PURIFICATION AND SOME PROPERTIES OF A PROTEIN INHIBITOR OF SUCROSE SYNTHETASE FROM RIPENING WHEAT GRAIN

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A heat-stable protein with a molecular mass of 30 kDa inhibiting the reverse reaction of sucrose synthetase has been isolated from ripening wheat grain and purified to the homogeneous state by the methods of gel filtration on Sephacryl S-200, ion-exchange chromatography on DEAE-cellulose 52, and chromatofocusing in a pH 6-4 gradient. The influence of this protein on K_M for sucrose has been shown which is expressed in a decrease in the affinity of the substrate for the enzyme. An inverse correlation has been found between the activity of sucrose synthetase and the inhibitor in the process of ripening of the wheat grain. On the basis of the results obtained, a hypothesis has been put forward of the participation of the inhibitor protein in the regulation of sucrose synthetase in the ripening of wheat grain.

The sucrose synthetase of cereal grains is one of the key enzymes in carbohydrate metabolism [1]. Recently, great attention has been devoted to the study of the properties and regulation of this enzyme, which is explained by its participation in the synthesis of starch precursors [2]. The maximum activity of sucrose synthetase in ripening wheat grain, corresponds to the maximum synthesis of starch [3] and then, in the phase of waxy ripeness, the activity of the enzyme falls sharply, which is connected with the presence in the grain of an inhibitory protein [4], but the properties of the purified inhibitor have scarcely been studied.

Our task was to develop a method of purifying the inhibitor protein to the homogeneous state and to study some of its properties. The inhibitor protein was isolated by a method proposed by Pontis [4]. The protein obtained was subjected to purification by gel filtration on a column (1.6 × 80 cm) on Sephacryl S-200. The fractions with inhibitory activity were combined and deposited on a column (1.6 × 8.0 cm) of DEAE-cellulose 52. The protein was eluted from the column with 0.08 and 0.2 M solutions of NaCl in 0.025 M Tris-HCl buffer, pH 8.0. The inhibitory protein was eluted by the 0.2 M NaCl. In Pontis's work [4] on the separation of an inhibitor from dormant wheat grain, three fractions with inhibitory activity were obtained. In the purification of the inhibitor from the ripening grain (end of waxy ripeness) using ion-exchange chromatography, we isolated a single fraction with inhibitory activity which, according to the results of SDS-electrophoresis, was distinguished by heterogeneity. The following stage of purification was the method of chromatofocusing. The fraction from the highest peak, which was eluted at pH 4.6-4.7, possessed activity suppressing the reverse reaction of sucrose synthetase and was homogeneous according to the results of SDS electrophoresis.

We studied the action of various amounts of inhibitor on the activity of sucrose synthetase in the forward and reverse reactions. The maximum suppression of the activity of the sucrose synthetase was achieved at a ratio of enzyme to inhibitor of 1:4 (as protein). Here the inhibitor suppressed the reverse reaction – the cleavage of sucrose to fructose and UDPG – while the forward reaction (the synthesis of sucrose) was slightly activated (by 5-10%). The inhibitor proved to be heat-stable. Heating the protein at 90°C for 10 min did not lower its activity. The optimum time of preincubation (at 30°C) of the enzyme with the inhibitor was 40 min. The molecular mass of the inhibitor protein, according to the results of gel filtration on Sephacryl S-200 using low-molecular-mass marker proteins, was 30 kDa.

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We studied the influence of the inhibitor on the K_M value of sucrose. An increase in K_M for sucrose from 50 to 200 mM was observed. Thus, the inhibitor decreases the affinity of the substrate, sucrose, for the enzyme, which shows the concrete nature of inhibition. This type of interaction of the inhibitor with the enzyme permits the assumption of its participation in the regulation of the activity of sucrose synthetase in the process of the ripening of wheat grain. Furthermore, we observed an increase in inhibitory activity as the grain ripened. Thus, for example, on the seventh day after flowering it amounted to 30%, on the 16th day to 60%, and in the phase of waxy ripeness to 80%. These results correlate well with those on the fall in sucrose synthetase activity in the ripening grain.

EXPERIMENTAL

Inhibitory activity was determined on a purified preparation of sucrose synthetase from wheat grain of milky ripeness (16-22 days after flowering). The isolation and purification of the enzyme was carried out by the method described previously [5].

The activity of the sucrose synthetase in the forward reaction was determined in an incubation mixture with the following composition (μmole): Tris-HCl buffer, pH 7.5 – 30; UDPG – 0.5; fructose – 2.0; enzyme preparation – 100 μl (5-7 μg of protein). The reaction product, sucrose, was determined by Roe's method [6]. The activity of the enzyme in the reverse reaction was determined in an incubation mixture including (μmole): citrate buffer, pH 6.4 – 2.0; UDP – 0.5; sucrose – 5.0; enzyme preparation – 100 μl (5-7 μg of protein). The reaction product, fructose, was determined by the Nelson–Somogyi method [7].

Electrophoresis in PAAG in the presence of Na-DDS was carried out by the method proposed by Laemmli [8]. The marker proteins used were phosphorylase B – 94,000; bovine serum albumin – 67,000; ovalbumin – 43,000; carboanhydrase – 30,000; soybean trypsin inhibitor – 20,000; and α-lactoalbumin –14,000 Da (Pharmacia).

Chromatofocusing was performed with the use of the ion-exchange resin PBE 94 and polybuffer 74 (Pharmacia) in a pH gradient of 4.0-6.2 using as the starting buffer 0.02 M histidine-HCl, pH 6.2.

The molecular mass of the inhibitor was determined by gel filtration on a column (1.6 × 80 cm) of Sephacryl-200 using as marker proteins ovalbumin – 45,000; soybean trypsin inhibitor – 20,000; myoglobin – 17,800; and cytochrome C – 14,000 Da. Protein was determined by Lowry's method [9].

SUMMARY

An inhibitor protein suppressing the reverse reaction of sucrose synthetase has been isolated from ripening wheat grain and purified to the homogeneous state and it has been characterized and some of its properties have been determined.

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