binding lectins. Here, we report the expression of the human 14K-type β-galactoside-binding lectin in *E. coli*. We inserted the cDNA cloned from a human lung library into a plasmid carrying a *taq* promoter, and succeeded in producing up to 2 mg of the recombinant protein from 1-l culture by a simple procedure. The recombinant lectin was indistinguishable from the placental lectin except that the N-terminal amino acid is not blocked. The expression system thus established should provide a basis for extended studies on β-galactoside-binding lectins from vertebrates.

2. EXPERIMENTAL

2.1. *The construction of the expression plasmid pH14GAL*

A 0.39-kb DNA fragment encoding most of the amino acid sequence of the human 14K-type lectin was prepared from the *Hin*fI and *Nco*I digest of a full-length cDNA clone LD9-3 (0.9 kb) [8], which contains a unique *Hin*fI site 27 nucleotides downstream of the initiation codon and a unique *Nco*I site 12 nucleotides downstream of the termination codon. Two single-stranded DNA fragments were synthesized with an Applied Biosystems 381A synthesizer; one is a 35-mer of sequence 5′-GATCC ATG GCT TGT GGT CTG GTC GCC AGC AAC CTG-3′ containing the initiation codon ATG at positions 6–8, and the other is a complementary 34-mer, 5′-ATT CAG GTT GCT GGC GAC CAG ACC ACA AGC CAT G-3′. These fragments were annealed and ligated with the 0.39-kb cDNA fragment. Downstream of the *Nco*I site, we inserted a 90-bp fragment obtained from another cDNA clone LA1-2 (0.4 kb) [8] by cutting at the same *Nco*I site as that in clone LD9-3 and at a *Pst*I site that originated from pUC18. This procedure produced a fragment (0.51 kb) which has *Bam*HI and *Pst*I sites at both termini. The fragment was inserted immediately downstream of the *taq* promoter of a *Bam*HI/*Pst*I-cut expression vector pUC540 (Kan^R), which is a derivative of pUC540 with added kanamycin resistance [15]. The size and orientation of the insert were examined by comparing the restriction map produced by *Hin*fI, *Eco*RI and *Bam*HI with that of pUC540 (Kan^R). Nucleotide sequences around the ligation sites were confirmed by the dideoxy chain termination method [16] using the synthetic 35-mer and a commercially available M13 primer.

2.2. *Western-blotting analysis*

E. coli strains of SCS1 and Y1090 were transformed with pH14GAL by the procedure of Hanahan [17]. Cells were grown in an appropriate medium containing ampicillin (50 μg/ml), kanamycin (30 μg/ml) and isopropyl-β-D-thiogalactopyranoside (IPTG, 0.1 mM). To prepare either lactose extract or lactose-free extract, cells were collected by centrifugation, and were suspended in the original volume of EDTA/PMSF/MEPBS (2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 4 mM 2-mercaptoethanol, 20 mM sodium phosphate, pH 7.2, 0.15 M sodium chloride) or 0.1 M lactose in EDTA/PMSF/MEPBS. Cells were disrupted by sonication with a Branson Sonifier. After removal of the cell debris, each extract was analyzed by polyacrylamide gel electrophoresis in the presence of SDS. For Western-blotting experiments, separated proteins were electrophoretically transferred to a polyvinylidenedifluoride (PVDF) membrane, Immobilon (Millipore). After extensive blocking with 1% skim milk (Morinaga Co. Ltd, Tokyo, Japan), the membrane was sequentially treated with 1000-fold-diluted anti-human placental lectin antiserum [10] (16 h, at room temperature), and with 1000-fold-diluted horseradish peroxidase-labeled (goat) anti-rabbit IgG (Miles) (2 h, at room temperature). After extensive washing, the membrane was stained with a Konica Immunostain Kit (Tokyo, Japan) as described previously [8].

