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## Facile purification of a β-glucan hydrolase by chromatography on DEAE-cellulose

Ion-exchange chromatography on substituted celluloses and, to a lesser extent, dextrans has become a common procedure for the purification of proteins[1–3]. This method has the particular advantage of being suitable for handling large quantities of material and, usually, the ability to recover the purified protein in high yield. One disadvantage lies in the fact that purification by this procedure generally results in only partial separation of the components of mixtures so that repeated chromatography or subsequent purification by other methods is usually necessary.

During attempts to develop methods for the large-scale purification of low-molecular-weight β-D-glucan hydrolases the behavior of some enzymes of this type on DEAE-cellulose has been examined. In the present communication the use of this material to obtain a single-step purification to homogeneity of a β-1,4-glucanase (β-1,4-glucan 4-glucanohydrolase, E.C. 3.2.1.4) is described. This takes advantage of an extremely strong, but reversible, binding of the enzyme to the ion exchanger and results in elution of the enzyme at exceptionally high concentrations of salt, long after all other proteins displaced by a salt gradient have left the column.

*Experimental*

A crude enzyme extract from the extracellular culture filtrate of a species of Cytophaga (available from BDH Ltd., Poole, Dorset, Great Britain, catalog No. 39072) dissolved in citrate–phosphate buffer (25 m*M*, pH 8.0) and centrifuged (50,000 *g*, 45 min) to remove insoluble material, was applied to a column (2.8 × 9 cm) of DEAE-cellulose (Whatman microgranular DE-52) equilibrated with the same buffer. Protein was displaced from the exchanger by elution with a salt gradient (0–1.0 *M* sodium chloride), followed by further elution with 1.0 *M* sodium chloride in the same buffer. The protein distribution is shown in Fig. 1. Activity towards soluble CM-cellulose (Cellofas B, medium viscosity, Imperial Chemical Industries, Ltd.) measured by increase in reducing power[4,5] is also shown in this figure. It is seen that high concentrations of sodium chloride are required for elution of the enzymic activity, this being eluted as a broad peak and associated with amounts of protein below the limit of the measurement procedure employed[6].

In the experiment shown, a solution containing 75 I.U. of β-1,4-glucanase (425 mg protein) was applied to the DEAE-cellulose column. 68 I.U. of activity was present in the effluent (91% recovery). The specific activity increased from 0.176 to 4.0 during the process, a purification of 23-fold. After concentration of the active fractions in an ultrafiltration cell fitted with a UM-2 Diaflo membrane (Amicon Corp., Lexington, Mass., U.S.A.) 17 mg of purified enzyme was obtained, which was homogeneous as judged by analytical polyacrylamide gel electrophoresis[7,8].

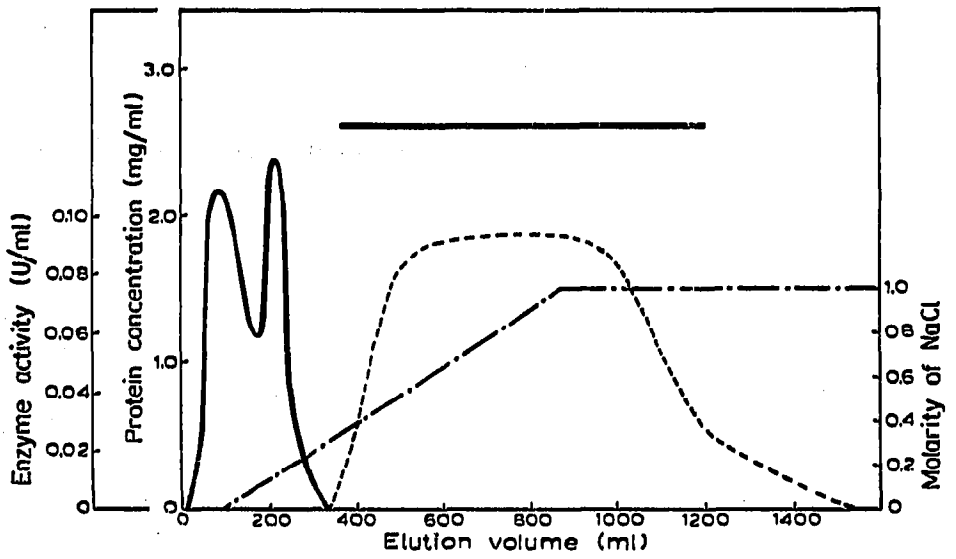


Fig. 1. Chromatography of Cytophaga enzyme preparation on DEAE-cellulose. Elution was performed with a gradient of sodium chloride (· — · — ·) 0–1.0 *M* over 750 ml in 25 m*M* citrate–phosphate buffer pH 8.0, followed by further elution with 1.0 *M* NaCl in the same buffer. Fractions of volume 11.0 ml were collected automatically. Protein in the fractions (——) was determined by the method of LOWRY *et al.* [6]. β-Glucan hydrolase activity (- - - - -) was measured using CM-cellulose as substrate. Fractions indicated by the heavy bar were combined as purified β-glucanase and concentrated.

*Discussion*

The procedure described has the particular advantage of being simple and giving a high recovery of activity. There are few other reports of single-step purifications using this medium. While it was initially thought that the powerful binding is due to the affinity of the enzyme for the matrix of the ion exchanger, such as has already been observed in the case of other closely-related enzymes[9], this is now considered unlikely. The enzyme exhibits similar behavior during chromatography on DEAE-Sephadex A-50 under the same conditions. The phenomenon may be the result of the enzyme being unusually acidic. If this is the case then it is unexpected since other enzymes of this type, like lysozyme, are generally basic in nature[10]. However, this problem is expected to be resolved when the results of studies on the composition and structure of the enzyme, currently in progress in collaboration with Prof. B. KEIL, become available.

As an alternative to prolonged washing with high concentrations of salt, the enzymic activity may be eluted using a pH gradient, when it appears in the effluent at about pH 4.4.

We have detected no hydrolysis of the ion exchanger during the process when performed at either 2° or room temperature. This technique promises to be a useful method for obtaining quantities of this glucan hydrolase and possibly other related enzymes. The detailed properties of the enzyme will be described elsewhere.

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