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Evidence for an increase in positive surface charge and an increase in susceptibility to trypsin of *Sarcophaga* lectin (from the flesh fly, *Sarcophaga peregrina*) on its interaction with galactose, a hapten sugar of the lectin

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When *Sarcophaga* lectin (from the flesh fly, *Sarcophaga peregrina*), an insect humoral lectin, was eluted from a column of DEAE-cellulose in the presence of galactose (a hapten sugar of this lectin), it emerged at a lower salt concentration than when galactose was absent. In the presence of galactose the lectin was, in addition, more susceptible to trypsin digestion. The lectin was found to have an affinity for basic proteins such as histone H3 and sarcotoxin IA, but this property was lost in the presence of galactose. These results suggested that the lectin changes its conformation on interaction with galactose. This change is suggested to result in the exposure of some hidden lysine and/or arginine residues.

INTRODUCTION

Sarcophaga lectin is a galactose-binding lectin induced in the haemolymph of third-instar larvae in response to body injury [1]. The same lectin was purified from the culture medium of an embryonic-cell line of *Sarcophaga* [2]. This lectin is a C-type lectin [3] with molecular mass of 190 kDa, consisting of 32 kDa and 30 kDa subunits in a molecular ratio of 2:1 [1]. These two subunits are essentially the same protein derived from a single gene [4,5]. This lectin is a defence protein of *Sarcophaga* and has been shown to participate in the elimination of foreign substances introduced into the abdominal cavity of this insect [6]. Recently it was also found to be essential for the morphogenesis of adult structures during development of the imaginal discs. Moreover, it was shown to participate in imaginal-disc development in an autocrine manner, namely, it was secreted by the imaginal discs themselves in the presence of 20-hydroxyecdysone and was indispensable for their further development [7]. Therefore this lectin seems to play two independent roles: in the defence system and in imaginal-disc development [8].

As galactose significantly inhibited the hemagglutinating activity of *Sarcophaga* lectin, the receptor for this lectin was suggested to contain a galactose moiety [1]. In fact, galactose inhibited both the elimination of sheep red cells introduced into the abdominal cavity of *Sarcophaga* larvae [6] and the terminal differentiation of imaginal discs cultured in the presence of 20-hydroxyecdysone *in vitro* [7].

The present paper describes increases in positive surface charge and susceptibility to trypsin of *Sarcophaga* lectin on its interaction with galactose. On binding to receptors via galactose moieties the confirmation of the lectin seems to change in such a way that positively charged amino acid residues become exposed. This conformational change may be related to the biological activity of this lectin.

MATERIALS AND METHODS

Sarcophaga lectin and its radioiodination

Sarcophaga lectin was purified to homogeneity from the culture medium of NIH-Sape-4 cells, an embryonic cell line of *Sarcophaga* [9], as described in [2]. Radioiodination of *Sarcophaga* lectin was performed essentially by the method of Hunter & Greenwood [10] as described previously [11]. The specific radioactivity was usually about 10^6 c.p.m./μg of protein.

Binding of *Sarcophaga* lectin to basic proteins

The binding of the lectin to basic proteins was examined by dot-blot analysis. For this, 5 μg of a basic protein was blotted on to a nitrocellulose filter (Schleicher and Schuell), and the filter was treated in skim-milk solution [20 mM-Tris/HCl buffer, pH 7.9, containing 5% (w/v) skim milk (Difco)] for 1 h at room temperature. Then the filter was soaked in 5 ml of rinse solution [10 mM-Tris/HCl buffer, pH 7.9, containing 0.25% skim milk, 150 mM-NaCl, 1 mM-EDTA, 0.1% (w/v) Triton X-100 and 0.01% (w/v) NaN_3] and gently shaken for 4 h at 4 °C in the presence of 1 μCi of radioiodinated *Sarcophaga* lectin. Galactose or glucose was added to this reaction mixture, when necessary, at a final concentration of 25 mM. Then the filter was washed well with the rinse solution and autoradiographed.

Analysis of *Sarcophaga* lectin by anion-exchange h.p.l.c.

Radioiodinated *Sarcophaga* lectin (1×10^5 c.p.m.) was dissolved in 100 μl of 50 mM-phosphate buffer, pH 7.5. Galactose or glucose was added to this solution at a final concentration of 100 mM when necessary. Each sample was applied to an h.p.l.c. column of DEAE-3SW (7.5 mm × 75 mm; Tosoh, Tokyo, Japan) connected to a Gilson h.p.l.c. system and eluted with a linear gradient of 0–0.26 M-NaCl prepared in 50 mM-phosphate buffer, pH 7.5. When indicated, galactose or glucose was added to this elution buffer at a final concentration of 100 mM. Fractions (1 ml) were collected, and the radioactivity of each fraction was measured.

