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Availability of L-fucose-like sites on the surface membrane of normal and transformed mammalian cells

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SUMMARY

A L-fucose-binding glycoprotein isolated by affinity chromatography from *Lotus tetragonolobus* seeds contains two types of L-fucose-binding molecules. Binding experiments with labeled molecules have shown that this glycoprotein did not bind specifically to the surface membrane of either normal or malignant transformed cells even after the cells had been treated with trypsin. These cells were also not agglutinated by this protein. Thus, although normal and transformed mammalian cells contain glucose or mannose, *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine-like binding sites, as measured by the binding of lectins with these specificities, L-fucose-like binding sites for these two types of molecules are not available on the surface membrane of these cells.

Studies on the binding of molecules with different carbohydrate-binding sites can be of value in elucidating the changes in the surface membrane associated with the malignant transformation of normal cells. Previous results have shown changes in the surface membrane in malignant cell transformation including differences in the locating of binding sites for Concanavalin A[1-9]. These studies, and those with wheatgerm[10,11] and soybean[12,13] agglutinins, have shown that carbohydrates bound by these three lectins are present as binding sites on the surface membrane of normal and transformed mammalian cells. A glycoprotein which binds L-fucose has been purified from *Lotus tetragonolobus* seeds[14], and L-fucose is present on the cell surface membrane[15]. The present experiments were undertaken to determine, with the L-fucose-binding glycoprotein (which will be referred to as L-fucose-binding protein), whether L-fucose-like binding sites are available on the surface membrane and whether this protein can agglutinate normal or malignant transformed cells.

The normal cells used were from secondary cultures of golden hamster and rat embryo cells, and the untransformed mouse cell line 3T3. The transformed cells were cultured cell lines transformed by polyoma virus, simian virus 40, Rous sarcoma virus, or after treatment with the chemical carcinogen dimethylnitrosamine, and the ascites form of a Moloney virus-induced lymphoma grown *in vivo* in A strain mice[7]. Cells were grown *in vitro* in Eagle's medium with a 4-fold concentration of amino acids and vitamins with 10% fetal calf serum, as described[7]. The fucose-binding protein was prepared by affinity chromatography by Miles-Yeda. It was stored as a solution in phosphate-buffered saline, pH 7.2, at −20°C. Agarose ϵ-aminocaproylfucosamine (Miles-Yeda) stored at +4°C was used as affinity column for the fucose-binding protein. Concanavalin A (Miles-Yeda) was stored as a solution in saturated NaCl at room temperature. Agglutination and binding experiments were performed as previously described[1,2]. The fucose-binding protein was labeled with [^{3}H]acetic anhydride by the method of Dr. I.R. Miller, Weizmann Institute (personal communication). Cell protein was determined by the method of Lowry *et al.*[16].

Specificity of the fucose-binding protein for L-fucose. Different carbohydrates, including those found on cell surfaces, were tested for their ability to inhibit the binding of the fucose-binding protein to agarose amino caproyl fucosamine. The results show (Fig. 1) that the fucose-binding protein binds specifically to L-fucose. 50% inhibition was obtain with 8 μg L-fucose and only 5–20% inhibition was obtained with 100 μg of other carbohydrates tested.

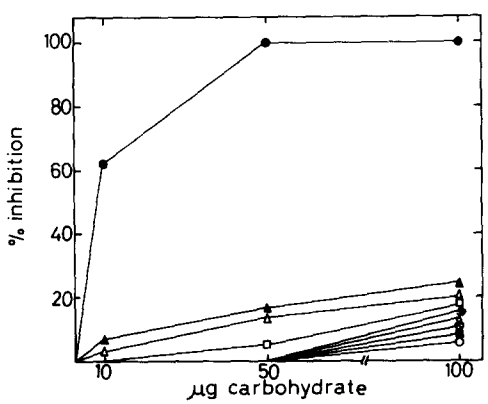

Fig. 1. Specificity of the fucose-binding

Fig. 1. Specificity of the fucose-binding protein for L-fucose. ●, L-fucose; O, D-fucose; ■, D-glucose; ◆, D-galactose; x, α-methyl-D-glucopyranoside; ⊛, α-methyl-D-mannopyranoside; △, *N*-acetyl-D-glucosamine; □, D-ribose; ▲, D-arabinose. For the experiments, 1 ml phosphate-buffered saline containing varying amounts of carbohydrate was incubated with 1 mg protein for 30 min, the mixture was then added to 0.5 ml resin with a binding capacity of 3.2 mg/ml. After 30 min incubation at 24°C the supernatant was measured at $A_{280\,nm}$, and the inhibition was calculated.

