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HYDROPHOBICITY OF LECTINS

I. THE HYDROPHOBIC CHARACTER OF CONCANAVALIN A

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Summary

The hydrophobicity of Concanavalin A has been estimated by its tendency to adsorb to hydrophobic adsorbents. Experiments varying temperature, salt concentration and hydrophobicity of the adsorbent were consistent with accepted criteria of hydrophobic interaction between biomolecules and hydrophobic ligands. The biological significance of the hydrophobic character of Concanavalin A is also discussed.

Introduction

When studying erythrocyte stroma as an affinity adsorbent for purification of lectins from the two Mexican strains *Phaseolus vulgaris* var. 'Garbancillo' and *Phaseolus coccineus* var. 'Alubia' [1] we realized that the purification obtained was partly due to hydrophobic interaction between the adsorbent and the lectins. This observation prompted us to start looking at the chromatographic behaviour of various other lectins on a series of hydrophobic supports under physiological conditions. The aim is to establish to what extent hydrophobic effects may be involved in the interaction between immobilized lectins and cell surface receptors as well as glycoproteins in solution [2,3].

For a start, a model system involving Concanavalin A and different neutral alkyl agaroses has been studied. Concanavalin A was chosen for the following reasons: first, Concanavalin A is the most extensively characterized lectin to date and the only one for which the three-dimensional structure has been

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worked out in detail [4,5]; and second, observations by several research groups indicate that Concanavalin A shows considerable hydrophobicity [6—12].

Materials and Methods

Alkylagarose derivatives were synthesized according to the method of Hjertén et al. [13] or obtained, as octyl-Sepharose, from Pharmacia Fine Chemicals, Uppsala, Sweden. The degree of substitution for the gels used in this study, as determined by NMR [14], ranged between 50—60 mmol of substituent/mol galactose if nothing else is stated.

Sepharose CL-4B and freeze-dried Concanavalin A were purchased from Pharmacia Fine Chemicals, Na ^{125}I was obtained from Amersham, and lactoperoxidase and glucose oxidase were from Sigma. All other chemicals were of analytical purity.

Labelling of Concanavalin A with ^{125}I

This was performed by the lactoperoxidase method [15]. 25 mg Concanavalin A dissolved in 2 ml of phosphate saline buffer (0.02 M K_2HPO_4/KH_2PO_4, pH 7.4, containing 0.9% NaCl and 0.02% NaN_3) being 1 M in D-glucose was mixed with 200 μl lactoperoxidase (0.4 mg/ml), 5 μl Na^{125}I ($8.3 \cdot 10^8$ dpm) and 200 μl glucose oxidase (0.5 mg/ml) and left to react overnight. After addition of NaCl to a concentration of 4 M the solution was applied to an octyl-Sepharose^R column (1.4 × 30 cm). Concanavalin A adsorbed to the column and excess iodide and glucose was washed away with 4 M NaCl, and the labelled Concanavalin A was then eluted with distilled water. The specific activity was calculated after measuring radioactivity in a Beckman LS 100 C scintillation counter using Searle Gamma Vials and found to be $2.4 \cdot 10^{-3}$ mCi/mg of protein.

Column chromatography

Experiments were performed in duplicate using Pasteur pipettes as columns. These allowed the use of only small amounts of gel and the high flow rates obtainable made it possible to complete an entire experiment involving packing, equilibration, chromatography, and desorption in 4 h. The chromatographic conditions pertaining to each particular case are given in the corresponding figure legend. Experiments to determine the effect of temperature were run in a temperature chamber, whereas all other experiments were done at room temperature.

In each column experiment a known amount of ^{125}I-labelled Concanavalin A was applied, and the distribution of radioactivity between irreversibly bound material, unbound substance, and desorbed material was determined as described above. The reported values are the averages of at least three experiments. The standard deviation is about 5%.

Results

Effects of temperature and hydrophobicity of the matrix

The adsorption behaviour of Concanavalin A and other lectins on alkylagar-

oses at varying temperature, salt concentration, and hydrophobicity of the alkyl gels may give some information about the extent to which these proteins are involved in hydrophobic interactions in biological systems. Fig. 1a shows that under physiological conditions (phosphate saline buffer and 37°C) Concanavalin A adsorbs to alkylagarose derivatives with a chain of 4 to 10 carbon atoms. At lower temperatures, the affinity of Concanavalin A for the gel decreases, although decyl agarose (C-10) is still an efficient adsorbent at 4°C. The hydrophobic mechanism of adsorption of Concanavalin A to these gels is further supported by the fact that an increase in the degree of substitution (Fig. 2) or in the length of the alkyl-residue attached to the gel (Fig. 1a) increases the amount of adsorbed Concanavalin A. The participation of cooperative electrostatic interactions in the binding were excluded using neutral alkyl derivatives of agarose [16,17]. Thus, it can be concluded that the adsorption of Concanavalin A to this type of adsorbents is an indirect estimation of the hydrophobicity of Concanavalin A.