2.3. *Purification of the recombinant lectin from* E. coli

Transformed cells were grown in 4–20 l of an appropriate medium containing antibiotics and IPTG (0.1 mM), collected by centrifugation, and suspended in approximately 10 ml/l culture of EDTA/PMSF/MEPBS. Cells were disrupted by sonication (3 min, at 0°C, three times at 1 min intervals) and the resultant suspension was centrifuged to remove debris. Then, clear supernatant solution was applied to an asialofetuin-agarose column (1.8 × 15 cm, 9 mg fetuin/ml gel) prepared as described [18], and after extensive washing of the column with EDTA/MEPBS, an adsorbed material was eluted with EDTA/MEPBS containing 0.1 M lactose.

Hemagglutinating activity was measured as described [19]. Protein concentration was determined by the method of Bradford [20]. Lipopolysaccharide (LPS) content of the purified sample was determined with a colorimetric endotoxin determination reagent, Pyrodick (Seikagaku Kogyo Co. Ltd, Tokyo, Japan). The N-terminal amino acid sequence was determined with an Applied Biosystems 477A pulsed-liquid sequencer.

3. RESULTS AND DISCUSSION

We constructed an expression plasmid pH14GAL from the plasmid pUC540 (Kan^R) [15] and a cDNA for a 14K-type lectin derived from a human lung cDNA library [8] as described in section 2. The initiation codon ATG was linked immediately downstream of the *taq* promoter via a synthetic *Bam*HI linker (see fig.1).

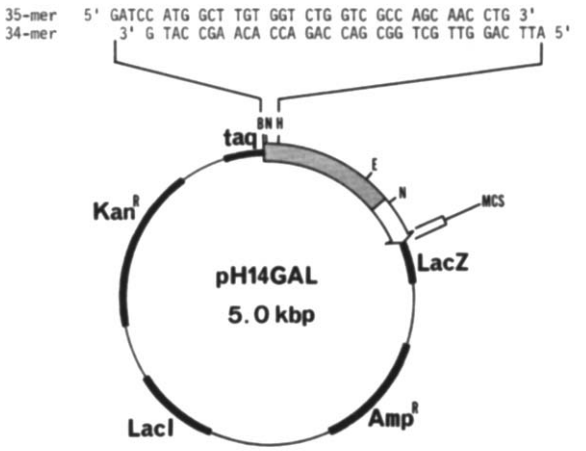

Fig.1. Construction of the expression plasmid pH14GAL. A segment map and the sequence in the ligated region of the synthetic 35/34-mer are shown. The lectin cDNA shown by a thick arrow was inserted immediately downstream of the *taq* promoter/operator region of pUC540 (Kan^R). The lectin-coding region is shaded. B, *Bam*HI; N, *Nco*I; H, *Hin*fI; E, *Eco*RI sites. MCS is a multi-cloning site that originates from pUC8 and pUC18.

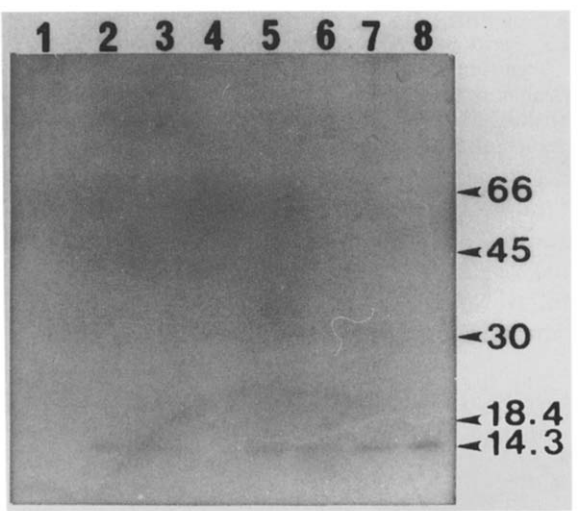

Fig.2. The result of Western-blotting analysis. *E. coli* SCS1 was transformed with pUC540 (Kan^R) (lane 1) and pH14GAL (lanes 2–6). In every case, materials equivalent to 10 μl culture were subjected to the analysis. Lanes: 1, 2, whole culture fractions; 3, pellet (cell) fraction; 4, supernatant (medium) fraction; 5, lactose extract; 6, lactose-free extract; 7, purified recombinant lectin (50 ng); 8, purified placental lectin (50 ng). Arrowheads indicate the eluting positions of bovine serum albumin (66 kDa), chicken ovalbumin (35 kDa), bovine carbonic anhydrase B (30 kDa), bovine β-lactoglobulin (18.4 kDa) and chicken lysozyme (14.3 kDa).

