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INTERACTIONS OF LECTINS WITH MEMBRANE GLYCOPROTEINS EFFECTS OF CONCANAVALIN A ON 5′-NUCLEOTIDASE

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SUMMARY

Concanavalin A causes a biphasic modification of the activity of the plasma membrane enzyme 5′-nucleotidase. The first stimulatory phase occurs from 0 to 0.05 μM concanavalin A, the second inhibitory phase at higher concentrations. The curve relating binding of ^{125}I-labelled concanavalin A and concentration of native lectin is similarly biphasic. The two phases likely result from occupation of distinct families of binding sites. When the enzyme is extracted from the membrane, the stimulatory phase disappears. Thus, the high affinity binding sites responsible for this phase depend upon the intact membrane structure while the others do not.

The use of lectins as tools for the investigation of a wide variety of phenomena at cell surfaces as well as many properties of the surface plasma membranes themselves has mushroomed in recent years [1–6]. We have recently reported that ^{125}I-labelled concanavalin A binds to isolated liver plasma membranes in a manner which is saturable, time and temperature dependent and inhibited by haptenes and high concentration of native protein [7]. The present communication describes the influence which this binding has on the activity of the plasma membrane enzyme, 5′-nucleotidase.

Plasma membranes were isolated by the method of Ray [8] from male Wistar rats (130–170 g) which had been starved for about 15 h. Concanavalin A from Sigma was labelled with ^{125}I using lactoperoxidase [9]. Binding experiments were performed by a method identical to that used by Rodbell et al. [10] for binding of ^{125}I-labelled glucagon to liver plasma membranes. 5′-Nucleotidase was extracted from membranes with 0.7 % solutions of either Triton X-100 or Lubrol-PX in 50 mM Tris–HCl, pH 7.8, with a resultant increase in specific activity of about 60 %. Activity of 5′-nucleotidase was assayed in the presence of 10 mM 5′-AMP, 10 mM $MgCl_2$, 1 mM $CaCl_2$ and 50 mM Tris–HCl, pH 7.5, at 37 °C. Protein was measured according to Lowry et al. [11] and inorganic phosphate by a modification of the method of Allen [12]. Specific activities were expressed as μmoles P_i liberated per mg protein per h.

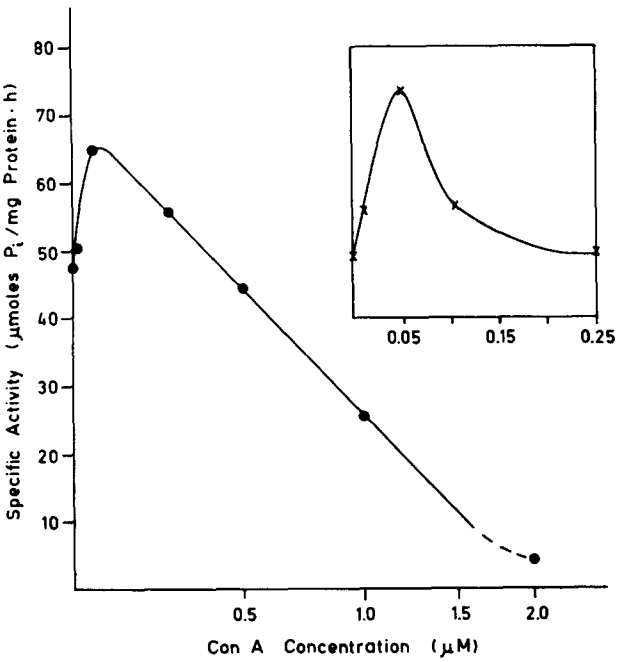

Fig. 1. Change in specific activity of 5'-nucleotidase of isolated liver plasma membranes due to exposure to concentrations of concanavalin A (Con A) indicated (log scale). Inset: curve for a similar experiment to emphasize increase in specific activity at low concanavalin A concentrations (linear scale). The labelling of abscissae and ordinates applies to both curves. 0.3 M α-methyl-D-glucoside prevented all changes in specific activity shown.