Tryptic digestion of *Sarcophaga* lectin

The susceptibility of *Sarcophaga* lectin to tryptic digestion was examined in 20 μl of 10 mM-Tris/HCl buffer, pH 7.9, containing 680 ng of *Sarcophaga* lectin and various amounts of trypsin (Worthington). Galactose or glucose was added to this mixture at a final concentration of 100 mM, as indicated. The reaction mixture was incubated for 17 h at 37 °C. Then 10 μl of 50 mM-Tris/HCl buffer, pH 7.9, containing 3% (w/v) SDS, 6% (v/v) 2-

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mercaptoethanol, 30% (v/v) glycerol and 0.015% (w/v) Bromophenol Blue was added, and the mixture was heated at 75 °C for 20 min. Samples were then subjected to SDS/PAGE.

Other procedures

Sarcotoxin IA, an antibacterial protein of *Sarcophaga*, was synthesized chemically as reported in [12]. *Sarcophaga* histone H3 was extracted from NIH-Sape-4 cells with high salt and purified to homogeneity by h.p.l.c. Protein was determined by the method of Lowry *et al.* [13]. The molecular mass of *Sarcophaga* lectin was determined by gel filtration on an h.p.l.c. column of G3000SWXL (7.8 mm × 300 mm; Tosoh). The elution buffer used was 100 mM-phosphate buffer, pH 7.0, containing 100 mM-Na_2SO_4.

RESULTS

Northern-blotting analysis showed that the *Sarcophaga* lectin gene was expressed transiently in the embryonic and pupal stages of *Sarcophaga* [14]. In fact, a significant amount of *Sarcophaga* lectin was detected in *Sarcophaga* embryos [14]. Assuming that specific binding sites for this lectin are present on the surface of embryonic cells, we tried to identify and isolate a *Sarcophaga*-lectin-binding protein from NIH-Sape-4 cells, which are a cell line from a *Sarcophaga* embryo. During the present study we detected two proteins with affinity to *Sarcophaga* lectin and purified them to homogeneity. The binding of *Sarcophaga* lectin to both of these proteins was completely inhibited in the presence of 25 mM-galactose, suggesting that the binding was specific.

Therefore we assumed that these proteins are specific binding proteins for this lectin and we characterized them. As the amino acid sequences of the two tryptic fragments of one of these proteins were very similar to those of *Drosophila* histone H1, and a tryptic fragment of the other protein was very similar to that of calf thymus histone H3, we concluded that these proteins are probably histone H1 and H3 respectively. These results suggested that the negatively charged region of *Sarcophaga* lectin has non-specific affinity to the basic regions of histone H1 and H3, but that the conformation of *Sarcophaga* lectin changes in the presence of galactose in such a way that the surface charge becomes more electropositive, resulting in loss of affinity to these proteins.

To examine this possibility we purified *Sarcophaga* histone H3 to homogeneity and examined whether *Sarcophaga* lectin bound to it. The results of dot-blotting (Fig. 1) show that *Sarcophaga* lectin bound to histone H3 and sarcotoxin IA and that binding was completely inhibited in the presence of 25 mM-galactose but not in the presence of the same concentration of glucose. Sarcotoxin IA is another basic protein of *Sarcophaga*; it has a molecular mass of 4 kDa and potent antibacterial activity against Gram-negative bacteria [15]. These results indicated that the binding of *Sarcophaga* lectin to various basic proteins is due to non-specific ionic interaction, although it is inhibited specifically by galactose.

As the *Sarcophaga* lectin lost its affinity to basic proteins in the presence of galactose, its surface charge may be greatly affected by the presence or absence of this sugar. To test this possibility we compared the elution profiles of radioiodinated *Sarcophaga* lectin on h.p.l.c. on an anion-exchange column in the presence or absence of galactose or glucose respectively. The superimposed elution profiles are shown in Fig. 2. The elution profile obtained in the presence of galactose was clearly different from the other two profiles. *Sarcophaga* lectin was eluted from the column as a sharp single peak at about 0.15 M-NaCl in the absence of sugar or in the presence of glucose. The peaks obtained under these two conditions did not coincide exactly, but were essentially the same. However, in the presence of galactose, the lectin was eluted in three broad peaks and was mostly eluted at much lower NaCl concentrations than under the other two conditions. These results indicate that the surface charge of *Sarcophaga* lectin becomes more electropositive in the presence of galactose, a finding consistent with the conclusion drawn from Fig. 1.