Both transformed *E. coli* strains, SCS1 and Y1090, produced the recombinant lectin in the presence of 0.1 mM IPTG. The result of Western-blotting analysis of proteins produced by the pH14GAL-transformed SCS1 is presented in fig.2. Lane 2 shows that the whole culture of pH14GAL-transformed cells gave a specific 14 kDa band stained with anti-human placental lectin antiserum. However, no corresponding band was detected in lane 1 (transformed with the vector only). Lanes 3 and 4 show the results of analysis of both cells and medium. Only the cell fraction gave the lectin band. This is consistent with the fact that

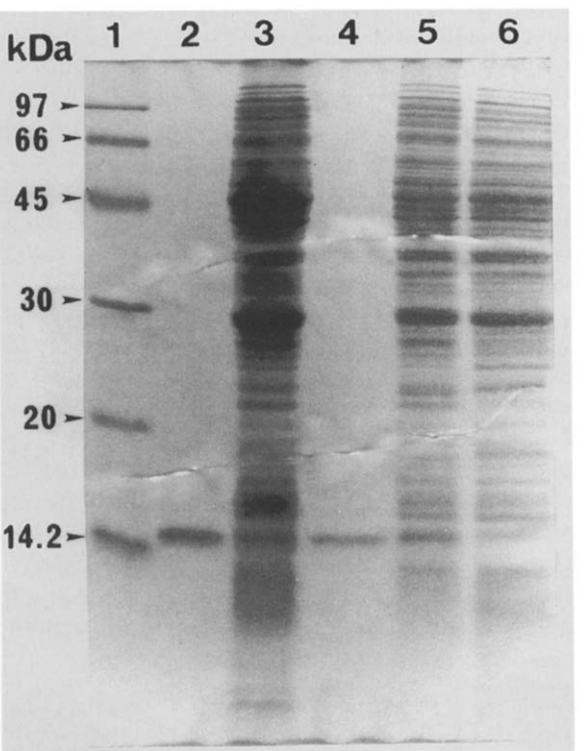

Fig.3. The result of 15% polyacrylamide gel electrophoresis in the presence of SDS. Proteins were stained with Coomassie blue. Lanes: 1, molecular mass markers (1 μg each); 2, purified placental lectin (1 μg); 3, lactose-free extract of SCS1 cells transformed with pH14GAL (100-μl culture equivalent); 4, purified recombinant lectin (0.5 μg); 5 and 6, whole culture fractions of SCS1 transformed with pH14GAL and pUC540 (Kan^R), respectively (both 10-μl culture equivalents). Arrowheads indicate the eluting positions of marker proteins of rabbit phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), chicken ovalbumin (45 kDa), bovine carbonic anhydrase B (30 kDa), soybean trypsin inhibitor (20 kDa) and bovine α-lactalbumin (14.2 kDa).

the hemagglutinating activity was not detected in the culture medium.

The effect of a hapten sugar on the efficiency of extraction of the recombinant lectin was also examined. The lectin was extracted from the cells either in the presence or absence of lactose, which usually facilitates the extraction of the 14K-type lectins from vertebrate tissues [9]. However, the lactose-free extract showed a hemagglutinating activity as high as that of the lactose extract (the activity was measured after the removal of lactose by dialysis). In both fractions, hemagglutinating activity was inhibited almost completely (more than 90%) by 7.5 mM lactose. Western-blotting patterns of both fractions were essentially identical (lanes 5 and 6 in fig.2). Moreover, almost equal amounts of the lectin were purified from both extracts (data not shown). This suggests that the recombinant lectin did not bind to insoluble materials of *E. coli* via the sugar-binding site. *E. coli* does not seem to have an endogenous receptor for the lectin. No evidence has appeared of the existence in *E. coli* of β-galactoside-binding lectins corresponding to those of vertebrates [21,22].