We first observed that the addition of relatively high concentrations (25–50 μg/ml) of concanavalin A to the assay medium caused considerable inhibition (20–60 %) of the 5'-nucleotidase activity of isolated plasma membranes. However, consistent with the extremely low rate of dissociation of bound ^{125}I-labelled concanavalin A from these membranes [7], a similar inhibition was observed when membranes preincubated in the presence of the same concentrations of concanavalin A and washed were assayed in the absence of the lectin. The results of one such experiment are shown in Fig. 1. The response is biphasic, showing stimulation of activity at low concentrations of concanavalin A and inhibition at higher concentrations. The character of the stimulatory phase is more clearly represented by the inset curve (separate experiment). At 0.05 μM, concanavalin A caused about a 50 % enhancement of activity. The principle curve in Fig. 1 reveals that at higher concanavalin A concentrations activity decreases exponentially to about 10 % of the control value by 2 μM. At 5 μM, inhibition is complete.

Although a satisfactory explanation of this biphasic response is not immediately apparent, it seems that increasing concentration of concanavalin A up to a critical level (0.05 μM) alters the enzyme either directly or via interaction with other membrane glycoproteins so as to cause increased rates of phosphohydrolase activity, whereas, above this critical value activity is reduced.

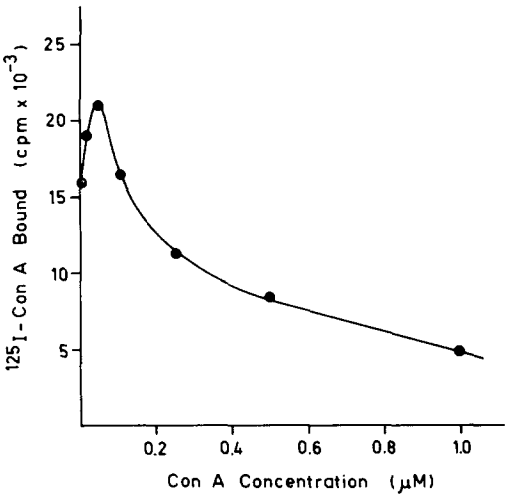

Fig. 2. The effect of native concanavalin A on the binding of ^{125}I-labelled concanavalin A (Con A) (53 000 cpm; 0.05 pmole at each point) to isolated plasma membrane (24.75 μg protein at each point). Corrections were made for "non-specific" binding which occurred in the presence of 0.3 M α-methyl-D-glucoside.

Fig. 2 shows that native concanavalin A alters the extent of binding of ^{125}I-labelled concanavalin A to these membranes in a similarly biphasic manner. Low concentrations of the unlabelled protein are capable of enhancing the binding of labelled concanavalin A; higher concentrations of the former effectively displace the latter. During the first stimulatory phase, binding of the native molecule results in increased affinity or exposure of membrane sites for ^{125}I-labelled concanavalin A, presumably due to interactions between sites. The similarity between the curves in Fig. 1 and Fig. 2 is particularly striking since the peaks occur at 0.05 μM concanavalin A in both cases. While not conclusive, this relationship is highly suggestive that the first phase of the modification of 5'-nucleotidase by concanavalin A may also be a result of the co-operative behaviour of high affinity binding sites.

To learn more about the second inhibitory phase, the influence of a fairly high dose of concanavalin A on the 5'-nucleotidase activity at varying substrate concentrations was determined and the results are represented by the reciprocal plots in Fig. 3. Although most obvious is the 50 % reduction in V, the K_m also increased, indicating a "mixed type" of inhibition [13]. This finding together with the lack of influence of changing the sequence of addition of concanavalin A and the substrate [7] suggest that inhibition does not occur simply as a result of a blocking of the substrate site by the large lectin molecule.

In light of the fact that Scatchard plots of the ^{125}I-labelled concanavalin A binding data revealed the presence of more than one population of sites [7] we feel that the high affinity sites, which when occupied at low concanavalin A concentrations cause both enhancement of further binding and of 5'-nucleotidase activity, are distinct from those which are occupied at higher concentrations, causing displacement of ^{125}I-labelled concanavalin A and inhibition of 5'-nucleotidase.

