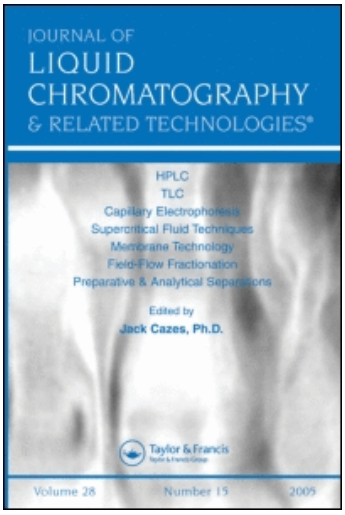

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A Simple Approach to Recovery of Proteins from Polyacrylamide Gel Electrophoresis Rods

R. Bhushan[a]; K. R. N. Reddy[a]
[a] Department of Chemistry, Universityn of Roorkee, Roorkee, India

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A SIMPLE APPROACH TO RECOVERY OF PROTEINS FROM POLYACRYLAMIDE GEL ELECTROPHORESIS RODS

R. BHUSHAN AND K.R.N. REDDY
Department of Chemistry
Universityn of Roorkee
Roorkee-247667 India

Abstract

A simple approach to the recovery of proteins from polyacrylamide gel electrophoresis rods without using any special apparatus is reported. The recovery of proteins has been 92%. The bands from the polyacrylamide gel electrophoresis rods atre cut , homogenised with sodium chloride solution and centrifuged. The supernatent is dialysed and lyophilysed. Polypeptides, subunits and small fragments due to enzymatic hydrolysis can be easily isolated.

Introduction

The recovery of protein from section of polyacrylamide gel as reported in the literature (1) is either by simple diffusion or by means of second stage of electrophoresis which is often reffered as electrophoretic elution. Recovery of more than small amounts of separeted material from the gel is a major difficulty and sets a limit to the usefullness of simple electrophoretic apparatus. Therefore, lengthy experimental procedures and complex and special apparatus are required.

During the amino acid sequence determination of certain plant proteins in our laboratory (2-4) difficulties were encountered in the quantitative recovery of protein, polypeptides, subunits and their smaller fragments from PAGE. The present communication reports a simple method for the isolation and recovery of conarchin (a peanut protein) subunits from the PAGE rods for their subsequent use in amino acid composition and sequence analysis. The method can be applied to several other proteins or polypeptides.

EXPERIMENTAL

Materials

Electrophoresis grade acrylamide and N-N´- methylene bis acrylamide, Tris and TEMED (AR grade) were obtained from Spectrochem, India; SDS, 2-mercaptoethanol and other reagents were from BDH, Coomassie Brilliant Blue R-250 was from Pierce Chemical Co. USA; dialysis membrane was of Spectrum Medical Industries Inc., UV spectrophptometer was Beckman model DU-6.

Isolation of protein and subunits

Theprotein conarchin was isolated from peanuts (*Arachis hypogea* L.). Peanuts were powdered and completely defatted with n- hexane. The defatted powder was extracted with sodium chloride solution (10%) . Arachin (3) and conarchin were fractionally precipitated (5) by sodium sulfate. The precipitated conarchin was separeted and purified as reported (6). The homogenity of conarchin thus obtainrd was established by DEAE-cellulose chromatography and PAGE.
The subunits of conarchin were separeted under varying conditions of buffer pH and acrylamide concentrations. The samples of protein (1mg/ml) pretreated with urea (7M) or SDS (0.1%) were prepatred in presence or absence of 2- mercaptoethanol (0.2M).A constant current of 2mA per tube was applied for 90-120 minutes. The gels were fixed in a mixture of ethanol- acetic acid- water (25:10:65, v/v) for one hour and stained with a solution of coomassie brilliant blue R- 250 (0.1%) in methanol- acetic acid- water (45:10:45, v/v).Gels were destained by acetic acid (7%). The gel tubes of 10.5 cm x 0.5 cm were used and the length of the gel column was 8 cm. In all 120 such tubes were run electrophoretically on which a total of 10 mg protein was applied for subunit isolation.

Recovery of subunits

the various stained bands (seven in all) on the gel rods were carefully cut and were grinded thoroughly in a Tenbroek homogeniser with sodium chloride solution (10%). The contents were first centrifuged and the supernatant liquids were dialysed for 36 hours against double distilled water. These were then lyophilised.

Determination of Protein

The residues left in the lyophiliser were dissolved in phosphate buffer (0.1M, pH 7.0). The absorbancefor each solution was read at 280 nm. Various standard solutions of bovine serum albumin (in the range of 0.05-1.0 mg / ml) were prepared and their absorbance were recorded at 280 nm, and a standard plot between concentration and absorbance was drawn to determine the concentration of solutions of subunits. Besides, protein determination was also carried out by Lowry´s method (7).

