Purification of Pepsin by Gel Filtration

Bertil Gelotte and Anna-Britta Krantz

Research Laboratories, Pharmacia, Uppsala, Sweden

A new separation method called gel filtration has recently been developed [1]. It is an extension of the observations made by Lathe and Ruthven [2] that molecules of different size can be separated, when filtered through a column of starch. In these experiments the cross-linked polysaccharide Sephadex G-50 (manufactured by Pharmacia, Uppsala, Sweden) was used as bed material.

Sephadex consists of small grains, which swell in aqueous solutions. In the swollen state the gel grains are packed to a column in a glass tube. When a solution is filtered through the bed, solutes of large molecular size are prevented from entering the gel phase and will thus move in the aqueous phase surrounding the grains. Low molecular weight solutes, however, penetrate the gel phase and will pass the column in the water inside as well as outside the grains. They will therefore be eluted from the column later than the larger ones.

The method is very useful for desalting colloid solutions, for fractionation of polymer homologues and for group separation of biological extracts [1,3-5]. It can also be used as a step in a purification procedure, which is exemplified by a purification of pepsin.

A crude pepsin (Pepsin 1:10 000 from A/S Orthana, Copenhagen) was used as starting material. The proteolytic activity was tested spectrophotometrically at 280 mμ with hemoglobin as substrate and expressed as activity per mg of nitrogen. The activity of our preparations was compared with that of a twice crystallized pepsin (Worthington Biochemical). The gel filtration experiments were made on a column 7.5 × 49 cm equilibrated with 0.2 M sodium acetate pH 4.9.

100 ml of the acetate buffer containing 37 g of crude pepsin was filtered through the column with the same buffer as eluent, and with an elution rate of 30 ml/min. After partial concentration in vacuum the eluted pepsin was desalted by a second gel filtration on the same column using distilled water as eluent. The saltfree pepsin was then lyophilized.

A good purification and a quantitative recovery was obtained in the filtration experiments. Unfortunately, there was some loss of activity in the concentration steps. Therefore, in another series of experiments, the concentration was made with Sephadex. The pepsin solution was mixed with the dry powder, which takes up water but leaves the protein molecules outside the grains. By filtration with careful suction a concentrated enzyme solution was obtained without any loss of activity.

The experimental results are summarized in Table 1.

Table 1.

Treatment	Yield, %		Pepsin activity	
	Nitrogen	Activity	Conc. in vacuum	Conc. with Sephadex
Orig. solution	100	100	23	23
1st gel filtration	35	99	65	59
Concentration	35	84	55	64
2nd gel filtration	23	84	84	87
Concentration	23	72	72	95
2 × cryst. pepsin			117	

1. Porath, J. and Flodin, P. *Nature* **183** (1959) 1657.
2. Lathe, G. H. and Ruthven, C. R. J. *Biochem J.* **62** (1956) 665.
3. Flodin, P. *In preparation.*
4. Porath, J. *Biochim. et Biophys, Acta. In press.*
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