Complete Recovery of Proteins in Concentrators and Some Drawbacks, Revealed by Polyacrylamide Gel Electrophoresis

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Summary. Two types of concentrators, collodion bags and Minicon-B-15-cells have been investigated. The recovery of proteins from collodion bags after the concentration of diluted solutions was complete in about 90% of the experiments. But some collodion bags showed a loss of proteins. This was due partially to a higher membrane cut-off and hence dependent on the molecular weight and to a higher extent due to adsorption of basic proteins checked by a synthetic charge isomere. A test for proper performance of collodion bags is described using carbamylated chymotrypsinogen A or potato proteins. The Minicon-B 15-cells revealed, besides some general loss of proteins, a higher proportion of a decrease of basic proteins.

Analyse von Proteinen; Elektrophorese, Gel; Konzentrierung, Kollodiumhülsen / Minicon-Zellen.

Introduction

Collodion bags have proved to be an excellent tool for the concentration of diluted solutions under reduced pressure. They have been used mainly in clinical laboratories from which no adverse effects have been reported so far. However, cellulose nitrate
membranes may adsorb small amounts of proteins under distinct conditions. This property can be used for quantitative estimations [4, 5].

In our laboratory we have used about 200 collodion bags for concentrating a great number of sometimes extremely diluted fractions obtained by preparative electrophoresis of potato proteins. The bags were re-used for at least 10 times with the same performance. The recovery of proteins was mostly complete, but in a few bags we observed unexpected losses. In this paper we like to stress the fact that most bags did not show losses, but that every bag should be tested before use by the method described. The reason for these losses are not examined. However, the type of loss has been demonstrated.

Recently a new simple type of concentrator-bags, the Minicon® cell was introduced by Amicon. These cells are fitted with a Diaflo® membrane, a new noncellulosic, neutral material, and are recommended for qualitative assay, because nonspecific binding of protein components may occur on the membranes [1]. We have done a few experiments with the Minicon-B-15-cells, the type with the lowest membrane cut-off (15000 MW) and we have compared the results with those obtained with collodion bags.

Materials and Methods
Collodion bags (SM 12300 or S & S UH 100) and the glass apparatus (SM 15304 or S & S 100/2)1 were purchased from Sartorius Membranfilter GmbH, D-34 Göttingen, or from Schleicher & Schüll, D-3354 Dassel, respectively, the Minicon cells from Amicon, N. V., Oosterhout (N. B.), Holland. Chymotrypsinogen A, 6 times crystallized, was obtained from Serva, D-69 Heidelberg, urea and ammonium sulphate, reagent grade, from Merck, D-61 Darmstadt.

Carbamylation of chymotrypsinogen A was done according to Bobb and Hofstee [2] with some modifications: 500 mg of chymotrypsinogen A were dissolved in 25 ml of 8 M urea. The solution was divided into portions of 0.5 ml in 50 small test tubes. These were placed in a boiling waterbath for exactly 2 min (10 samples), 4 and 8 min (20 samples each) and immediately afterwards cooled in an icebath. All samples of each set were combined and subjected to dialysis against a 0.04 M Tris/EDTA-Na/borate buffer, pH 8.2 [9] until the urea was entirely removed. The solution was then cleared at 15 000 g in a centrifuge (Ergirotiyet, PHYWE AG, D-34 Göttingen) and frozen at −20°C in portions of 1 ml. Further purification was not necessary.

Protein Preparation from Potato Tubers. To 100 ml of crude sap [9] 100 ml of a saturated solution of ammonium sulphate were slowly added at 20°C under stirring. The mixture was allowed to stand for 2 h at 4°C. After centrifugation the sediment was dissolved in 10 ml of the above mentioned buffer, pH 8.2 and dialyzed against the same buffer. Finally the preparation was centrifuged and stored in portions of 1 ml at −20°C.

Test of Collodion Bags. The suction vessels of the glass apparatus were filled with 0.03 M Tris/borate buffer, pH 7.9 [9]. Then the collodion bags were inserted according to the directions of the manufacturers. 0.2 ml of the carbamylated chymotrypsinogen A or of the potato proteins were diluted with 12 ml of the same buffer and the solution filled into each bag to be tested. The samples were concentrated at 4°C under reduced pressure of 500 mm Hg to a volume of about 0.05 ml. The concentrated samples were transferred from the bags into graduated test tubes by means of a pipette, fitted with a polyethylene capillary at the tip to avoid damage of the bags. The bags were rinsed with a small amount of buffer and the samples were made up to the original volume. The concentrated samples were compared with the untreated material by gel electrophoresis in slabs 3 mm thick.

Electrophoresis in polyacrylamide slabs was carried out vertically in the apparatus of Stegemann [8]. Chemicals, buffers, gel composition as well as electrophoretic conditions, staining and destaining methods were described by Stegemann et al. [9]. Sodium dodecyl sulphate (SDS) electrophoresis was performed according to Shapiro et al. [7] with minor variations reported by Koenig et al. [3].

Results and Discussion
Carbamylation of Chymotrypsinogen A
Our modified method of carbamylation was very reproducible and yielded a product in which the stepwise carbamylation could be traced by gel electrophoresis. If the samples heated for different times were dialyzed and checked separately by electrophoresis the course of the reaction could be followed (Fig. 1). However, for our experiment it was advantageous to have a product with evenly distributed pI-values. Therefore the samples were mixed in appropriate portions before dialysis. In contrast to Bobb and Hofstee [2] we obtained a considerable part of the carbamylated chymotrypsinogen A already after heating for 2 and 4 min. This might be due to the small volume we used so that the reaction temperature of 100°C was reached instantly. Bobb and Hofstee did not mention the quantities in their experiments.

Experiments with Collodion Bags
In most experiments the protein patterns obtained after diluting and concentrating the protein preparation (see Methods and Materials) were identical with the starting material. But some exceptions were observed in single batches from both manufacturers. Fig. 2, B—D, shows a test in three different batches with potato proteins: In the bags of the first one no proteins were lost in contrast to the third one where only those proteins were regained.