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Note

Behaviour of concanavalin A on DEAE-Sephadex and CM-Sephadex[a]

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Concanavalin A (Con A) is a lectin isolated from jack bean. It is composed of four subunits each having a molecular weight of 26 500 and containing one Ca^{2+} and one Mg^{2+} per subunit. It is conventionally purified either by ion-exchange chromatography on CM-cellulose [1] or by affinity chromatography on Sephadex [2]. The Sephadex ion exchangers are composed of an ion-exchange group and dextran. The individual roles of these two moieties in the adsorption and desorption of dextran binding proteins are not well defined, but it has been generally assumed that the ion-exchange group is responsible for retention. The present investigation was undertaken to test this hypothesis. Con A has the ability to interact with both the ion-exchange group and dextran. Hence the adsorption and elution behaviour of this protein on DEAE-Sephadex and CM-Sephadex under different conditions was studied.

EXPERIMENTAL

Concanavalin A (CSIR Centre for Biochemicals, New Delhi, India), CM-Sephadex C-50 and DEAE-Sephadex A-50 (Pharmacia, Uppsala, Sweden) were used. All other chemicals were of analytical-reagent grade. The purity of Con A was ascertained by polyacrylamide gel electrophoresis (PAGE) under native and denaturing conditions [3]. The bands were revealed by staining the gels after electrophoresis with Coomasie Brilliant Blue G-250.

The influent protein solution was prepared by dissolving Con A in 50 m*M* Tris–HCl buffer (pH 8.3) or 50 m*M* acetate buffer (pH 5.0), depending on the pH required for the binding. The adsorption of protein on DEAE-Sephadex was carried

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out under different conditions, *viz.*, low salt (0.01 *M* NaCl), low salt with glucose (0.01 *M* NaCl, 0.1 *M* glucose) and high salt (1 *M* NaCl).

The resin was washed with the loading buffer until the absorbance of the effluent at 280 nm was negligible (<0.1). Elution of the protein was carried out with different eluting agents such as low salt with glucose, high salt and high salt with glucose (1 *M* NaCl, 0.1 *M* glucose). The solutions were prepared in 50 m*M* Tris–HCl buffer (pH 8.3) or 50 m*M* acetate buffer (pH 5.0). The retention and elution of Con A (in 5-ml fractions) were determined by measuring the absorbance at 280 nm.

RESULTS AND DISCUSSION

The Con A preparation showed a single band in native PAGE and multiple bands in sodium dodecyl sulphate PAGE, as reported by Wang *et al.* [3]. The protein was therefore considered to be electrophoretically pure. Retention of Con A on DEAE-Sephadex was almost identical under conditions of low salt and low salt with glucose. However, 1 *M* NaCl could elute 50% of the protein retained in the presence of low salt and only 20% in low salt with glucose (Table I). The results further show that elution with 1 *M* NaCl–0.1 *M* glucose leads to a better and faster desorption of protein (Fig. 1). Moreover, no protein was seen in the effluent when the elution was effected with low salt with glucose. It was also observed that Con A was retained on DEAE-Sephadex under high-salt conditions, and the protein could not be eluted with high salt with glucose.

Con A desorbed by a high salt concentration may bind to Sephadex and hence elution is faster in the presence of glucose. In addition, as the sugar–Con A complex is more basic than free Con A [4], it is not expected to be retained on anion exchangers. The firm retention of Con A on DEAE-Sephadex under high-salt conditions may be attributed to affinity interactions which are stronger, probably owing to the dissociation of the tetrameric protein.

The results of experiments carried out using CM-Sephadex show that the amount of protein retained per gram of resin was higher for CM-Sephadex than

TABLE I

ELUTION OF CONCANAVALIN A FROM DEAE-SEPHADEX AND CM-SEPHADEX WITH DIFFERENT ELUENTS

Parameter	DEAE-Sephadex (pH 8.3)			CM-Sephadex (pH 5.0)			CM-Sephadex (pH 8.3)	
	Low salt[a]	Low salt–glucose[a]	High salt[a]	Low salt[a]	Low salt–glucose[a]	High salt[a]	Low salt[a]	Low salt–glucose[a]
Protein retained (mg/g resin)	84	85	63	145	143	58	8.0	27.0
Recovery (%)								
Low salt–glucose[a]	0	–	–	13.0	–	–	0.0	5.0
High salt[a]	48.0	20	–	47.0	42.0	–	62.0	–
High salt–glucose[a]	63	–	9.0	58.0	–	24.0	–	–

[a] Low salt = 0.01 *M* sodium chloride; low salt–glucose = 0.01 *M* sodium chloride–0.1 *M* glucose; high salt = 1 *M* sodium chloride; high salt–glucose = 1 *M* sodium chloride–0.1 *M* glucose.

