Concanavalin A, a receptor protein with apparently co-operative binding characteristics

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Laser nephelometry is a suitable technique for the quantitative determination and differentiation of both lectins and glycoconjugates in the low-picomolar range. Simultaneously this method renders possible investigations on the specificity and mode of interaction between lectins and different ligands. The results demonstrate that the degree of co-operativity between concanavalin A and the respective glycoconjugate is dependent on the presence of hydrophobic binding sites and can be substantially altered by conformational changes of the ligand. The transition from apotransferrin to Fe^{3+} -transferrin induces a transformation of the sigmoidal-shaped binding curve to a hyperbolic one. Hence, at low concentrations, Fe^{3+} -transferrin is bound far better than apotransferrin, whereas maximal binding is nearly identical. After removal of *N*-acetylneuraminic acid, concanavalin A is less efficient in differentiating between the Fe^{3+} -charged and Fe^{3+} -free (apo) forms of transferrin.

Studies on membrane glycoproteins and glycolipids have been facilitated by the use of lectins, which are known to bind specifically to saccharides and thus provide important information about the properties of cell surfaces. Though these macromolecules have originally been isolated from various plant seeds, lectin-like substances have been demonstrated in lower vertebrates and even in mammalian tissue (Sharon & Lis, 1975; Stockert *et al.*, 1974; Köttgen *et al.*, 1979a). Much attention has been focused on the binding sites responsible for complex-formation between lectin and carbohydrate groups. However, there has been a suggestion that concanavalin A, the best-explored lectin, possesses an additional site that can be occupied by a hydrophobic ligand (Edelman *et al.*, 1972). Owing to the high sensitivity of laser nephelometry, minute amounts of glycoprotein and lectin can be determined, and, by use of this method, detailed studies on lectin–glycoprotein interaction are possible (Köttgen *et al.*, 1979b). The present results reveal co-operative binding properties of concanavalin A in the presence of hydrophobic ligands. Consequently, a change of conformation causing the exposure or masking of hydrophobic glycoprotein areas should have a substantial effect on the affinity between lectin and glycoconjugate. The possible biological

significance of this finding is demonstrated by using the human Fe^{3+} -transferrin/apotransferrin system.

Materials and methods

Complex-formation between the lectin concanavalin A and different glycoconjugates causes an increase in relative light scattering, which was determined with a Hyland PDQ nephelometer equipped with a helium/neon laser (wavelength 633 nm) (Hyland Laboratories, Costa Mesa, CA, U.S.A.). Concanavalin A was purchased from Pharmacia (Uppsala, Sweden); fetuin, ovalbumin and methyl α -D-mannopyranoside were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.); highly purified transferrin and immunoglobulin G came from Behring A.G. (Marburg/Lahn, Germany); all other chemicals of analytical grade were supplied by Merck A.G. (Darmstadt, Germany). All glycoproteins used were homogeneous on polyacrylamide-gel electrophoresis.

Measurements were performed at 25°C in 1.0 ml of 0.1 M-Tris/HCl buffer, pH 7.0, containing CaCl_2 (5 mM) and poly(ethylene glycol) 5000 (5%, w/v). Before use, all solutions were passed through a Millipore filter (pore size 0.45 μm) to avoid any possible interference by contaminating light-scattering material. The complex-formation reaches equilibrium within 3–4 h, after which time no further

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change in light scattering is observed. Blank values obtained in the absence of lectin or glycoconjugate were subtracted. The results are given as c (relative light scattering, given in volts), which is directly proportional to the amount of complex formed.

Results and discussion

Previous investigations have shown that complex-formation between lectin and glycoconjugate exhibits a great deal of similarity with enzymic reactions, and lectins have been called 'enzymes with reduced function' (Hankins & Shannon, 1978). Therefore the Michaelis-Menten equation, or the equivalent linear transformations, often referred to as 'double-reciprocal plots', are useful tools for describing and evaluating our results.

