Binding of *Lens culinaris* lectin to sea urchin embryo chromatin

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Summary. Lentin binds specifically to sea urchin embryo chromatin. This binding is saturable and inhibited by α-methyl-mannose. Scatchard plot analysis of the binding reaction suggests a single binding site.

The binding of a number of plant lectins to specific sugar residues of membrane glycoproteins was shown to be highly specific[1]. The lectin-binding property was also recently demonstrated in the chromatin from rat liver, where it was ascribed to nonhistone glycoproteins[2]. This corroborates the evidence of the similarity between the nuclear membrane and the nonhistone proteins presented by Jackson[3] and Comings and Harris[4]. In a previous work, we also pointed out the similarity between glycoproteins in membrane and chromatin, and the possibility that the differences between them are rather topographical than chemical (unpublished work). In view of the high specificity of the lectin-binding reaction, and the need of specific proof for the stated similarity between chromatin and nuclear membrane proteins, we tested the ability of sea urchin embryo chromatin to bind specifically the *Lens culinaris* lectin, here termed lentin.

Material and methods. Embryos of sea urchin *P. lividus* at the hatching blastula stage were collected by low speed centrifugation, washed in 1 M glucose and suspended in a buffer containing 0.075 M NaCl, 0.024 M EDTA, 8 mM diisopropyl fluorophosphate (DFP), pH 7.6. Homogenization was done with a Dounce homogenizer on an ice-bath. Crude nuclei were sedimented at 800×g and washed 3 times with the same solution. Nuclear envelopes were removed by washing nuclei with 1 M glucose containing 0.43% (w/v) Na-deoxycholate and 0.86% (w/v) Tween 40. This treatment was shown to remove efficiently the nuclear envelope with granules attached to it[5,6] and to enable separation of nuclei into chromatin and nuclear membrane fraction[7,8]. The suspension was centrifuged at 16,000×g and the sedimented chromatin washed 3 times with 10 mM Tris buffer, pH 8.0. The crude chromatin was layered on 25 ml of 1.7 M sucrose in 10 mM Tris buffer and centrifuged in an SW 25.1 rotor for 3–4 h at 23,000 rev/min at 4 °C. Purified chromatin was dialyzed overnight against 10 mM Tris buffer at 4 °C and sedimented at 12,000×g.

^{131}I-lentin was dissolved in Hanks solution and stored at 4 °C. Chromatin was adjusted to about 2 A_{260} units with Hanks solution, and 1 A_{260} unit aliquots were incubated with ^{131}I-lentin in centrifuge tubes. The volume was made up to 1 ml with Hanks solution containing 50 mg% bovine serum albumin. The mixture was incubated for 60 min at room temperature and then centrifuged for 20 min at 12,000×g at 4 °C. After decantation of the supernate into counting vials, the pellet was dissolved in 1 ml of 0.1% sodium dodecyl sulfate in 10 mM Tris, 0.1 M NaCl, 1 mM EDTA, pH 7.5, and transferred quantitatively into counting vials.

Results and discussion. Chromatin prepared by the procedure described satisfied most criteria for a purified preparation. The ratio of maximum (260 nm) to minimum (240 nm) absorbance was 1.4–1.5, whereas protein:DNA and RNA:DNA weight ratios were approx. 1.7 and 0.12, respectively. The treatment of nuclei with a nonionic detergent (Tween 40) in combination with an ionic surfactant (Na-deoxycholate) was shown to provide an optimal condition for removal of the outer and the inner membranes without grossly distorting the nuclei[5–8].

Figure 1 shows that lentin binds sea urchin embryo chromatin preparations and that the binding reaction proceeds in a time-dependent manner. The specificity of the reaction

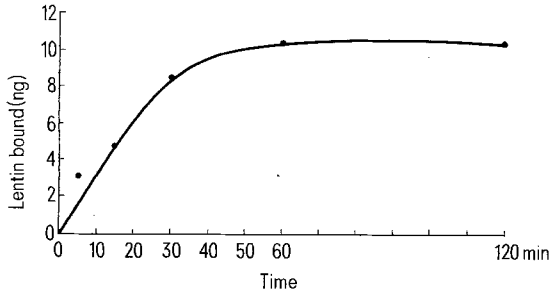

Fig. 1. Time course of lentin binding to chromatin. ^{131}I-lentin (50 ng) was incubated with 1 A_{260} unit of chromatin for different periods of time at room temperature and the amount bound to chromatin was calculated as described under 'material and methods'.

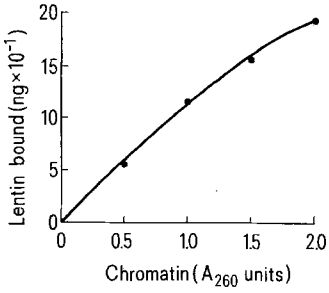

Fig. 2. Relationships of bound lentin amounts and chromatin concentrations. Various amounts of blastula chromatin were incubated for 1 h with 600 ng of lentin at room temperature (final volume 1.0 ml).