There is some evidence that this enzyme is a glycoprotein [14]. However, the question of whether the observed effects of concanavalin A are the results of direct interaction of the lectin with the enzyme or are mediated via binding to other membrane glycoproteins cannot be unequivocally answered until purification of

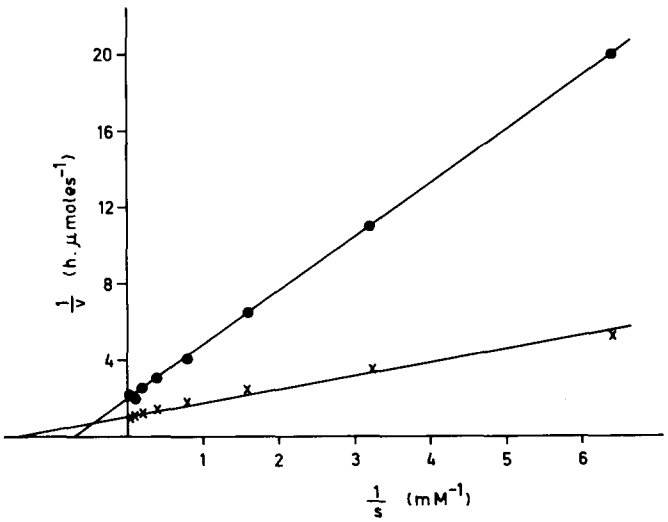

Fig. 3. Lineweaver–Burk plots of reciprocal of 5′-nucleotidase specific activity (v) versus reciprocal of 5′-AMP concentration(s) in the presence (●) and absence (×) of 1.5 μM concanavalin A.

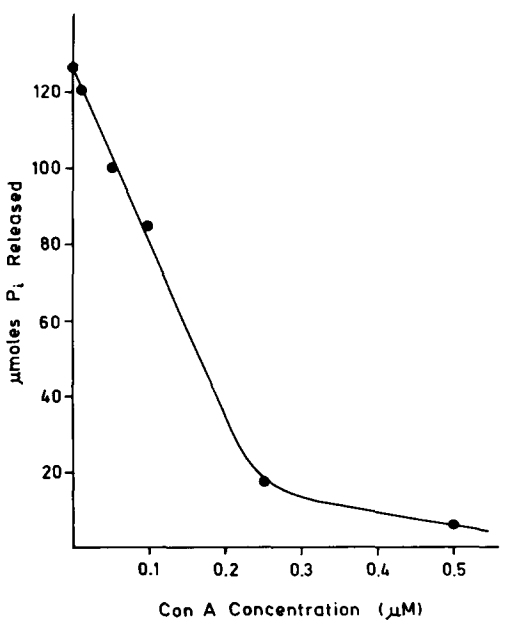

Fig. 4. Effect of concanavalin A (Con A) on inorganic phosphate released from 5′-AMP by the 5′-nucleotidase extracted from isolated plasma membrane (37.5 μg protein at each point) by a 1-h incubation in 0.7 % Triton X-100 (50 mM Tris–HCl, pH 7.8) at 37 °C.

the enzyme has been completed. We are presently engaged in this task. In the meantime, we have studied the influence of concanavalin A on the enzyme after it was extracted from the membrane with a non-ionic detergent. The result shown in Fig. 4 is distinctly different from that found with intact membranes. Not only is the stimulatory phase completely absent, but the solubilized enzyme is much more sensitive to inhibition by concanavalin A: at 0.05 μM, a 10 % inhibition has occurred in contrast to a 50 % stimulation with particulate enzyme (Fig. 1); at 0.5 μM inhibition was virtually complete, whereas, with intact membrane activity was only slightly lower than that of the control (Fig. 1). Hence, removal from the membrane renders the enzyme non-stimulatable by the low concentrations of concanavalin A which occupy high affinity binding sites on the intact membrane. The extracted enzyme also either has more inhibitory sites exposed or is more susceptible to the inhibitory alteration once binding has occurred. These observations would be consistent with a stimulation of activity due to indirect interaction of lectin with enzyme and an inhibition as a result of a direct association.

We hope that further experiments with lectins specific for other monosaccharides commonly found in glycoproteins and some modified forms of these will help distinguish whether the modifications of 5'-nucleotidase activity were due to general perturbations of carbohydrate moieties or specifically due to the binding of the tetravalent concanavalin A, presumably to mannose residues.

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