Sarcophaga lectin is rich in lysine residues [5]. If hidden lysine and/or arginine residues of *Sarcophaga* lectin become exposed as a result of a conformational change on its interaction with galactose, it may become more sensitive to tryptic digestion. To examine this possibility, we digested *Sarcophaga* lectin with increasing amounts of trypsin in the presence or absence of galactose. As Fig. 3 shows, *Sarcophaga* lectin was not digested by trypsin at 0.75 μg/ml under any of the conditions tested. However, it was digested by trypsin at 7.5 μg/ml and the amount of lectin digested was greater in the presence of galactose than under other conditions. Moreover, a unique degradation fragment with molecular mass of 15 kDa was detected only under this condition (Fig. 3*b*, lane 2). These results again support the conclusion that the conformation of *Sarcophaga* lectin changes when it interacts with galactose. We isolated the 15 kDa tryptic fragment obtained by digestion with 7.5 μg of trypsin/ml from

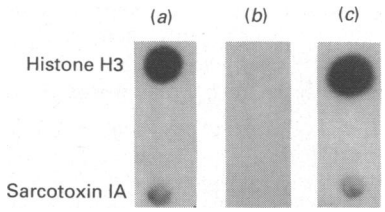

Fig. 1. Effect of galactose and glucose on the binding of *Sarcophaga* lectin to basic proteins

Histone H3 isolated from NIH-Sape-4 cells and sarcotoxin IA (each 5 μg) were blotted on to a nitrocellulose membrane filter, and binding of radioiodinated *Sarcophaga* lectin was examined in the absence or presence of 25 mM-galactose or -glucose. (*a*) Without sugar; (*b*) with galactose; (*c*) with glucose.

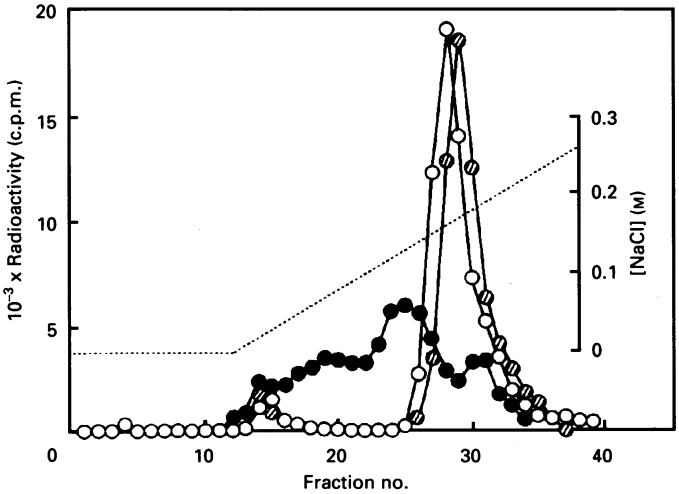

Fig. 2. Chromatographic elution profiles of *Sarcophaga* lectin

Radioiodinated *Sarcophaga* lectin (10^5 c.p.m.) was subjected to h.p.l.c. on an anion-exchange column of DEAE-3SW in the presence or absence of 100 mM-galactose or -glucose. Fractions were collected and their radioactivities were measured. The three elution profiles obtained in independent experiments are superimposed. ○, Without sugar; ●, with galactose; ⊘, with glucose; ------, NaCl concentration.

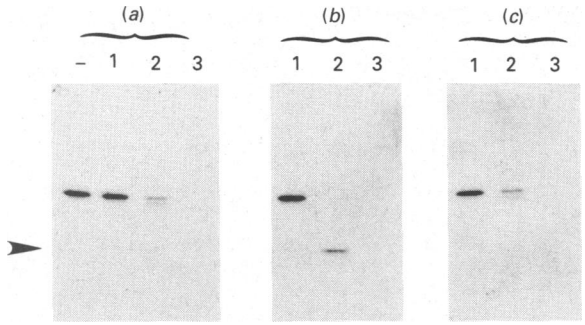

Fig. 3. Effect of galactose on tryptic digestion of *Sarcophaga* lectin

Sarcophaga lectin (680 ng) was digested with increasing amounts of trypsin in the presence or absence of 100 mM-galactose or -glucose. Then the digestion products were subjected to SDS/PAGE and stained with Coomassie Brilliant Blue. (*a*) Without sugar; (*b*) with galactose; (*c*) with glucose. Amounts of trypsin used: lane 1, 0.75 μg/ml; lane 2, 7.5 μg/ml; lane 3, 75 μg/ml. Lane '-' contained intact *Sarcophaga* lectin. The arrow indicates a 15 kDa fragment.