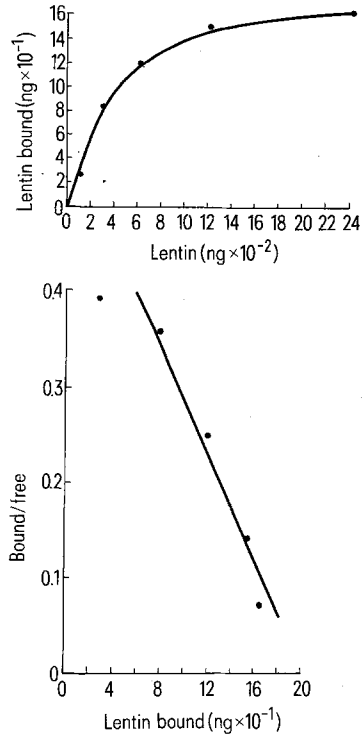

Fig. 3. Scatchard plot analysis of the lentin binding to blastula chromatin. Various concentrations of lentin were allowed to interact with 1 A_{260} unit of chromatin and specific binding was determined. The upper graph shows the direct binding curve used to plot the data according to Scatchard.

was proven by inhibitory effect of α-methylmannose. In the presence of 100 µM of α-methylmannose, nonspecific binding was found to be about 2% of the total binding. The values obtained for the total binding were therefore routinely corrected by this amount.

Figure 2 shows that at a concentration of 600 ng/assay mixture, binding of lentin to blastula chromatin was directly proportional to chromatin concentration up to about 1 A_{260} unit, and that, at higher concentration of chromatin, it tended to level off.

Data presented in the inset of figure 3 show that the binding of lentin to chromatin is saturable. Maximal binding of about 150 ng of lentin is achieved at approx. 600 ng of lentin/1 A_{260} unit of chromatin. Scatchard plot analysis of the binding reaction suggested a single type of binding site.

In view of a strong affinity of lectins towards the sugar residues, one would expect the presence of bound carbohydrates in chromatin. These compounds were indeed detected in chromatin by a variety of techniques[9-12]. We also found that nonhistone proteins (isolated from chromatin prepared by the procedure used here) exhibit a positive reaction with periodic acid-Schiff (PAS) reagent. The glycoproteins were found to reside among the high (> 67,000) and the low (< 18,000) mol.wt proteins[13]. The high mol.wt nonhistone proteins were identified as concanavalin A receptors by Rizzo and Bustin[2]. While these authors propose the use of lectins as structural probes for the study of organization of a restricted class of components in chromatin, we suggest that the lectin-binding property of chromatin could be exploited to provide new insights into the role of nonhistone glycoproteins in the regulation of gene activity.

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Tsetse fly reactions to light and humidity gradients

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Summary. Tsetse flies are positively phototactic below about 30 °C and negatively phototactic above it. The flies show a preference for the wet end of a humidity gradient and the bright end of a dorsal light intensity gradient. Studies of activity levels indicate that tsetse flies should aggregate in damp situations where the activity level is minimal, whereas in practice the flies are distributed throughout the whole of a gradient. Analyses of the water and fat content of experimental flies indicates that the reactions of individual flies is determined by their physiological condition and the conditions under which the flies have previously been kept. Previous ecological studies on the reactions of flies to humidity and light stimuli need to be reassessed in the light of these findings.

Buxton and Lewis[2] showed that temperatures are minimal and humidity maximal at dawn in northern Nigerian thickets. From dawn to about mid-day the temperature rises and the relative humidity falls, after which the temperature remains constant, and the humidity rises slowly. After dusk, the temperature declines and the humidity rises until dawn of the following day, when the cycle is repeated. Under natural conditions a dorsal light intensity gradient develops at the junction of the riverine vegetation and adjacent savannah woodland or at the margins of the *Isoberlina doka* ecotone in northern Nigeria. Between dawn and noon, a humidity gradient was also observed to develop in these situations. The experimental investigations described below attempt to assess the reactions of *Glossina morsitans* and *G. austeni* to such diurnal environmental changes.

Materials and methods. The flies used in the experiments were reared from pupae of *G. morsitans* Westwood and *G. austeni* Newstead supplied by the Tsetse Fly Laboratories of the University of Bristol.

The reactions of tsetse flies to light intensity gradients. The reactions of tsetse flies to light intensity gradients were investigated using 17 specimens of *G. morsitans* in an artificial dorsal light gradient 120 cm long ranging from 1250 to 130 lux at 25 °C. The experiments were performed with a relative humidity (RH) of 100% and then repeated with a fresh batch of flies at 50% RH. The results are given in table 1.

The reactions of tsetse flies to a humidity gradient. Both sexes of teneral specimens of *G. morsitans* were placed in a 120 cm long humidity gradient ranging from 100 to 20% RH at 25 °C under an illumination of 210 lux. 3 series of experiments were performed. In the first, the tsetse flies were kept in a humidity of 100% 48 h before the start of the experiment, and in the second series at 50%. In the third series *Calliphora erythrocephala* (Meigen) previously kept at 80% RH for 48 h were used. In a fourth series 12 gorged specimens of *G. austeni* were placed in a circular humidity gradient ranging from 0 to 100% RH and the positions of the flies recorded at 3-min intervals until 120 records had been obtained. The experiment was then repeated after 24 and after 48 h. The results are given in table 2 and in the text.

Measurements of activity levels. The activity levels of teneral male and female flies of *G. morsitans* were determined by placing the flies in a geigy cage at 25 °C in an illumination of 210 lux in an atmosphere of either 20% or 100% RH. The number of spontaneous flights or walks per fly per min was then determined. The results are given in the text.