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APPLICATION OF POLYACRYLAMIDE GRADIENT GEL ELECTROPHORESIS TO THE STUDY OF MULTIPLE COMPONENTS OF CONCANAVALIN A AND RELATED LECTINS

BJÖRN KARLSTAM
Department of Biomedical Research, Pharmacia AB, Box 181, S-751 04 Uppsala (Sweden)
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SUMMARY

Polyacrylamide gradient gel electrophoresis was used for analysing different Concanavalin (Con A) preparations. The results indicated one predominant band and various minor extra bands which were not revealed by polyacrylamide disc gel electrophoresis. None of the bands disappeared after repeated affinity chromatography on Sephadex, which suggests that the heterogeneity may depend on the complexity of Con A itself. Con A with different metal content also showed the same electrophoretic components.

The major faster migrating component, which is identical in position with the single band in disc gel electrophoresis, represents intact dimers. The minor components are suggested to be different molecular species, which are separated by means of polyacrylamide gradient gel electrophoresis, probably depending on a combined effect of electrophoretic mobility and molecular sieving.

INTRODUCTION

Electrophoresis has been continuously developed by the introduction of improved supporting media, *e.g.*, polyacrylamide. Polyacrylamide gel electrophoresis[1–3] was first applied to serum proteins using a weakly basic buffer and subsequently to the fractionation of basic proteins and peptides by performing the electrophoresis in acidic buffers[4]. For the characterization of jack bean phytoagglutinin Concanavalin A (Con A), the latter conditions were applied and one band was found at pH 4.5 whereas three bands were observed in the presence of 8 *M* urea at the same pH[5]. Further studies on Con A in sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis gave evidence for several components at the subunit level corresponding to the subunit itself and some naturally occurring fragments[6,7]. The occurrence of different electrophoretic components possessing nearly equal hemagglutinating abilities has been demonstrated by isoelectric focusing[8,9].

In an earlier paper it was shown that different batches of highly purified Con A with different metal content were indistinguishable in polyacrylamide gel electrophoresis, all being represented by one band[10].

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In the present study polyacrylamide gradient gel electrophoresis was applied and revealed multiple components of Con A and related lectins. This is a simple, accurate and sensitive method for testing protein homogeneity[11] and the significance of the results is discussed.

EXPERIMENTAL

Materials

Jack bean meal (*Canavalia ensiformis*) was obtained from Sigma (St. Louis, MO, U.S.A.) and Worthington Biochemicals (Freehold, NJ, U.S.A.). Whole beans of *Canavalia rosea* were obtained from Paul Müggenburg (Hamburg, G.F.R.). Commercial Con A was obtained from Calbiochem (Los Angeles, CA, U.S.A.) (grade A, lyophilized in sodium chloride, batch 940022), Miles-Yeda (Rehovot, Israel) (recrystallized twice from saturated sodium chloride solution, batch 79-001), Sigma (grade III, lyophilized in sodium chloride solution, batch 910-5010 No. C-2631) and Pharmacia (Uppsala, Sweden) (lyophilized powder, batches 3059 and 4000). All salt-containing samples were dialysed against distilled water and subsequently against the appropriate electrophoretic buffer. Demetallized Con A was produced by extensive dialysis of the native protein against 0.05 *M* formate buffer (pH 3)[10]. Three different proteins with respect to their metal contents and carbohydrate-binding abilities (A, B and C) were isolated and lectin was isolated from *C. rosea* seeds by specific adsorption on Sephadex according to previous work[10]. Polyacrylamide gradient gel slabs (PAA 4/30) were obtained from Pharmacia.

Polyacrylamide gradient gel electrophoresis

Polyacrylamide gel electrophoresis was carried out in a gradient system with a monomer gradient of 4–30%. A complete system for polyacrylamide gel electrophoresis developed by Pharmacia was used, including pre-formed gel slabs 2.7 mm thick in glass cassettes (82 × 82 × 4.9 mm). Gradients of 8.5–31% were also tried. All slabs were pre-equilibrated with the appropriate electrophoretic buffer. To protein solutions containing 10–30 mg/ml of lectin was added up to 10% of sucrose and 5–15 μl were applied to the sample well using a Hamilton microlitre syringe. Two buffer systems were compared by performing the electrophoresis either in 0.02 *M* acetate buffer (pH 4.0) or according to Reisfeld *et al.*[4]. In the latter instance the gel slabs were equilibrated with 0.06 *M* potassium hydroxide–0.37 *M* acetic acid buffer (pH 4.3) and the electrode compartments were filled with 0.35 *M* β-alanine–0.14 *M* acetic acid buffer (pH 4.5). The running time was 3–5 h at 40 mA.