Purification of the recombinant lectin was greatly facilitated, because lactose was unnecessary for extraction of the lectin, and so no step for the subsequent removal of lactose was required. The *E. coli* extract containing the lectin was directly applied to an asialofetuin-agarose column, and the recombinant lectin was eluted with lactose after extensive washing of the column. No hemagglutinating activity was detected in a flow-through fraction. The eluted protein gave a single band in polyacrylamide gel electrophoresis, and had the same mobility as the placental lectin, corresponding to a monomeric molecular mass of 14 kDa (lane 4 in fig.3). It was also reactive with antiserum raised against the placental lectin (lane 7 in fig.2). Specific hemagglutinating activity of the recombinant lectin was almost the same as that of the placental lectin; the minimum concentration required for the hemagglutination was approximately 1 μg/ml. The yields were 0.9 and 2.0 mg/l culture for SCS1 and Y1090, respectively. Thus, it might be possible to prepare more than 100 mg of the lectin in a week. Asialofetuin-agarose chromatography was also effective for the removal of endotoxin lipopolysaccharide (LPS) that originated from *E. coli*; the LPS content in the purified lectin was as low as 60 ng/mg protein. Although the N-terminus of the placental lectin is acetylated [6], N-terminal sequence analysis showed that the recombinant lectin was not blocked at its N-terminus. This result is consistent with the general observation that N-terminal acetylation is rare in bacteria and fungi [23]. However, the sequence was identical with that of the placental lectin [6] up to 39 residues (fig.4). Since the hemagglutinating activity of the recombinant lectin was almost the same as that of the placental lectin, the acetyl group is not essential for the correct folding of the protein or the formation of the sugar-binding site.

This system for production of the recombinant lectin should not only provide a basis for extended studies, e.g. site-directed mutagenesis, but also make available large quantities of the human lectin, which might be interesting from the medical viewpoint.

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Placenta                    5                   10                  15                  20
lectin      Ac-Ala-Cys-Gly-Leu-Val-Ala-Ser-Asn-Leu-Asn-Leu-Lys-Pro-Gly-Glu-Cys-Leu-Arg-Val-Arg-

Recombinant
lectin      H-Ala-( )-Gly-Leu-Val-Ala-Ser-Asn-Leu-Asn-Leu-Lys-Pro-Gly-Glu-( )-Leu-Arg-Val-Arg-

Placenta                   25                  30                  35                  40
lectin        Gly-Glu-Val-Ala-Pro-Asp-Ala-Lys-Ser-Phe-Val-Leu-Asn-Leu-Gly-Lys-Asp-Ser-Asn-Asn-

Recombinant
lectin        Gly-Glu-Val-Ala-Pro-Asp-Ala-Lys-Ser-Phe-Val-Leu-Asn-Leu-Gly-Lys-Asp-Ser-Asn-( )-
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Fig.4. The result of N-terminal sequence analysis of the recombinant lectin and comparison with the sequence of a human placental lectin. No cysteine residue was detected (ND), as the intact recombinant protein was directly applied to a sequencer. The sequence of the placental lectin was taken from [6].

During preparation of this manuscript, Couraud et al. presented results on the expression of a 14 kDa β-galactoside-binding lectin isolated from human promyelocytic leukemia cells HL60 [24]. Their results were essentially similar to those of ours. Since no 14 kDa lectin could be detected in macrophages, cells of a more advanced differential stage ([25] Oda and Kasai, unpublished observation), the presence of the 14 kDa lectin in HL60 cells may imply that the lectin plays a significant role in cell differentiation.

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