As demonstrated in Fig. 1, a direct relationship exists between the amount of glycogen added and the degree of concanavalin A-polysaccharide complex-formation (curve *a*). Increasing amounts of methyl mannoside cause a dissociation of the complex already formed, a finding known from various studies on concanavalin A (curves *b-d*) (So & Goldstein, 1968). Furthermore, from the intercept with the abscissa, the dissociation constant of

Fig. 1. Double-reciprocal plots of concanavalin A-glycogen complexes

The concentration of concanavalin A was kept constant ($0.083 \mu\text{M}$) while increasing amounts of glycogen were added (abscissa). (a) Without the competitive inhibitor α -methyl mannoside; (b-d) in the presence of 0.02, 0.08 or 0.1 M α -methyl mannoside respectively. c is the degree of complex-formation, i.e. the relative light scattering. From the intercept with the abscissa the dissociation constants can be calculated.

the individual system can be calculated. Likewise, the inhibitor constant of each sugar can be accurately determined from the Dixon plot (Segel, 1975).

However, if glycogen is replaced by human apotransferrin, a well-characterized serum protein, the shape of the curves shows an entirely different picture. The double-reciprocal plots are comparable with enzymic reactions that obey apparently co-operative kinetics. A linear transformation is achieved by plotting $1/c$ against $1/[S]^4$ instead of against $1/[S]$. This is known from enzymology, and is quite characteristic of allosteric (i.e. co-operative) binding reactions (Segel, 1975).

Under conditions generally used, concanavalin A is present as tetramer (Kalb & Lustig, 1968; Huet & Claverie, 1978), whereas concanavalin A dimers show, as expected, a co-operativity decreased by half. On the basis of the same data, the Hill plot has been applied, because this double-logarithmic plot is linear over the entire range of ligand concentration. Moreover, the number of binding sites can be read directly from the slope of the curves (Segel, 1975).

If various glycoconjugates are tested with concanavalin A, a different number of binding sites and a different degree of co-operativity is found: h (Hill coefficient) = 4 for both human apotransferrin and ovalbumin; $h = 2$ for fetuin and immunoglobulin G; $h = 1$ for glycogen and α -mannan (Fig. 2). These findings are supported by a published assumption of a Ca^{2+} -induced conformational change and co-operative binding of Mn^{2+} ions by concanavalin A (Alter *et al.*, 1977).

As shown by Clegg *et al.* (1977) and Williams *et al.* (1978), univalent ligands like 4-methylumbelliferyl α -D-mannopyranoside and *p*-nitrophenyl α -D-mannopyranosyl- α -D-mannopyranoside are bound to concanavalin A without evidence of co-operative interactions. Both compounds, however, are rather small molecules, with hydrophobic and hydrophilic areas in close vicinity to each other, with the consequence that these glycosides show a predominantly hydrophilic behaviour. Conversely, serum (glyco)proteins, though they are in general easily soluble, possess distinct hydrophobic regions. Hydrophobic binding sites have been described for ovalbumin (McClure & Edelman, 1966), immunoglobulins and serum albumin (Singer, 1965). Likewise, the relatively high content of hydrophobic amino acids, e.g. leucine and valine, indicate that the transferrin molecule belongs to the same category (Mann *et al.*, 1970).

In order to examine the co-operative mechanism and hydrophobic binding site more accurately, the complex-formation between concanavalin A and the above-mentioned glycoconjugates was studied in the presence of 40% (w/v) ethylene glycol, which occupies, and therefore efficiently blocks, hydro-

Fig. 2. Cooperative-binding properties of concanavalin A in the presence of different glycoconjugates

The number of co-operative binding sites can be read directly from the slope of the double-logarithmic Hill plots: $h = 1$ (glycogen or α -mannan), no co-operativity; $h = 2$ (fetuin or immunoglobulin G), intermediate degree of co-operativity; $h = 4$ (human apotransferrin or ovalbumin), high degree of co-operativity. By applying polyacrylamide-gel disc electrophoresis it could be demonstrated that, under assay conditions (pH 7.0, 25°C), concanavalin A is predominantly present as a tetramer (see also Huet & Clavier, 1978). If temperature (Huet *et al.*, 1974) or pH value (Kalb & Lustig, 1968) are lowered, concanavalin A is subject to a tetramer-dimer transition. At 2°C, concanavalin A dimers prevail, which show a co-operativity decreased by half ($h = 2$). $C_{\max}$ is the maximal complex-formation calculated from the double-reciprocal plot (intercept with the ordinate).