At the end of the electrophoretic run the gel slabs were stained with 0.5% Amido Black in 7% acetic acid for 10–30 min. Some slabs were stained with Coomassie Brilliant Blue (0.16 mg/ml) in 7.5% acetic acid and heated for 1.5–2 h at 80–85°C.

Slabs stained with Amido Black were electrophoretically destained in 7% acetic acid at 2 A and 36 V for 30 min. Slabs stained with Coomassie Brilliant Blue were destained in water–methanol–acetic acid (6:3:1) for 24 h.

Protein analyses

All protein determinations on purified lectins were estimated from the absorbance at 280 nm using $A_{1\,cm}^{1\%} = 11.4$[12].

Metal analyses

Analyses for manganese and calcium were performed by atomic-absorption spectrometry on a Varian-Techtron Model AA-5 instrument using manganese chloride and calcium chloride as standards.

RESULTS

Homogeneity in polyacrylamide gel electrophoresis is usually taken to be a good indication of the purity of proteins. Using 7.5% polyacrylamide gel electrophoresis (pH 4), all preparations included in this study showed homogeneity and never revealed more than a single band. Samples containing various amounts of metal ions (Mn^{2+} plus Ca^{2+}) have previously been reported to be indistinguishable by polyacrylamide gel electrophoresis, although they could be separated into three subclasses by means of affinity chromatography on Sephadex[10].

In this study polyacrylamide gradient gel electrophoresis was applied, which is a more sophisticated separation method in which proteins are driven through pores of progressively decreasing size until they are brought nearly to a stop according to their size[11]. Fig. 1 shows such an electrophoretic pattern of six different samples of Con A, clearly demonstrating several bands for each species. Irrespective of the metal content of the proteins, all preparations gave one major band and several minor extra bands. The faster migrating predominant component, identical in position with the single

Fig. 1. Polyacrylamide gradient gel electrophoresis of six different samples of Con A containing different amounts of metal. The electrophoresis was performed in a monomer gradient of 4–30% gel in 0.02 *M* sodium acetate buffer (pH 4.0) at 40 mA for 4 h. The plate was stained with 0.5% Amido Black in 7% acetic acid and destained at 2 A and 36 V for 30 min in 7% acetic acid. The samples had the following metal contents in percent from left to right: (1) 0.25 Mn^{2+}, 0.23 Ca^{2+}; (2) 0.16 Mn^{2+}, 0.18 Ca^{2+}; (3) 0.0007 Mn^{2+}, 0.001 Ca^{2+}; (4) 0.003 Mn^{2+}, 0.009 Ca^{2+}; (5) 0.0007 Mn^{2+}, 0.003 Ca^{2+}; (6) 0.17 Mn^{2+}, 0.21 Ca^{2+}.

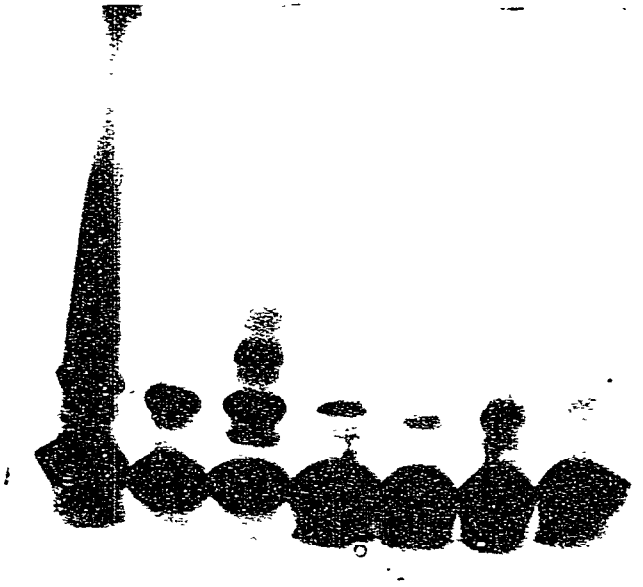

Fig. 2. Polyacrylamide gradient gel electrophoresis of commercial Con A preparations and *C. rosea* lectin. Conditions for electrophoretic run, staining and destaining as in Fig. 1. The samples were as follows from left to right: (1) Calbiochem grade A; (2) Miles-Yeda recrystalized twice; (3) *C. rosea* lectin prepared by affinity chromatography on Sepahdex; (4–6) different Pharmacia preparations; (7) Sigma grade III.