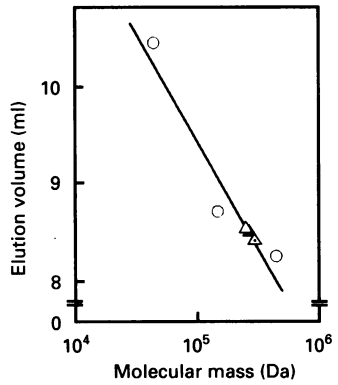

Fig. 4. Estimation of molecular mass of *Sarcophaga* lectin in the presence or absence of sugars

Sarcophaga lectin (8.5 μg) was subjected to h.p.l.c. on a molecular-sieve column in the presence or absence of 100 mM-galactose or -glucose. The column was calibrated with ovalbumin (45 kDa), γ-globulin (150 kDa) and ferritin (450 kDa), and molecular masses are plotted against elution volumes (○). The molecular masses of *Sarcophaga* lectin under these conditions are plotted as triangles: △, without sugar; ▲, with galactose; △, with glucose.

the gel and partially determined its amino acid sequence. The sequence of 11 amino acid residues from its *N*-terminus was VPQLQKALDGR, and this sequence coincided with that of 11 amino acid residues of the *N*-terminus of the intact *Sarcophaga* lectin [5], indicating that this fragment is the *N*-terminal half of this lectin.

As reported previously, *Sarcophaga* lectin has a quaternary structure comprising two subunits [1]. If these subunits dissociate on interaction with galactose, the molecular mass of *Sarcophaga* lectin should decrease. We tested this possibility by measuring the molecular mass of this lectin in the presence or absence of galactose by molecular-sieve h.p.l.c. As Fig. 4 shows, the molecular mass of *Sarcophaga* lectin did not change appreciably in the presence of galactose, indicating that its quaternary structure is maintained, although its conformation is changed.

DISCUSSION

In the present paper we suggest that a conformation change of *Sarcophaga* lectin takes place on its interaction with galactose. On addition of galactose, *Sarcophaga* lectin becomes more basic and tends to be eluted from a column of DEAE-cellulose at a lower salt concentration. This lectin was eluted from the column as a sharp, single peak in the absence of galactose, but as three broad peaks in the presence of galactose. The elution profile in the presence of galactose suggests the presence of multiple states of *Sarcophaga* lectin with slightly different surface charges. An increase in the positive surface charge of *Sarcophaga* lectin in the presence of galactose is likely to be due to exposure of lysine and/or arginine residues rather than to a decrease in negative surface charges, because *Sarcophaga* lectin becomes more sensitive to tryptic digestion in the presence of galactose. This result contrasts with that of the dimeric 14 kDa bovine heart lectin [16]. In that case the carbohydrate ligand actually causes a marked resistance to digestion by the proteinase.

When the lectin was subjected to tryptic digestion we found that a 15 kDa fragment was obtained only in the presence of galactose, and we identified this fragment as the *N*-terminal half of the lectin molecule. *Sarcophaga* lectin is a C-type lectin and its consensus carbohydrate-recognition domain is located in this region [3]. Probably this fragment becomes resistant to tryptic digestion on interaction with galactose and becomes detectable on SDS/PAGE.

Judging from the present results, it is likely that C-type lectins with this consensus carbohydrate-recognition domain change in conformation on interaction with their hapten sugars. The conformations of some C-type lectins are known to change with the Ca^{2+} concentration or pH of the medium [17–19]. However, this is the first demonstration of a change in surface charge of a C-type lectin on its interaction with a hapten sugar.

This finding is important, because the same conformational change probably occurs when *Sarcophaga* lectin binds to its receptor on the surface of imaginal discs via their galactose moieties. *Sarcophaga* lectin is essential for the morphogenesis of adult structures during development of imaginal discs [7]. Possibly only the more electropositive form of *Sarcophaga* lectin can transmit a signal to imaginal-disc cells that is able to induce their further differentiation.

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