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Short Communications

Fractionation of Barley Globulins on Dextran Gel Columns

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Dextran gels allow the fractionation of macromolecules according to molecular size. It was of interest to re-examine barley globulins by this new technique, because Quensel [1] and Djurtoft [2] have determined the molecular weights of these proteins accurately by ultracentrifugal analysis.

We have earlier [3] reported the fractionation of the water-soluble proteins of barley grain by chromatography on Sephadex G-25, G-50 and G-75 gels at low ionic strength. By increasing the ionic strength the interference of adsorption and ion-exchange can be avoided and a pure molecular sieving effect obtained. Therefore in the present study chromatographies were performed on Sephadex G-100 and G-200 gels in buffered salt solution. The fractions obtained were analysed further by paper and disc electrophoresis.

Preparation of globulins. 200 g of finely ground and acetone-treated barley (Balder) was extracted for 1 h with 400 ml of water [4]. The slurry was centrifuged and the procedure repeated for times to wash out the albumins. The globulins were then extracted for 1 h with 400 ml of 2.5 % NaCl solution [4] containing 10 mM phosphate buffer, pH 7.5. The extraction was performed at + 4°C.

After centrifugation, the globulin solution (about 200 ml) was concentrated by ultrafiltration of filter Lsg-60 (Membranfiltergesellschaft, Göttingen). The protein was collected from the

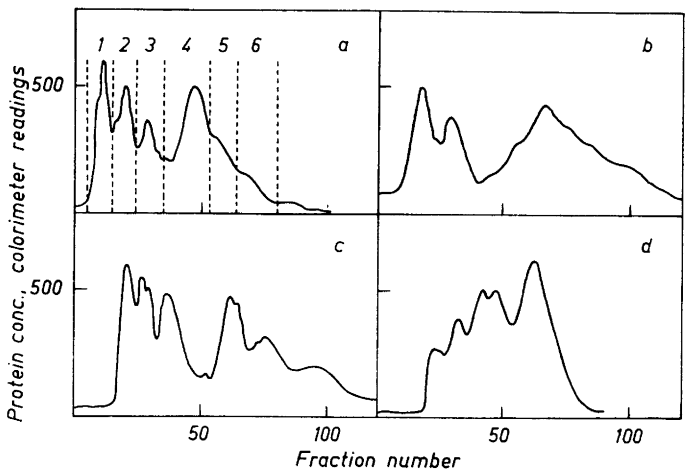

Fig. 1. The elution curves of chromatographies on Sephadexes

a. G-100 chromatography at pH 7.5
b. » » at pH 9.0
c. » » at pH 6.6
d. G-200 » at pH 7.5

The dotted lines in Fig. 1a indicate the groups subjected to electrophoretic analyses.

filter with 5—8 ml of the same buffer solution as was used for the gel filtration.

Gel-filtration. The gels were packed according to Porath [5] into columns (4 × 60 cm) in a 2.5 % NaCl solution containing 10 mM phosphate buffer. In one case the gel filtration was performed in a tris buffer, pH 9.0. The concentrated sample was pipetted on to the column. Elution was performed with this solution at the rate of 1 ml/min. The first 200 ml of effluent was discarded and then 5 ml samples were collected. The fractions obtained by gel filtration were concentrated by ultrafiltration and subjected to electrophoresis.

Protein determination. The protein determinations were performed according to Lowry *et al.*[6], using a Klett-Summerson colorimeter.

Paper electrophoresis. Paper electrophoresis was performed by the technique we have reported earlier [7], but with a higher buffer concentration, *viz.* $\mu = 0.2$ in the globulin analysis.

Disc electrophoresis. Disc electrophoresis was performed at pH 9.0 with the technique and buffer system of Ornstein and Davis as described by Chang *et al.*[8] The small-pore gel contained 11 % acrylamide and 0.2 % methylene bisacrylamide. In the electrophoretic destaining a current of about 5 mA per tube was used. Stronger currents eluted some of the bands; apparently the stain-globulin complexes are not stable at higher temperatures.

Results and discussion. Fig. 1a shows that at pH 7.5 the globulins separate on Sephadex G-100 in 10 groups of components with decreasing molecular weights. There are 6 primary and 4 secondary groups. The first and second primary groups, each include one and the third group two secondary groups which are seen as shoulders in the elution curve. The curves depend on the pH used. Figs. 1b and 1c show clearly that some groups disappear at higher pH's (Fig. 1b) whilst some primary groups separate more distinctly and some secondary groups disappear at lower pH's (Fig. 1 c). This is probably due to the dissociation/association of certain globulins of defined molecular size.

The elution curve of Sephadex G-200 (Fig. 1d) indicates five groups of components. The first four groups are apparently of large molecular size and the last corresponds to the groups 4, 5 and 6 in the G-100 chromatography. This was confirmed by re-chromatography on Sephadex G-100. Chromatographies at more extreme pH's were not performed because of the danger of denaturation.