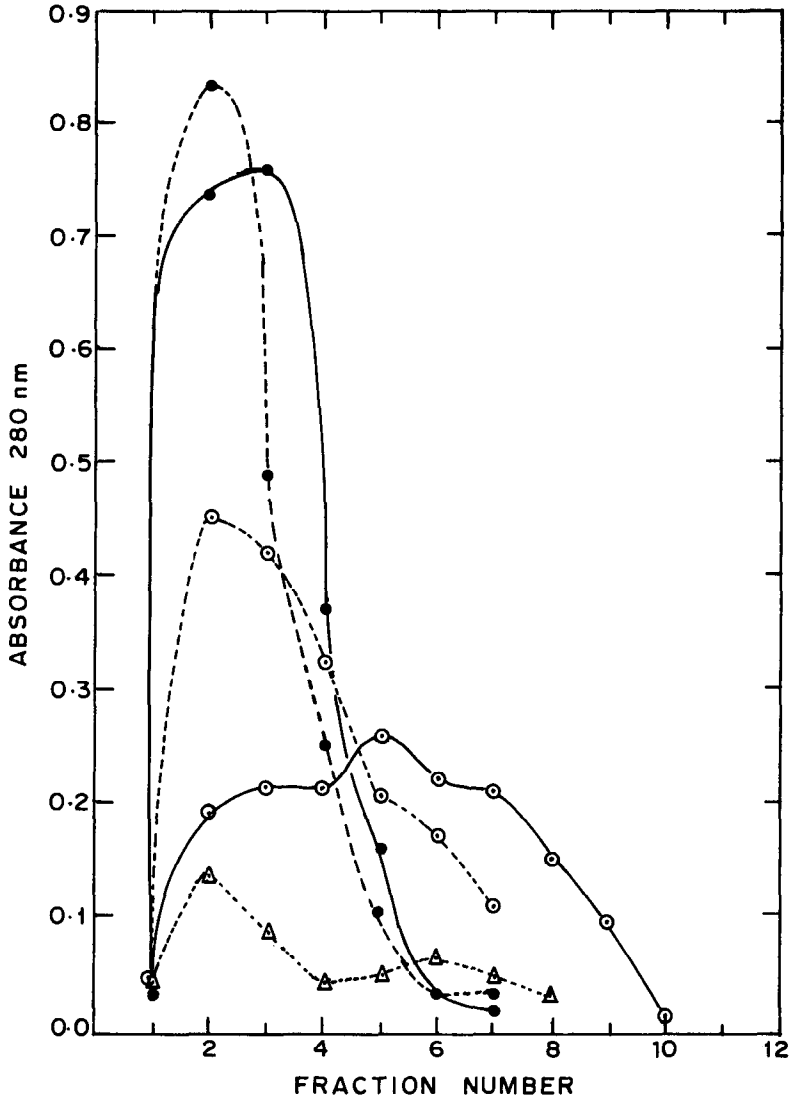

Fig. 1. Chromatographic elution profiles of Con A on (solid line) DEAE-Sephadex (pH 8.3) and (dashed line) CM-Sephadex (pH 5.0). (●) 1 *M* sodium chloride and 0.1 *M* glucose; (○) 1 *M* sodium chloride; (△) 0.01 *M* sodium chloride and 0.1 *M* glucose.

DEAE-Sephadex (Table I). However, as with DEAE-Sephadex, the amount of protein retained on the matrix under low-salt binding conditions was the same in the presence or absence of glucose. About 12% of the protein retained could be eluted by low salt with glucose. Similarly, Con A was desorbed faster when high salt with glucose was the eluent. The protein could also be retained on CM-Sephadex under high-salt conditions, and an appreciable amount of Con A thus retained could be eluted using

high salt with glucose. At pH 5.0, Con A exists in its dimeric form [5], which would explain the adsorption–elution behaviour observed above.

These results indicate that Sephadex may participate in the retention of Con A on CM-Sephadex. It is evident that as the protein can be eluted with glucose alone, the sugar-binding sites are not involved in the ionic coupling. The fraction of Con A eluted by low salt with glucose is bound only to the dextran moiety of the matrix. The protein so eluted is also not due to the microheterogeneity of Con A, if any. It can therefore be concluded that the retention of Con A on CM-Sephadex is due to both charge- and sugar-specific interactions. However, the protein seems to bind preferentially to the ion-exchange group, which may be due to the capacity of the ion exchanger.

Further experiments with CM-Sephadex showed that Con A was retained on the matrix at pH 8.3, although the retention was relatively less (Table I). This retention could be improved by the presence of glucose, probably owing to the increased basicity of the protein. We expected that at pH 8.3 Con A would be retained mainly by affinity interactions. However, it was found that low salt with glucose was unable to bring about significant elution of the protein, whereas high salt provided a better eluent. This may be a result of the weaker affinity interactions and ionic forces at pH 8.3 in comparison with those existing at pH 5.0. The observation that the protein loaded in the presence of glucose could not be desorbed by high salt cannot be explained satisfactorily. It should also be mentioned that in all the experiments 100% recovery of the protein was not observed, which may be due to the poor sensitivity of the method used.

In the above study, Con A was used as a model representing those proteins which bind to dextran. It can be concluded that in such cases glucose–sodium chloride would be a good eluting agent.

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