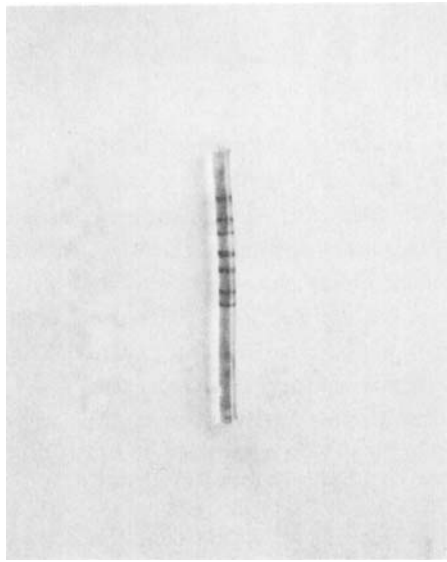

Fig. 1. Electropherogram of conarachin on polyacrylamide gel in the presence of SDS.

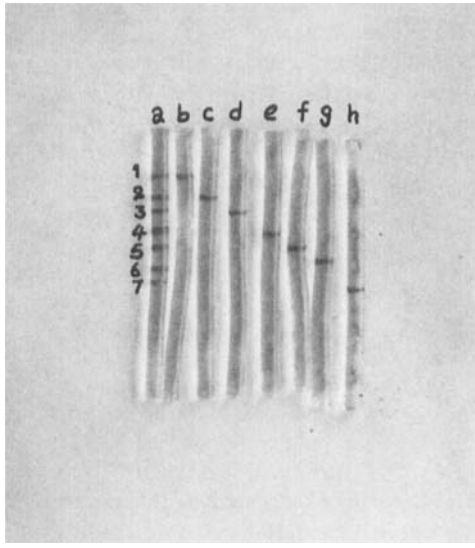

Fig. 2 Electropherograms of conarachin (a) and isolated subunits (b,subunit 1; c,2; d,3; e,4; f,5; g,6; and h,7;) in the presence of SDS on polyacrylamide gel.

Results and Discussion

Seven subunits from protein conarchin were separated using polyacrylamidegel (11.5%).The bands (Fig. 1) were cut and homogenised with sodium chloride solution followed by dialysis and lyophilisation. The protein passed easily into sodium chloride solution as the gel was insoluble in it, and any monomer of acrylamide left was removed during dialysis together with traces of dye. The determination of concentration of each subunit by UV and Lowry's method in the combined extracts of 120 gels accounted for the sum of the concentrations of seven subunits as 9.26mg against 10mg of total protein applied to all these gel rods.Thus therecovery of the protein by the above method was a little more than 92%.The comparison between the seven purified subunits and the native conarchin on PAGE in presence of SDS are shown in Fig. 2.The subunits recovered and purified ,as above, clearly corresponded to those of parent conarchin and were found to be homogenous with respect toelectrophoretic mobility, molecular weight and amino acid composition.

To ascertain the efficiency of the method the same experimental procedure was applied to the recovery of arachin (another pea nutprotein) subunits and to the recovery of small polypeptides of different subunits of arachin obtained by cleavages due to CNBr and trypsin.The yields were more than 90% in each case. It is a successful and simple approach for the recovery of even small amounts of polypeptides from PAGE rods using simple electrophoretic apparatus, and without electrophoretic elution or using a diffusion approach(1). It can safely be recommended for other proteins, however, the recovery may depend on the nature of individual protein.

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References:

1. Gordon,A.H.(1983).in Laboratory Techniques in Biochemistry and Molecular Biology Vol I, Part I, Elsevier- North Holland, Amsterdam.
2. Bhushan,R., Goyal,R.N.and Agarwal,A.(1984). J.Protein Chem., *3*, 395.
3. Bhushan,R.,Goyal,R.N. and Agarwal,A. (1985). Biochem. Intl., *11*, 477.

4. Bhushan,R. and Pant,R. (1987). J. Indian Chem. Soc., *64*, 19.
5. Brohuet,S. and Sandergen,E. (1954). in The Protein, Vol 2,(Eds. Neurath,H. and Bailey,K.), Academic Press, New York.
6. Dechary,J.M., Talluto,K.I., Evans,W.J., Carney,W.B. and Altschul,A.M. (1961). Nature, *190*, 1125.
7. Lowry,O.H., Rosebrough,N.J., Farr,A.L. and Randall,R.J. (1953). J. Biol. Chem., *193*, 265.