Tests for L-fucose-binding sites on the cell surface membrane. Normal and transformed cells (Table I) were tested for agglutination by the L-fucose-binding protein and by Concanavalin A at 24°C. The results show (Table I) that although all the transformed cells were agglutinated by Concanavalin A, these cells were not agglutinated by the L-fucose-binding protein even after the cells had been treated with trypsin. Normal cells that were agglutinated by Concanavalin A after treatment with trypsin were also not agglutinated by the L-fucose-binding protein.

TABLE I

AGGLUTINABILITY OF NORMAL AND TRANSFORMED CELLS BY THE L-FUCOSE-BINDING PROTEIN AND BY CONCANAVALIN A

The cells grown *in vitro* were dissociated with 0.02% disodium versenate or 0.25% trypsin solution[2] (15–30 min at 37°C) 4 days after subculture, and tested for agglutination after 30 min incubation at 24°C with L-fucose-binding protein and Concanavalin A. Similar results were obtained with hamster and 3T3 cells transformed by polyoma virus, hamster cells transformed by simian virus 40, Rous sarcoma virus, or after treatment with the chemical carcinogen dimethylnitrosamine[7]. The ascites form of a Moloney virus-induced lymphoma grown *in vivo*[7] was also agglutinated by Concanavalin A, but not by the L-fucose-binding protein. The agglutination assay was performed as described[2].

Cells	*Agglutination with 500 μg/ml at 24° C*			
	Treatment with disodium versenate		*Treatment with trypsin*	
	L-Fucose-binding protein	*Concanavalin A*	*L-Fucose-binding protein*	*Concanavalin A*
Hamster normal	–	–	–	++++
Hamster dimethylnitrosamine transformed	–	++++	–	++++
Rat normal	–	–	–	++++
Rat polyoma transformed	–	++++	–	++++
3T3 untransformed	–	–	–	++++
3T3 simian virus 40 transformed	–	+++	–	+++

The ability of the L-fucose-binding protein to bind to the cell surface membrane was tested by labeling the protein with [^{3}H]acetic anhydride. The labeled protein, with a specific activity of 32 5000 cpm per mg, had the same activity as the native protein on the affinity column (Fig. 2). [^{3}H]Concanavalin A labeled by the same method, was identical to ^{63}Ni-labeled Concanavalin A[1] and native Concanavalin A in chromatography on Sephadex G-50 and the ability to agglutinate cells. Cells were incubated with 250 μg ^{3}H-labeled L-fucose-binding protein at 24°C for 30 min[1], and the results (Table II) show no significant L-fucose specific binding either in normal or transformed cells even after the cells had been treated with trypsin. Untrypsinized group O human red blood cells were also not agglutinated by the L-fucose-binding protein and also showed no specific binding.

Chromatography and electrophoresis of the L-fucose-binding protein. The protein we used contained two types of molecules, A and B[14], as measured by chromatography

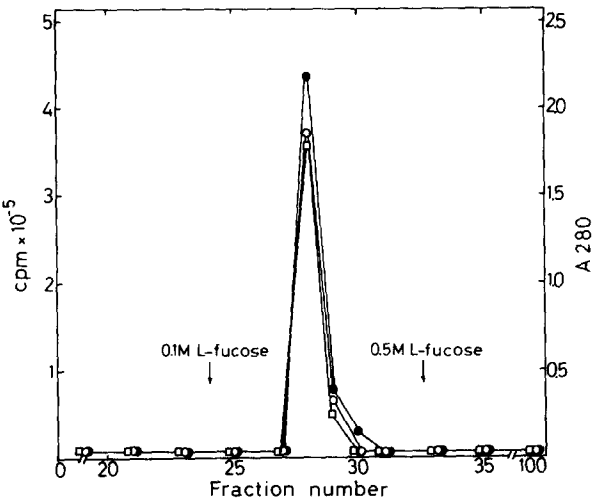

Fig. 2. Affinity chromatography of native and ^{3}H-labeled L-fucose-binding protein. The column (agarose ϵ-aminocaproylfucosamine from Miles-Yeda) was equilibrated with phosphate-buffered saline, pH 7.2, and developed with the same buffer containing L-fucose at 24°C. Arrows show the addition of 0.1 M and 0.5 M L-fucose. □, $A_{280\ nm}$ labeled protein; O, A_{280nm} labeled protein; ●, radioactivity.