Salt dependence

Fig. 1b shows the tendency of Concanavalin A to adsorb to a series of hydrophobic gels as a function of the salt concentration. It can be seen that C-6 is the most satisfactory adsorbent for this lectin since the bulk of adsorbed material can be released simply by reduction of the ionic strength of the medium. The C-8 and C-10 derivatives adsorb Concanavalin A too strongly, since a substan-

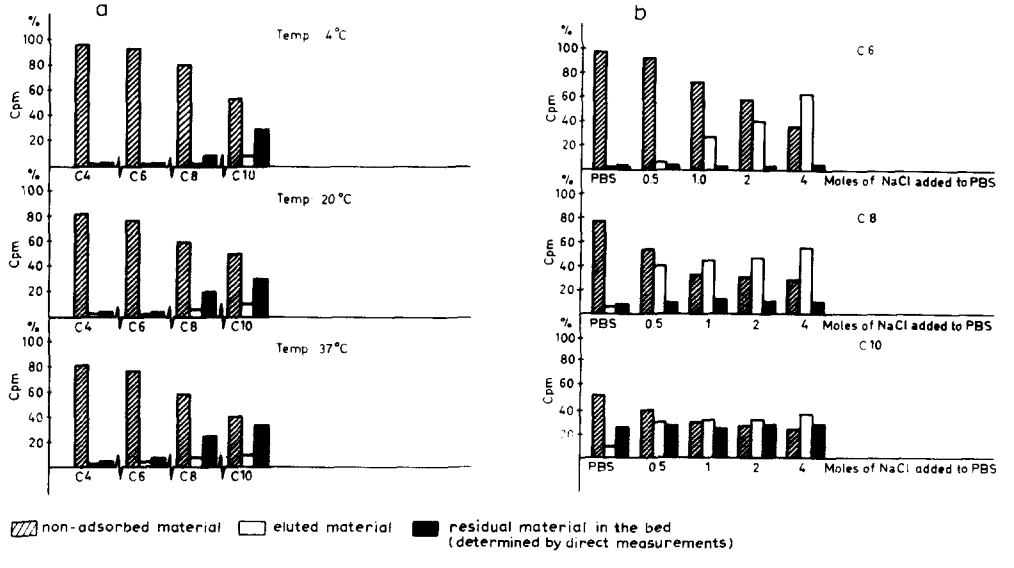


Fig. 1. Temperature (a) and salt concentration (b) effects on the adsorption of Concanavalin A to a series of alkylagarose. 35 μg of labelled Concanavalin A ($2 \cdot 10^5$ counts/min) in 0.5 ml of phosphate saline buffer were applied to columns (0.5 X 2.5 cm) equilibrated with the same buffer (except in the case of increasing salt concentration effects where the columns were equilibrated under the conditions signaled in b). Following the columns were washed using 10 ml of the adequate buffer in each case. Desorption was performed by reducing ionic strength with distilled water (10 ml) and the radioactivity determined in the pooled fractions as indicated in Materials and Methods. The percentages shown in the figures correspond to the total amount of radioactivity applied.

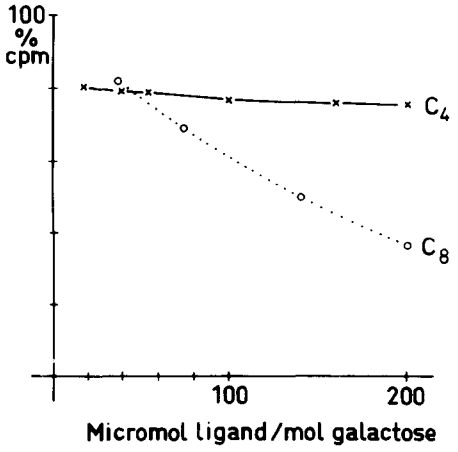


Fig. 2. Effect of the degree of substitution of C-4 and C-8 derivatives of agarose on their capacity to adsorb Concanavalin A. Similar samples of Concanavalin A used in the experiments described in Fig. 1 were applied in a series of adsorbents varying in their degree of substitution (D.S.). The procedure was the same as the one described in Fig. 1. The percentage of non-adsorbed material is plotted against the D.S. of the adsorbent. Other conditions were room temperature and phosphate saline buffer.

tial part of the applied material binds irreversibly. Fig. 1b also indicates that C-8 and C-10 reach their maximum adsorbent capacity at a much lower salt concentration than that required for C-6 to reach a similar level.

