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Note

In situ chromatographic separation of natural mixtures

Bovine heart proteins

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The process of adsorption chromatography consists essentially of two basic processes: firstly, the adsorption of a mixture of proteins on to a suitable carrier such as an ion-exchange resin or an oxide and, secondly, the elution of individual proteins or groups of proteins by eluents of a given composition, molarity and pH. If a natural mixture of proteins is attached somehow to insoluble particles, which in general need not be by forces of adsorption only, the first step of adsorption chromatography can be avoided. From such mixtures, groups of proteins or even individual proteins can be eluted by choosing a suitable eluent. This technique could be applied to such complex systems as homogenized muscle tissue, or for the elution of protein components from pulverized teeth or bones.

This type of separation, *i.e.*, the elution of various simpler constituents from a naturally occurring mixture, was utilized by Willstätter at the beginning of the century. However, the material (minced muscle) was too compact to permit effective elution and the control of pH and the constituents of eluting solutions were limited. With the increasing availability of centrifuges for the separation of the eluate from undissolved material, this method became used less and less. Today, with effective means of disruption of cell constituents, such as homogenization and ultrasonics, and a wide range of available filtration media, the old method seems to have gained a number of advantages that make it in some respects superior to separation by centrifugation.

The aim of this paper is to illustrate the above principle of *in situ* chromatographic separation with the elution of actomyosin from heart muscle.

EXPERIMENTAL AND RESULTS

Bovine heart muscle (30 g) was homogenized in a blender with distilled water (100 ml) for 10 min. A 10-ml volume of the homogenate, containing approximately 2.3 g of tissue, was placed into a 5 cm I.D. Büchner funnel equipped with Whatman No. 1 filter-paper. Above the filter-paper several layers of soft tissue paper were placed, in order to prevent the clogging of the filter-paper with cell debris. The

homogenate, spread in a thin layer above the tissue paper, was then carefully washed first with distilled water, then with 0.6 *M* potassium chloride solution and finally with 1.0 *M* potassium chloride solution. The eluate was collected below the funnel in 5-ml fractions. From each fraction 0.2 ml was pipetted into 3 ml of physiological saline and the absorbance of this solution was measured at 280 nm (Fig. 1).

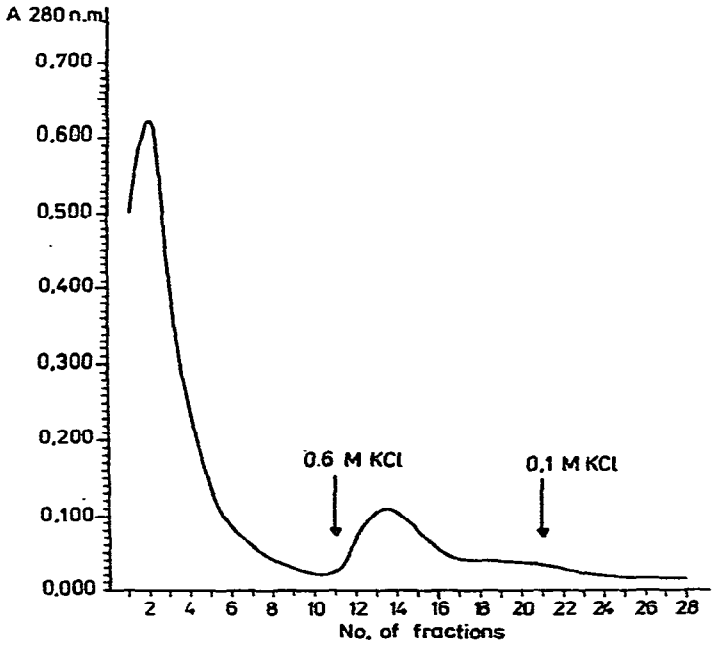

Fig. 1. Protein content of fractions eluted from homogenized bovine heart with distilled water and 0.6 *M* and 1.0 *M* potassium chloride solution.

The first fractions contain residual haemoglobin, myoglobin and other proteins that are soluble in pure water. We considered the elution of these contaminating proteins to be complete after a decrease in the absorbance of the eluted fractions to an approximately constant level (0.01 absorbance unit). Then, by washing the homogenate with 0.6 *M* potassium chloride solution, we eluted the actomyosin completely, again until the absorbance of the eluting solution reached a virtually constant, minimal level. The elution with 1.0 *M* potassium chloride solution yielded no significant amounts of proteins. Of course, by choosing a suitable eluent, such as pyrophosphate–ATP or potassium iodide, it is possible to elute other protein fractions, also.

DISCUSSION

The problem of the separation of proteins by selective elution has recently been discussed in a number of papers[1–3]. Von der Haar[1] described "affinity elution", a method for the specific elution of a given protein from a mixture, adsorbed on a

non-specific adsorbent. He also used[2] a non-selective precipitation of proteins on the surface of agarose gels in a solution of ammonium sulphate at a concentration slightly below that needed for the precipitation of the given proteins in solution. From these "interfacially salted-out" proteins, different groups of proteins are eluted by decreasing the concentration of ammonium sulphate in the eluent. Mizutani and Mizutani[3] described the selective adsorption of proteins on to the surface of controlled-pore glass, from which different fractions of proteins can be eluted by means of various solvents. Jennisen and Heilmeyer[4] eluted selectively some of the skeletal muscle enzymes which were previously adsorbed.

The above methods have two things in common: firstly, the selective adsorption of a mixture of proteins on to a suitable surface, and secondly, the specific elution of certain proteins or their groups by suitable eluting solvents. The method presented here used the second step only for a selective elution of individual proteins or their groups from naturally occurring material containing a number of proteins attached in some way to insoluble matrices.

The proposed method has several advantages over the method usually employed, in which the eluted fractions are separated by centrifugation[5–7]. Firstly, the proposed method could be used as a micro-scale method, permitting the elution of proteins from such small amounts of tissue as, for example, individual rat muscles. Secondly, the elution of contaminating proteins can be made much more complete than by even three-fold or more repeated washing and centrifugation. Thirdly, the elution of proteins that are to be isolated is more complete. In a micro-scale method there is a tendency to elute all available proteins of interest. To achieve comparable results, the elution–centrifugation cycle should be repeated several times. Fourthly, the method is simpler and much less time-consuming than the repeated elution–centrifugation. Fifthly, the published methods describing the isolation of individual proteins from complex homogenates usually deal with the isolation of one protein only. The proposed method can be used for the subsequent isolation of several different proteins from the same material by choosing a suitable solvent.

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