BBA 63478

Chromatography of a halophilic enzyme on hydroxylapatite in 3.4 M sodium chloride

The extremely halophilic bacteria require very high concentrations of NaCl for growth. Most halophilic enzymes studied are irreversibly inactivated in the absence of high concentrations of neutral salts, and this makes their purification and characterization difficult. In our studies of the proteolytic enzymes of *Halobacterium salinarium*[1], we have tried to purify an extracellular protease which this organism excretes into the growth medium. We have found that it is possible to get considerable purification of this enzyme by chromatography on hydroxylapatite. Hydroxylapatite differs from ion exchange in the respect that the adsorption of proteins is rarely affected by the presence of relatively high concentrations of NaCl [2]. The Halobacterium protease is adsorbed even in the presence of 3.4 M NaCl but can be desorbed by increasing the phosphate concentration of the eluting buffer. We have also carried out some experiments with a nonhalophilic protease which show that the introduction of acidic groups in this enzyme markedly affects its chromatographic behaviour on hydroxylapatite.

Halobacterium salinarium Strain 1 M was grown in Medium 73 under conditions described earlier[1], and the cell-free medium containing the protease was concentrated about 20 times by ultrafiltration through Diaflo PM 10 membranes. The concentrated enzyme solution was then applied with the aid of a peristaltic pump to a column (20 cm × 2 cm) with hydroxylapatite, prepared by the method of TISELIUS *et al.*[3], which had been equilibrated with 0.05 M Tris–HCl buffer (pH 8.0) containing 20% (w/v) NaCl. The column was washed with the same buffer until the effluent had negligible absorption at 280 nm. The protease could be eluted with a buffer

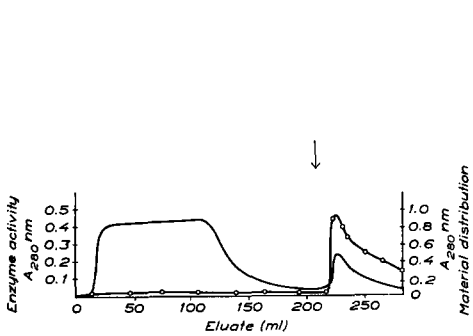

Fig. 1. Chromatography of concentrated cell-free medium of Halobacterium on a column of hydroxylapatite (20 cm × 2 cm). 100 ml concentrate was applied. Elution was performed with 0.05 M Tris–HCl buffer (pH 8.0) containing 20% (w/v) NaCl. Start of elution with a buffer containing 0.05 M potassium phosphate is indicated by the arrow. ———, material distribution; ○—○, protease activity.

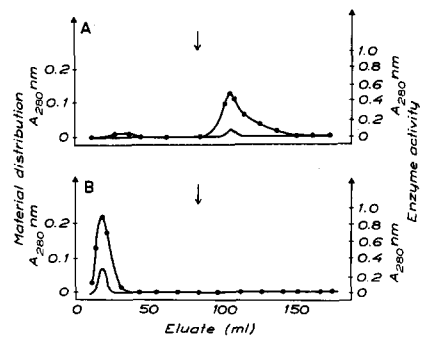

Fig. 2. Chromatography of succinylated (A) and untreated (B) Arthrobacter proteinase. The start of elution with 0.05 M Tris–HCl (pH 8.5) containing 20% NaCl 0.05 M phosphate is indicated by arrows. ———, material distribution; ●—●, enzyme activity.

which, in addition to the above mentioned salts, contained phosphate. Fig. 1 illustrates an experiment in which 0.05 M potassium phosphate was applied and shows that the enzyme was eluted in a single peak. Further increase of the phosphate concentration to 0.5 M eluted a small amount of material which did not contain any proteolytic activity. The specific activity of the enzyme calculated on a protein basis increased about 10 times and the recovery was about 50%.

It has been suggested that the adsorption of proteins to hydroxylapatite depends on their content of acidic groups[4]. This is confirmed by some experiments which we have done with a protease from the nonhalophilic bacterium Arthrobacter. This enzyme is a basic protein[5], and it was not adsorbed on hydroxylapatite under conditions used for the purification of the halophilic enzyme (Fig. 2B). However, after succinylation which only partly inactivated the Arthrobacter protease, it was adsorbed like the Halobacterium proteinase on the hydroxylapatite and could be eluted with 0.05 M phosphate (Fig. 2A). The succinylation was performed by the method of KLOTZ AND KERSZTES-NAGY[6] by adding 40 mg of succinic anhydride with intensive stirring, and during cooling, to a solution of 20 mg protease in 10 ml of water; the pH was kept at 7 by titration with 0.2 M NH_4OH. After 1 h, the solution was dialyzed against 0.05 M Tris–HCl (pH 8.0) containing 20% NaCl, before application on the hydroxylapatite.

Isoelectric focusing experiments in ampholine pH 3–6 showed that the succinylated Arthrobacter protease had an isoelectric point between pH 3.5 and 4.0 as compared with a value of about 8.5 for the untreated enzyme. Due to the rapid inactivation during electrophoresis, our determinations for the halophillic enzyme are less reliable. However, a preparation which had been further purified by gel filtration gave a single protein peak with an isoelectric point between pH 3.5 and 3.8.

The experiments show that it is possible to purify enzymes from extremely halophilic bacteria on hydroxylapatite in the presence of sodium chloride. Furthermore the results support the hypothesis of BERNARDI AND KAWASAKI[4] that the adsorption of proteins to hydroxylapatite depends on their content of carboxyl groups.

Institute of Biochemistry,
University of Uppsala,
Uppsala (Sweden)

PER NORBERG
BENGT V. HOFSTEN

1 P. NORBERG AND B. V. HOFSTEN, *J. Gen. Microbiol.*, 55 (1969) 251.
2 S. HJERTÉN, *Biochim. Biophys. Acta*, 31 (1959) 216.
3 A. TISELIUS, S. HJERTÉN AND Ö. LEVIN, *Arch. Biochem. Biophys.*, 65 (1956) 132.
4 G. BERNARDI AND T. KAWASAKI, *Biochim. Biophys. Acta*, 160 (1968) 301.
5 B. V. HOFSTEN, H. VANKLEY AND D. EAKER, *Biochim. Biophys. Acta*, 110 (1965) 585.
6 J. M. KLOTZ AND S. KERESZTES-NAGY, *Nature*, 195 (1962) 900.

Received June 29th, 1970

Biochim Biophys. Acta, 220 (1970) 132–133