Elution

Additional information about the hydrophobicity of a particular biomolecule may be obtained from studying the conditions required for its elution from hydrophobic adsorbents. In the present experiments distilled water has successfully been used as an eluant. Its efficiency compared to other agents usually

TABLE I

DESORPTION OF CANCANAVALIN A FROM DECYL AGAROSE (C-10) WITH DIFFERENT ELUANT SYSTEMS

Eluant system *	Percent of non adsorbed material **	Percent of eluted material	Percent of residual radioactivity	Total recovery
Ethylene glycol (50% in phosphate saline buffer)	28	50	20	98
Triton X-100 (0.1% in phosphate saline buffer)	30	34	38	102
Sodium deoxycholate (0.1% in phosphate saline buffer)	30	44	24	98
Distilled H_2O	29	36	36	101
Phosphate saline buffer	30	20	46	96
Phosphate saline buffer + 0.1 M glucose	30	22	42	94
Phosphate saline buffer + 1.0 M glucose	31	20	46	97

* % are respect to the total amount of radioactivity applied.

** 35 μg of labelled lectin ($2 \cdot 10^5$ cpm) were applied to a decyl agarose column (0.5 × 2.5 cm) equilibrated with phosphate salihe buffer containing 2 M NaCl at room temperature.

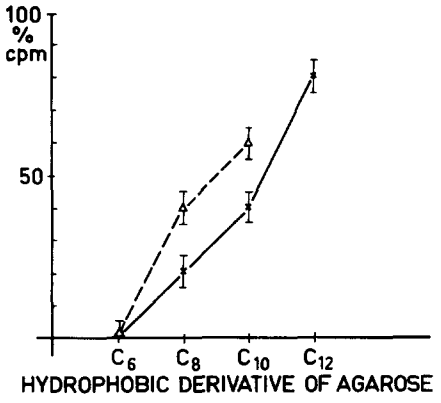


Fig. 3. Effect of D-glucose on the adsorption of Concanavalin A to alkylagaroses. Similar samples of Concanavalin A employed in the experiments described in Fig. 1, but in the presence of 0.1 M D-glucose, were applied to column (0.5 X 2.5 cm) equilibrated this time with sugar containing buffer (0.1 M D-glucose in phosphate saline buffer) at room temperature. The procedure was essentially the same than as described in Fig. 1. The percentages of total bound radioactivity are plotted against the nature of alkylagarose employed (with similar D.S.): - - - - -, radioactivity adsorbed in the presence of sugar; +———+, radioactivity adsorbed in the absence of sugar.

employed for desorption in hydrophobic interaction chromatography [16,17] is shown in Table I. The results indicate the following scale of decreasing desorption capacity in this particular system: ethylene glycol (50% in phosphate saline buffer) > deoxycholate (0.1% in phosphate saline buffer) > Triton X-100 (0.1% in phosphate saline buffer) > H_2O > phosphate saline buffer. Interestingly, water is almost as efficient as Triton X-100. The presence of a known inhibitor of Concanavalin A (D-glucose) in the eluant does not increase desorption efficiency, and this observation led us to a complementary experiment aimed at determining whether or not engagement of the sugar-binding site by carbohydrate prior to exposure of the lectin to a hydrophobic adsorbent would affect the overall extent of binding. A series of adsorptions in the presence of 0.1 M glucose are shown in Fig. 3. Surprisingly, the total amount of adsorbed material was increased by about 20% when glucose was present. The results with 1.0 M glucose were quite similar.

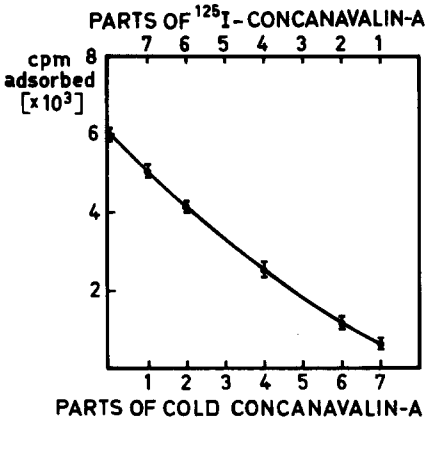
Discussion

Soon after introduction of hydrophobic adsorbents for separation purposes [18—20], a method to estimate the relative hydrophobicity of biomolecules became available. Since separation of water-soluble proteins, membrane proteins, viruses, and bacteria on this type of adsorbents is mainly due to discrimination between biomolecules differing in the number and/or size of exposed hydrophobic sites, alkylagaroses can be used for determining their relative hydrophobicity [16,17]. The biological importance of such a 'hydrophobic scale' has been illustrated by Edebo et al. [21] who distinguished between pathogenic and non-pathogenic Salmonella strains by looking at their adsorption to alkylagaroses.