band in disc gel electrophoresis, probably represents an intact dimer of Con A[10]. The minor components are suggested to be different molecular species consisting of different combinations of subunits and naturally occurring fragments. These could be distinguished on the basis of their different molecular weights and net charges. The same electrophoretic pattern was obtained when analysing Con A re-chromatographed on Sephadex, and repeated runs on the same sample of freeze-dried material were reproducible several years after preparation. This suggests good stability and is evidence of a very distinct electrophoretic pattern.

Fig. 2 shows some results for commercial Con A preparations and a related lectin from *C. rosea* isolated in our laboratory. The electrophoresis was carried out under the same conditions as in Fig. 1. Very small differences were found between most samples, except for the batch of Con A from Calbiochem and the *C. rosea* lectin. These two samples contained even more bands than the other species. It was impossible to associate these differences with differences in the biological activity. Ultracentrifugal and gel filtration studies on the *C. rosea* lectin have demonstrated that this lectin has a slightly higher molecular weight than Con A (to be published).

Fig. 3 shows the electrophoretic pattern of the same samples as in Fig. 2. These samples were developed in a modified disc electrophoretic system according to Reisfeld *et al.*[4] using a gradient of 8.5–31 %. The results confirmed the previous observations, *i.e.*, the occurrence of one major and a varying number of minor components.

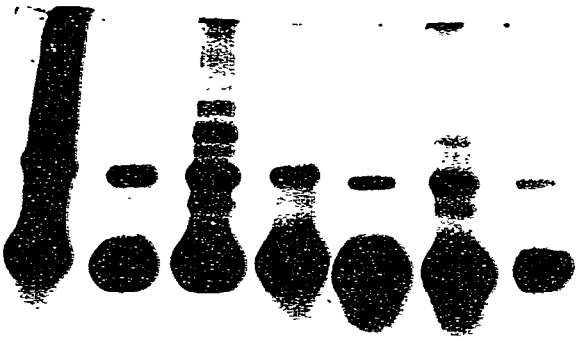

Fig. 3. Polyacrylamide gradient gel electrophoresis of the same samples as in Fig. 2 in a gradient of 8.5–31 %. The electrophoresis was performed in 0.14 *M* acetic acid–0.35 *M* β-alanine buffer (pH 4.5) according to Reisfeld *et al.*[4]. The plate was stained with Coomassie Brilliant Blue (0.16 mg/ml) in 7.5 % acetic acid and heated for 1.5–2 h at 80–85°C. Destaining was carried out in water–methanol–acetic acid (6:3:1) for 24 h.

DISCUSSION

All previously published polyacrylamide gel electrophoretic data on Con A can generally be divided into two main groups. The first includes runs performed in buffers without other additives[9,10,12,13] and the second includes runs performed in buffers in the presence of detergents[5–7]. Studies with highly purified Con A showed a single band in polyacrylamide gel electrophoresis when the pH was below 7[5,10,12]. Further, native and demetallized Con A and mixtures of these two forms are represented by the same band and are indistinguishable in polyacrylamide gel electrophoresis[10,14]. Using SDS polyacrylamide gel electrophoresis Wang *et al.*[6] demonstrated three electrophoretic components, one representing a uniform subunit with a molecular weight of 27,000 and the other two being naturally occurring fragments of this subunit. The occurrence of different electrophoretic components possessing nearly equal hemagglutinating abilities has been demonstrated by isoelectric focusing[8,9] and suggested the term "isophytohemagglutinins"[8].

In this work multiple components were revealed by using polyacrylamide gradient gel electrophoresis. This method has a very high resolution capacity owing to a combination of the electrophoretic mobility and the resistance of the supporting medium as a molecular sieve. The microheterogeneity was very apparent in all samples tested and no detergents were needed for the separation. To confirm that the extra minor bands were not impurities, several samples were re-chromatographed on

Sephadex and re-used in polyacrylamide gradient gel electrophoresis. None of the bands disappeared under this treatment, which suggests that the heterogeneity may depend on the complexity of lectin itself. It would be interesting to study this phenomenon in detail by preparing larger amounts of the extra bands and testing their chemical composition and biological activity.

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