When the primary G-100 groups were subjected to paper and disc electrophoresis, the heterogeneity of all groups was revealed. Figs. 2 and 3 show the paper and disc electrophoretic patterns of one experiment. A key (Fig. 4) has been added because it

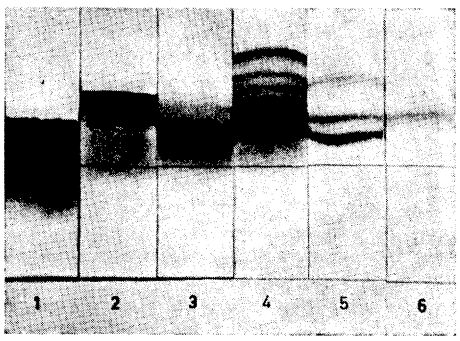

Fig. 2. Paper electrophoresis of groups from chromatography on Sephadex G-100 at pH 7.5 (Fig. 1a)

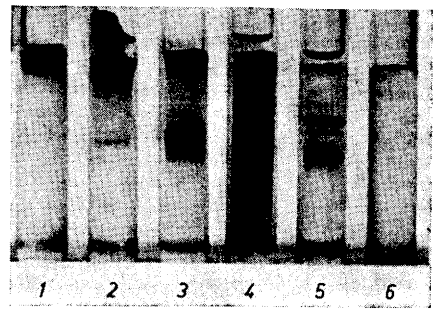

Fig. 3. The disc electrophoretic patterns of groups from Sephadex G-100 chromatography at pH 7.5. The numbers refer to the groups in Fig. 1a.

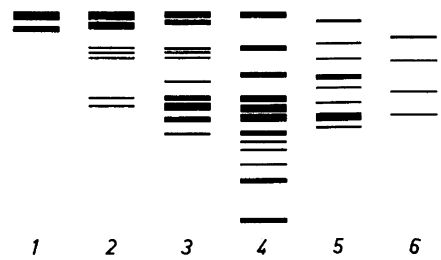

Fig. 4. Drawing of disc electrophoretic patterns of globulin fractions of barley.

is difficult to reproduce bands clearly on a photograph even though these can be seen unmistakably in the original gels.

The first G-100 group does not resolve at all in paper electrophoresis but forms a broad diffuse zone around the starting line. This is probably due to the low ionic strength of the buffer in the electrophoresis. In disc electrophoresis this group indicates two components of low mobility. Large amounts of the globulins remain at the start, probably because these molecules are too large to migrate into the gel, and the ionic strength in the gel is too low, in spite of the relatively high pH, to keep them in solution.

The other groups separate much better in electrophoresis than the first one. Taking into account the overlappings and mobilities of the different groups and fractions, it can be considered that the above mentioned 10 Sephadex groups of different molecular sizes include in all at least 8 paper electrophoretic fractions and 25 fractions separable by disc electrophoresis. The real number of components obtainable with this method lies somewhere between 22 and 43. Disc electrophoretic analyses, however, do not include those globulins which have their iso-electric points above pH 9.0.

A comparison of the Sephadex groups with the fractions obtained by Quensel and Djurtoft will be made.

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Dünnschicht-chromatographische Bestimmung der Askorbinsäure in Kartoffelknollen

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Herrn Professor Dr. *Hans von Euler* zum 90. Geburtstag gewidmet

In der Literatur finden sich divergierende Angaben über den Einfluss ionisierender Strahlung auf den Askorbinsäuregehalt in Kartoffelknollen. Einige Forscher, z.B.[1,2] fanden bei Bestrahlung mit 10 000 rad oder mehr eine Verminderung der Askorbinsäurekonzentration. Schwimmer und Mitarbeitern [3] haben jedoch eine Stunde nach der Bestrahlung eine Erhöhung im Askorbinsäuregehalt von Kartoffelknollen gefunden.

Die Bestimmung von Askorbinsäure in biologischem Material ist schwierig. Die meisten Untersuchungen bedienen sich der Reduktion von 2,6-Dichlorphenol-indophenol. Die unspezifische Reduktion, durch reduzierende Zucker, Sulfhydrylverbindungen etc., muss erst eliminiert werden; das pH muss genau bestimmt sein, in ungepufferten Lösungen kann Askorbinsäure zerstört werden; Puffersubstanzen können jedoch die Geschwindigkeit beeinflussen mit der die Farbintensität des 2,6-Dichlorphenol-indophenol abklingt [4,5]. Um solche Fehlerquellen zu vermeiden muss man sich also eine Eichkurve bekannter Askorbinsäurekonzentrationen unter genau denselben Verhältnissen wie sie in der Probe vorhanden sind, herstellen.

Die vorliegende Arbeit ist ein Versuch die Diskrepanz der Ergebnisse verschiedener Forscher bezüglich des Askorbinsäuregehaltes in gamma-bestrahlten Kartoffeln aufzuklären. Wir haben deshalb Parallelbestimmungen der Askorbinsäure mit zwei verschiedenen Methoden ausgeführt: 1) durch spektrophotometrische Bestimmung der Reduktion von 2,6-Dichlorphenol-indophenol (Modifikation der Methode nach Ponting [6,7]) vgl. Sereno und Mitarbeitern [8]; 2) mit Dünnschicht-Chromatographie.

Die Vorteile der Dünnschicht-Chromatographie brauchen kaum genannt zu werden. Die Methode ist schnell, weshalb eine Oxydation der Askorbinsäure vermieden wird. Die Askorbinsäure kann noch in Mengen von 0,2 μg bestimmt werden.