TABLE II

TEST FOR BINDING OF ^{3}H-LABELED L-FUCOSE-BINDING PROTEIN TO NORMAL AND TRANSFORMED CELLS

The cells grown *in vitro* were dissociated with 0.02% disodium versenate or 0.25% trypsin solution[2] and tested for binding with 250 μg ^{3}H-labeled L-fucose-binding protein per $2 \cdot 10^6$ cells after 30 min incubation at 24°C. Cells were incubated with labeled protein in the absence or presence of 0.1 M L-fucose. To calculate the amount of protein bound specifically (L-fucose specific), the amount bound in the presence of L-fucose was subtracted from the amount bound in the absence of L-fucose (Total). Similar results were obtained with hamster and 3T3 cells transformed by polyoma virus, hamster cells transformed by simian virus 40, Rous sarcoma virus, or after treatment with dimethylnitrosamine, and with the ascites form of a Moloney virus-induced lymphoma[7]. The specific activity of the ^{3}H-labeled L-fucose-binding protein was 325 000 cpm per mg. Binding experiments were performed as described[1].

Cells	*Total and L-fucose specific binding with 250 μg/ml at 24°C (cpm/1000 μg cell protein)*			
	Treatment with disodium versenate		*Treatment with trypsin*	
	Total	*L-Fucose-specific*	*Total*	*L-Fucose specific*
Hamster normal	1212	101	1779	94
Hamster dimethylnitrosamine transformed	1634	41	1884	48
Rat normal	963	30	908	80
Rat polyoma transformed	668	0	468	29
3T3 untransformed	1501	48	1483	91
3T3 simian virus 40 transformed	802	22	1044	0

on a DEAE-cellulose column and by electrophoresis on polyacrylamide gel or cellulose acetate (Fig. 3). Molecules of Type A have four binding sites and molecules of Type B have two binding sites[14]. Since three types of L-fucose-binding molecules, A, B and C, have been reported from *Lotus tetragonolobus*[14] it will be of interest to compare the activity of the three types of molecules.

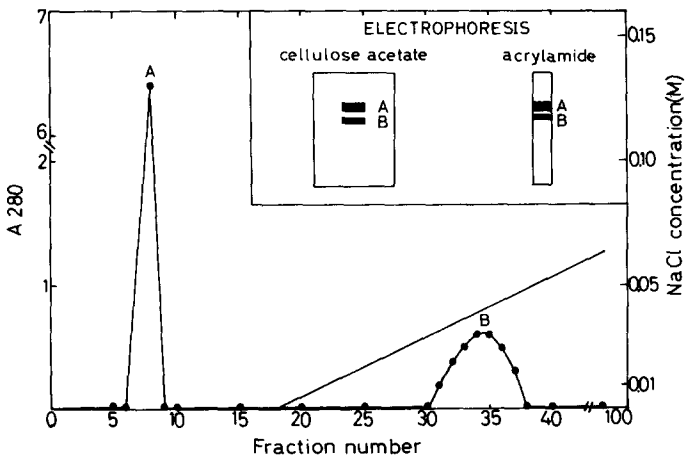

Fig. 3. Chromatographic separation of L-fucose-binding protein on a DEAE-cellulose column. Starting buffer, 0.01 M sodium phosphate, pH 7.6. Linear NaCl gradient was applied after elution of Protein A. Temperature, 4°C. Inset shows electrophoresis on polyacrylamide gel with β-alanine acetic acid, pH 4.3, 60 min at 4°C, and on cellulose acetate with 0.02 M sodium phosphate, pH 6.8, 20 min at 24°C [14].

Our results show that a L-fucose-binding protein isolated from *Lotus tetragonolobus* that contained two types of molecules with two and four binding sites, did not agglutinate normal or transformed cells even after the cells had been treated with trypsin. Binding experiments with ^{3}H-labeled L-fucose-binding protein also show no significant L-fucose specific binding in any of the cell types tested. The results indicate that although normal and transformed mammalian cells contain glucose or mannose, *N*-acetyl-D-glucosamine and *N*-acetyl-D-galactosamine-like binding sites on the surface membrane as measured by Concanavalin A[1,2], wheatgerm[10,11], and soybean[12,13] agglutinins, respectively, L-fucose-like binding sites for these two types of L-fucose-binding molecules are not available.

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