THE FRACTIONATION OF THE POLYSACCHARIDES OF RICE GRAIN STARCH

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Amylose and amylopectin are the main components of starch. The molecular weight of the linear polysaccharide — amylose — varies from hundreds of thousands to several millions [1-3]. Amylopectin (a branched polysaccharide) is the largest natural polymer. Its molecular weight may reach hundreds of millions [4, 5]. The sedimentation coefficients of amylose and amylopectin also differ considerably[6].

We have made an attempt to separate rice starch into fractions by centrifuging in a linear CsCl density gradient and by gel filtration on Sepharose. For these purposes we used starch for normal rice (Ushtobinskii and Kuban'-3 varieties) and waxy rice (K-4161, Glyutinoznyi 7744).

After centrifuging at 78,840g for only 2 h, the amylose had been separated from the amylopectin almost completely. Characteristics of the fractions obtained after 6-h centrifuging in a CsCl gradient of from 1.0 to 1.4 g/cm^3 were the optical absorption maxima of their complexes with iodine. Because of its high density, the amylopectin deposited on the bottom and the amylose was concentrated in the upper part of the gradient (1.00-1.15 g/cm^3). Several groups of molecules of the linear polysaccharide differing in their buoyant densities and giving complexes with iodine having different absorption maxima were observed. An inhomogeneity in the absorption of the individual sections of the spectrum by amylose—iodine complexes of maize starch has also been shown by gel filtration on Sepharose-2B [7].

On centrifuging in a density gradient of cesium chloride (1.4-1.8 g/cm^3), the amylopectin fractions both of the ordinary and of the waxy rice starch were concentrated in the density zone of 1.65-1.75 g/cm^3. Sedimentograms after centrifuging for 4 and 6 h were identical. In contrast to amylose, the amylopectin—iodine complexes proved to be homogeneous with respect to their optical absorption maxima: 570 and 550 nm for the amylopectins from normal and waxy rice, respectively. The starch from waxy rice consisted of amylopectin alone.

On gel filtration, the starch from ordinary rice was separated into two fractions corresponding to amylopectin and amylose. The starch from the waxy rice issued from the column as one compact peak. Our results are similar to those given in the literature for maize starch [2, 7].

Thus, the separation of amylose and amylopectin according to their densities requires only 2-4 h, while on gel filtration, when the molecules are separated on the basis of their molecular weights, a longer time is required (24-25 h).

EXPERIMENTAL

Preparation and Dissolution of the Starch. Starch was isolated from the grains of hulled rice by the method of Banks et al. [8]. The further procedure was as follows. A suspension of 50 mg of the starch in 0.2 ml of distilled water was treated with 9.8 ml of dimethyl sulfoxide, which is the best solvent for starch grains [1, 6]. The mixture obtained was shaken at +25°C for 5 h. The starch was dissolved in an atmosphere of nitrogen in order to prevent the degradative action of atmospheric oxygen [1, 9, 10]. After this, the solution obtained was treated with 40 ml of 96% ethanol and was centrifuged at 3000g for 15 min. The supernatant was taken off and the precipitate was dissolved in 5 ml of 0.5 N NaOH solution in an atmosphere of nitrogen. Then the solution was neutralized to pH 7.0-7.5 with 1 N HCl and the volume was made up to 10 ml (0.5% solution of starch) or to 50 ml (0.1% solution of starch).

Ultracentrifugation. A linear cesium chloride gradient was prepared with the aid of a gradientator in two variants: 1) from 1.0 to 1.4 g/cm^3; 2) from 1.4 to 1.8 g/cm^3. For cen-

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trifugation in a CsCl gradient (variant 1) 0.2 ml of a 0.5% starch solution was added to a test tube. On centrifuging by variant 2 dry CsCl was previously added to a solution of starch in the same solution to a density of 1.4 g/cm^3, and 0.1 ml was deposited on the gradient.

Centrifuging was carried out in a VAK-601 centrifuge (GDR) at 28,000 rpm (78,840g at the maximum radius) for 2, 4, and 6 h at a temperature of the rotor of 0°C (SW-40 rotor). After the end of centrifugation, the contents of the tubes were fractionated, beginning from the bottom, in 0.17-ml portions. The slope of the gradient was checked by determining the refractive index of the CsCl solution in the individual tubes by means of a RL-1 refractometer (Poland). To each fraction was added 2 ml of distilled water and 0.06 ml of a solution of iodine (2 g of iodine and 20 g of potassium iodide in one liter of water). In the samples obtained we determined the absorption maxima and the optical density at λ_{max} of the polysaccharide complexes in an SF-16 spectrophotometer.

Gel Filtration. For gel filtration we used Sepharose-2B (Sweden). The column was equilibrated with distilled water. The working volume of the column was 55 × 2.2 cm and the rate of elution 10 ml/h. The amount of 0.1% starch solution deposited on the column was 3 ml and the eluate was collected in 5-ml fractions. Then to each tube was added 0.1 ml of the iodine solution and the optical densities at 570 and 670 nm were measured on an SF-16 spectrophotometer.

SUMMARY

It has been shown that on centrifugation in a linear cesium chloride gradient the amylose and amylopectin of rice starch are concentrated in the density zones of 1.00-1.15 g/cm^3 and 1.65-1.76 g/cm^3, respectively.

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