Fig. 3. Complex-formation between concanavalin A and human apotransferrin or Fe^{3+} -transferrin

The addition of increasing amounts of Fe^{3+} (FeCl_3) to apotransferrin leads to a transition of the sigmoidal-shaped binding curves to hyperbolic ones, i.e. the apparently co-operative binding behaviour is decreased and finally disappears. (a) Apotransferrin alone; (b–d) 10 μM -, 50 μM - or 100 μM - Fe^{3+} respectively in the assay system. Especially at low concentrations, the Fe^{3+} -charged molecule is bound much better than apotransferrin. The determinations were performed while keeping the concentration of concanavalin A constant (0.083 μM).

phobic areas. Although the complex-formation between concanavalin A and glycogen is not affected, the binding of apotransferrin is inhibited in a mainly competitive mode. Similar results have been obtained when testing fetuin and immunoglobulin G, although, as could be predicted from the results presented in Fig. 2, the degree of inhibition was less pronounced. Similarly, Triton X-100 (2%, w/v) does not interfere with the concanavalin A–glycogen interaction, but lowers the binding of apotransferrin and fetuin. Since the complex-formation between glycogen and lectin is not inhibited, a possible denaturation of concanavalin A can be excluded. Hence it is reasonable to assume that: (a) the hydrophobic binding site of concanavalin A can be masked by ethylene glycol; (b) glycoproteins with hydrophobic regions can interact specifically with this site.

Studies by Davey *et al.* (1974) have shown that human interferon, a glycoprotein, can only be quantitatively recovered from a concanavalin A–Sephadex column if 50% (v/v) ethylene glycol is

added to the eluent. Methyl α -D-mannopyranoside alone is inefficient in the displacement of interferon, and the authors therefore conclude that interferon binds to concanavalin A, by both carbohydrate recognition and hydrophobic interaction.

Furthermore, conformational changes in the glycoconjugate should lead to altered binding to lectins. This can be shown by the example of apotransferrin/ Fe^{3+} -transferrin, because the attachment of Fe^{3+} alters the conformation of this transport protein (Aisen & Brown, 1975). Fig. 3 shows the effect of an increase in the Fe^{3+} concentration on the formation of the concanavalin A–apotransferrin complex. The sigmoidal binding curve becomes a hyperbolic one, owing to a decreased co-operative interaction between the reaction partners. Consequently, Fe^{3+} -transferrin is bound much better than apotransferrin in the low-micromolar concentration range of these ligands. This means that the receptor protein concanavalin A is able to distinguish the biologically relevant (i.e. the Fe^{3+} -charged molecule) from the less important one. It is worth comparing our results with the equivalent mammalian biological system,

the transferrin receptor on the reticulocyte cell surface. Both systems: (i) possess nearly identical affinity constants (K_a for concanavalin A/ Fe^{3+} -transferrin, $0.42 \mu\text{M}$; reticulocyte receptor/ Fe^{3+} -transferrin, $0.4 \mu\text{M}$); (ii) show a very similar dependency of the Ca^{2+} ionic strength; and, above all (iii) are characterized by an identical (3.6-fold) increase in the binding constants with the transformation from apotransferrin to Fe^{3+} -transferrin (Kornfeld, 1969; Van Bockxmeer *et al.*, 1978).

Our experiments also have significance for a different receptor system. Ashwell & Morell (1974) have presented evidence that the treatment of glycoproteins with neuraminidase (which removes terminal sialic acid groups) increases substantially the affinity between lectin-like receptors of hepatocytes and these desialylated glycoproteins. Further, the exposure of penultimate galactose residues is regarded as being responsible for this enhanced binding of asialoglycoproteins. Yet these findings might be seen from a different angle. The apotransferrin-concanavalin A system shows a high degree of co-operativity, whereas the co-operativity with asialo-apotransferrin is markedly decreased (Hill plot, $h = 2$). Hence, after desialylation, the receptor protein concanavalin A is less able to differentiate between the Fe^{3+} -free and Fe^{3+} -charged forms of transferrin, because, with the change from asialo-apotransferrin to asialo- Fe^{3+} -transferrin, the binding constant increases only 1.5-fold. In view of the fact that the removal of sialic acid apparently causes conformational changes within the glycoprotein molecules, the results of Ashwell & Morell (1974) could in part be attributed to these alterations.

Thus the laser nephelometry described here opens up not only new possibilities for precise studies of the carbohydrate specificity of lectins, but also renders possible new insights into the varied biological functions of these receptor proteins.

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