METAL CHELATE AFFINITY CHROMATOGRAPHY OF THE *DOLICHOS BIFLORUS* SEED LECTIN AND ITS SUBUNITS

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Received 8 June 1981

1. Introduction

Metal chelate affinity chromatography was first introduced by Porath and coworkers in 1975 [1]. This method is based on the principle that a protein capable of binding divalent cations may interact with these ions immobilized on chelate gels. Agarose gels containing iminodiacetic acid, a chelate forming ligand, have been used to fractionate human serum proteins [1], isolate lactoferrin from human milk [2], purify α_2-SH glycoprotein [3], interferon [4,5], granule proteins from granulocytes [6] and α_2-macroglobulin [7]. In all these reports Cu^{2+} or Zn^{2+} were chelated to the agarose gel used in the purification procedures.

The *Dolichos biflorus* seed lectin, which consists of apparently equal amounts of 2 types of subunits (I,II) [8] has been shown to bind ~4 Ca^{2+}/native tetrameric molecule [9]. However the distribution of these ions between the subunits is unknown. Here, we report the binding properties of the native lectin as well as its isolated subunits to a Ca^{2+} containing affinity column.

2. Materials and methods

2.1. *Isolation of the seed lectin and its subunits*

The *Dolichos biflorus* lectin was isolated by affinity chromatography on polyleucyl hog blood group A + H substance as in [10,11]. The lectin was dissociated into its subunits by treatment with urea, and subunits I and II were isolated by ion-exchange chromatography as in [8].

2.2. *Metal chelate affinity chromatography*

The gel, bis-carboxymethyl amino agarose (Sepharose 4B) [1] was kindly supplied by Dr J. Porath, University of Uppsala. The gel was packed in a column (9 × 100 mm) with a total volume of 6 ml and saturated with Ca^{2+} using 5 ml $CaCl_2$ (1 mg/ml). The bed was washed with 0.05 M Tris–acetate buffer (pH 8.2) containing 0.5 M NaCl and the flow rate was adjusted to 0.5 ml/min. The samples were applied in the same buffer as above and, after washing the column it was eluted with 10 mM EDTA in the same buffer.

3. Results and discussion

The *Dolichos biflorus* seed lectin is a metalloprotein containing Ca^{2+}, Mg^{2+}, Mn^{2+} and Zn^{2+} [9]. The relationship of the metal ion content to the carbohydrate binding activity of the lectin has been demonstrated. Removal of the metal ions results in a loss of carbohydrate binding activity of the lectin; this activity can be fully restored by remetallization with only Ca^{2+} [9]. Fig.1 shows that the native lectin binds to the metal chelate affinity column loaded with Ca^{2+} and is eluted with 10 mM EDTA. Other experiments showed that 0.1 M glycine–HCl buffer (pH 2.2) was incapable of eluting the bound protein.

The native lectin is a tetramer composed of apparently equal amounts of 2 types of subunits (I,II) [8]. It contains 2 carbohydrate binding sites/molecule and subunit I may be responsible for the carbohydrate binding activity of the lectin [12]. When isolated subunits I and II were chromatographed on the metal chelate affinity column they showed a distinct difference in their chromatographic properties (fig.2). Subunit I, which has carbohydrate binding activity [12], bound to the immobilized Ca^{2+} whereas subunit II

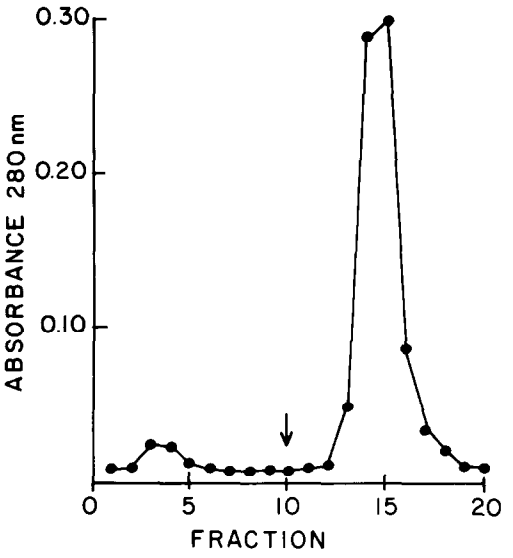

Fig.1. Metal chelate affinity chromatography of the native *Dolichos biflorus* seed lectin on a Ca^{2+}-containing gel. The lectin was applied to the column in a 0.05 M Tris–acetate buffer (pH 8.2), containing 0.5 M NaCl. The arrow indicates the point at which the column was eluted with 10 mM EDTA in the same buffer.

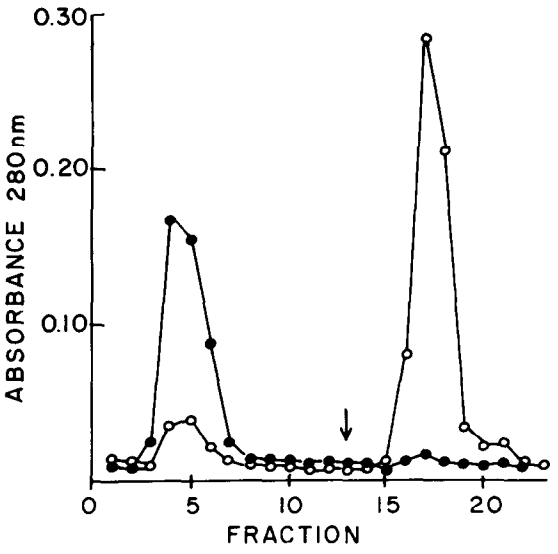

Fig.2. Metal chelate affinity chromatography of subunits I and II from the *Dolichos biflorus* seed lectin. Each subunit was applied individually to the column and the column profiles are superimposed on one another. The arrow indicates the point at which elution was begun with 10 mM EDTA: (○) subunit I; (●) II.

did not bind to the column. These subunits have identical NH_2-terminal amino acid sequences [13], similar amino acid and carbohydrate compositions and give reactions of identity in immunodiffusion against antisera to the lectin or its isolated subunits [8]. The only structural difference in the subunits so far detected is at their COOH-terminal ends where subunit I appears to be slightly longer than subunit II [8].

Attempts to determine the metal ion compositions of the native individual subunits have been unsuccessful because of the displacement of metal ions during the denaturation step required for subunit separation. This finding of a difference in the abilities of the 2 subunits to bind to the Ca^{2+} chelates indicates that they may also differ with respect to their affinities for Ca^{2+}. Since the carbohydrate binding activity of the native lectin is dependent upon the presence of metal ions [9], the differences in carbohydrate binding found between the 2 subunits [12] may be related to their different affinity for Ca^{2+}.

This study suggests a new application of metal chelate affinity chromatography as a rapid means of assessing metal ion-binding abilities of isolated protomers. Such information may be useful in determining possible sub-distribution of metal ions among subunits of a protein.

Acknowledgements

The metal chelate affinity gel was a personal gift from Dr J. Porath which is gratefully acknowledged. This work was supported by United States Public Health Service grant GM 21882 from the National Institutes of Health and United States Department of Agriculture grant SEA 5901-0-0242.

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