STUDIES ON SUCROSE SYNTHETASE
I. EFFECT OF TRYPSIN ON THE CLEAVAGE ACTIVITY

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1. Introduction

During the last years several authors have suggested that the synthesis and cleavage of sucrose catalyzed by sucrose synthetase (UDP-glucose:D-fructose 2-glucosyltransferase, EC 2.4.1.13) may be more complex than a simple reversible reaction [1–3].

Thus, the enzyme exhibits differences for both reactions regarding pH optima [1, 4–6], activation by mercaptoethanol [6] and by metal ions [6], buffers [1, 2], and temperature [2]. The kinetic characteristics of the reaction in the cleavage direction are also very complex [7]. Attempts to resolve the sucrose cleavage and synthetic activities and to subdivide the enzyme into catalytically active units have not been successful [6, 8].

Recently evidence has been reported showing that in Jerusalem artichoke (*Helianthus tuberosus*) sucrose synthetase could be resolved in two forms differing in pI and presenting different kinetic constants [9, 10]. This paper reports further work with one of these forms, showing that by submitting sucrose synthetase to the proteolytic action of trypsin it is possible to obtain an enzyme practically devoid of cleavage activity. Similar experiments carried out with sucrose synthetase from other plants gave the same results.

2. Materials and methods

Sucrose synthetase from Jerusalem artichoke tubers was prepared according to Wolosiuk and Pontis [9]. Peaks A and B were rerun in a preparative isoelectrofocusing column (LKB 440 ml column). Peak A, pI 5.85 used throughout this study, gave only one band by gel electrofocusing.

Ammonium sulphate purified sucrose synthetase from mung beans (*Phaseolus aureus*) and peas (*Pisum sativum*) was prepared by a procedure similar to the one used for Jerusalem artichoke tubers.

For the proteolytic reactions, 0.2 ml of enzyme (2 mg protein) was incubated with 0.2 ml of 200 mM HEPES buffer pH 7.1 containing 0.16 mg trypsin and incubated at 30°. At the times indicated (see fig. 1) aliquots were withdrawn and sucrose synthetase activity was assayed in the presence of 0.5 mg of soybean trypsin inhibitor.

Sucrose synthetase was determined by the methods already described by this laboratory [9, 11].

Enzyme activity: i) Synthesis: one unit of enzyme is defined as the amount which catalyzes the formation of 1 nmole sucrose per min at pH 8. ii) Cleavage: one unit of enzyme is defined as the amount which catalyzes the formation of 1 nmole fructose or UDP-glucose per min at pH 6.7.

3. Results and discussion

The effect of trypsin on the activities of sucrose synthetase which catalyzes the synthesis and cleavage of sucrose is shown in fig. 1. It can be seen that there is a clear difference in the rate of inactivation for both activities. After 15 min 70% of the synthetic activity remains, while only 30% of the cleavage activity can be measured.

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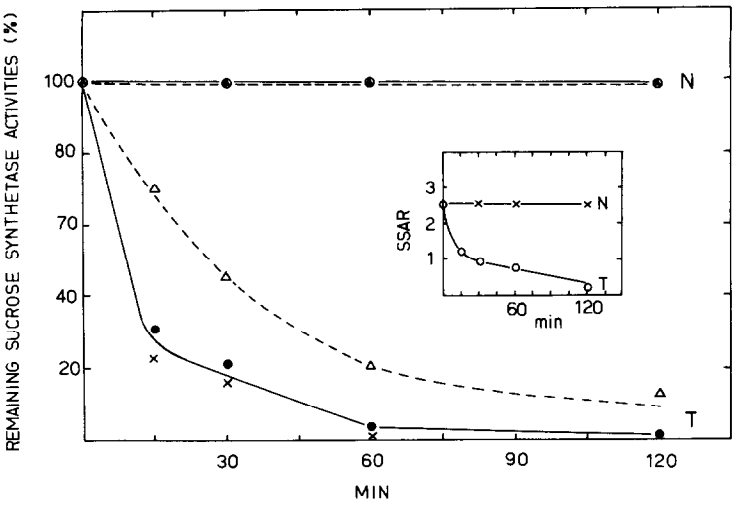

Fig. 1. Proteolytic action of trypsin on sucrose synthetase. Activities of the enzyme at zero time taken as 100%. Procedure described under Materials and methods. N, native enzyme: ▲, synthetic activity (sucrose formed); ○, cleavage activity (fructose formed); T, trypsin treated enzyme: △, synthetic activity (sucrose formed); ●, ×, cleavage activity (fructose and UDP-glucose formed, respectively).