The tendency of Concanavalin A to adsorb non-specifically to cell surfaces,

glycoproteins, and hydrophobic substances aside from its ability to bind specifically sugars of the mannosyl configuration, is now well recognized [6—12]. Concanavalin A immobilized on agarose has been a useful adsorbent for the purification of some glycoproteins [8—9,22]. However, it has also been noticed that only part of the adsorbed glycoproteins can be eluted with sugar [9]. Davey et al. [9], for instance, found that ethylene glycol in combination with mannosides considerably increased the recovery of adsorbed proteins from Concanavalin A-agarose. Zanetta et al. [22] and Lotan et al. [8] have reported that detergents greatly affect the total capacity of adsorption of immobilized Concanavalin A without changing its ability to recognize specifically mannosyl- and/or glycosyl-containing glycoproteins. In the present paper we show that Concanavalin A is indeed adsorbed to alkylagaroses by hydrophobic interaction. The adsorption increases with temperature, salt concentration, and hydrophobicity of the adsorbent in agreement with the theory of hydrophic interaction between biomolecules and hydrophobic ligands [16,17].

The labelling procedure does not appreciably modify the hydrophobic properties of Concavalin A. This conclusion is based on the observation that the fraction of labelled lectin binding to the adsorbent is largely independent of the ratio between labelled and unlabelled lectin jointly applied to octyl-Sepharose (Fig. 4). Fig. 5 indicates that affinity heterogeneity among binding sites on hydrophobic adsorbents is probably the main reason why only a fraction of the sample will be bound irrespective of the total amount of applied material. Such



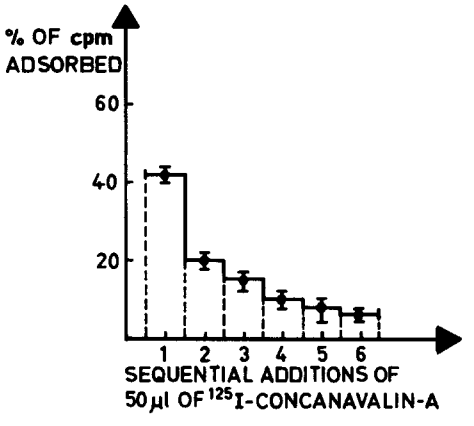


Fig. 4. Competitive binding of labelled and unlabelled Concanavalin A on octyl-Sepharose. Equimolar solutions of labelled and unlabelled Concanavalin A were mixed in different proportions and applied to an octyl-Sepharose column (0.5 X 1.0 cm) equilibrated with phosphate saline buffer 0.1 M in glucose at room temperature. The total amount of material applied in each case corresponds to 200 µg of protein in 200 µl of buffer. After application of the sample the column was washed with 4 bed volumes (800 µl) of buffer. The total amount of radioactivity adsorbed on the gel was determined as described in Materials and Methods and is plotted against the proportional mixtures of labelled and unlabelled Concanavalin A.

Fig. 5. Effect of sequential additions of 50 µl of ^{125}I-labelled Concanavalin A (1 mg/ml) to octyl-Sepharose. A total amount of 300 µl of ^{125}I-labelled Concanavalin A were added stepwise to an octyl-Sepharose column (0.5 X 1.0 cm) equilibrated with phosphate saline buffer 0.1 M in glucose at room temperature. After each step the column was washed with 4 times its bed volume (800 µl) of buffer. The amount of non-adsorbed radioactive material was determined as described, and the additional radioactive material adsorbed on the column after each step was plotted.

binding site heterogeneity has been explained on the basis of the physical structure of agarose beads [16].

The peculiar effect observed by the presence of the inhibiting carbohydrate during the adsorption step (Fig. 3) may be explained on the basis of the chemical structure of Concanavalin A. Since two tyrosyl and two asparagyl groups have been identified as being involved in the sugar-binding process [5,23,24], it is possible that the carbohydrate 'masks' those charged amino acids favouring the adsorption of the biomolecule to alkylagaroses. A sugaring-out effect [25] may also contribute to enhance the tendency of Concanavalin A to bind to hydrophobic adsorbents.

The tetrameric form of Concanavalin A may dissociate to dimers at 4°C [26]. This phenomenon could be responsible for a lower tendency of Concanavalin A to bind to hydrophobic adsorbents at low temperature. Therefore, it would be interesting to correlate this possibility with the known decrease in agglutinating ability which accompanies the transition from Concanavalin A tetramers to dimers, and the observation that the rate of agglutination is reduced with decreasing temperature [26]. The considerable affinity that Concanavalin A shows for alkylagaroses under physiological conditions is suggestive and some of the biological properties attributed to this lectin might stem both from its hydrophobic character and its sugar-binding capacity.

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