In order to follow the modification of the enzyme activities, we have defined the sucrose synthetase activity ratio (SSAR) as: *activity in the cleavage direction/ activity in the synthetic direction.* SSAR values above 1 indicate that the enzyme catalyzes sucrose cleavage much more rapidly than sucrose synthesis. Similarly, values below 1 indicate the reverse situation. The inset of fig. 1 shows the changes in SSAR owing to trypsin action, indicating the partial disappearance of the cleavage activity. Moreover, gel filtration of a partial tryptic digest shows the appearance of a peak running behind the position of the native enzyme, with a strongly diminished cleavage activity (fig. 2).

No difference in the rate of inactivation for the forward or reverse reactions were observed when sucrose synthetase was incubated with papain or chymotrypsin. This seems to indicate that in order to produce a change in SSAR, the proteolytic action must be very specific.

On the other hand, similar results to those obtained with the Jerusalem artichoke enzyme were obtained when ammonium sulphate purified sucrose synthetase from mung beans and peas was submitted to the proteolytic action of trypsin (table 1).

While the interpretation of the data presented in connection with the mechanism of the synthesis and cleavage of sucrose is at present difficult, several factors have emerged. The effect of trypsin on the sucrose

Table 1

Trypsin effect on sucrose synthetase from various sources.

Plant	Enzyme	SSAR
Jerusalem artichoke tubers	Native	2.60
	Trypsin treated*	0.93
Mung beans, seedlings 5 days old	Native	0.74
	Trypsin treated*	0.42
Peas, fresh, immature seeds	Native	1.03
	Trypsin treated*	0.31

*15 min incubation.

synthetase seems to be a general one, that is, it is independent of the enzyme source. The fact that the trypsin treated enzyme retained the synthetase activity, opens various possibilities regarding the native enzyme.

The simplest interpretation is that the action of trypsin modifies the conformation of the active center. On the other hand, it is tempting to visualize sucrose synthetase as a complex of two proteins or of a protein and a modifier. The high molecular weight reported for the enzyme [8] substantiates these possibilities.

No physiological implication of the results pres-

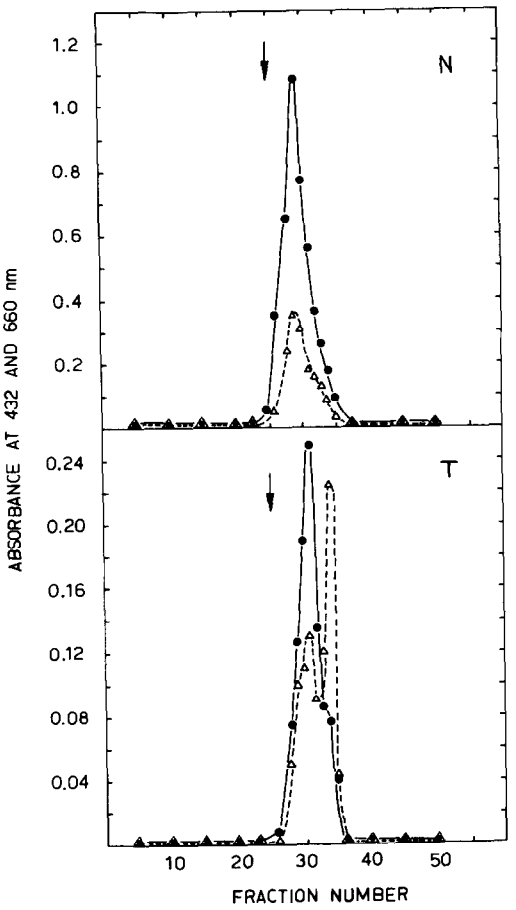

Fig. 2. Gel filtration of native and trypsin treated sucrose synthetase in a Bio-Gel P-300 column 2 × 45 cm, equilibrated with 50 mM Tris buffer pH 7.4, containing 1 mM EDTA. Flow rate: 0.08–0.1 ml per min. Fractions 1.15 ml. Upper figure, native enzyme; lower figure, trypsin treated enzyme, 15 min digestion. Equal amounts of enzyme protein (2 mg) were run through the column. The arrow indicated the fraction where blue dextran starts coming out. •, Cleavage activity, fructose formed determined by Somogyi-Nelson method at 660 nm; △, synthetic activity, sucrose formed determined by the thiobarbituric acid method at 432 nm.

ented here can be discussed at present. However, it is significant that different SSAR values are found according to the enzyme source.

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