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ENZYMES CONCERNED WITH SUCROSE SYNTHESIS AND TRANSFORMATIONS IN SEEDS OF MAIZE, BROAD BEAN AND CASTOR BEAN

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Abstract-A comparative study of the activities of sucrose synthetase, sucrose phosphate synthetase, sucrose phosphatase and other enzymes in developing and germinating plant seeds was carried out to gain further information on the pathways involved in sucrose synthesis and transformation. Sucrose phosphatase has not *previously been* detected in seeds. In developing cotyledons of broad bean *(Vicia faba* L.) and developing endosperm of maize (Zeu *mays* L.), tissues in which sucrose conversion to reducing sugars or sugar nucleotides is the predominant reaction involving sucrose, there was high activity of sucrose synthetase and relatively low activities of sucrose phosphate synthetase and sucrose phosphatase. In the cotyledons of germinating broad bean seeds, the scutella of germinating maize seeds and the endosperm of germinating castor bean seeds *(Ricinus communis* L.), tissues in which sucrose synthesis is more rapid than sucrose breakdown, there were high activities of sucrose phosphate synthetase and sucrose phosphatase. Sucrose synthetase activity was also high in the scutella of maize and endosperm of castor bean. The activity of invertase was very low in all tissues. The results support the hypothesis suggested by several workers that in some plant tissues sucrose synthetase catalyses the breakdown of sucrose and that sucrose phosphate synthetase and sucrose phosphatase catalyse the synthesis of sucrose via sucrose phosphate. However, sucrose synthesis catalysed by sucrose synthetase cannot be dismissed since tissues also contain this enzyme.

INTRODUCTION

KNOWLEDGE of enzymic synthesis and transformations of sucrose in higher plant tissues has been discussed recently.[1–5] It is clear that biosynthesis of sucrose may occur by two different pathways involving either sucrose synthetase (UDP-glucose: fructose-2-glucosyl transferase, E.C. 2.4.1.13) or sucrose phosphate synthetase (UDP-glucose : D-fructose-6-phosphate-2-glucosyl transferase, E.C. 2.4.1.14) and sucrose phosphatase (sucrose phosphohydrolase). Sucrose breakdown may also occur by two different pathways that involve either hydrolysis by invertase (β-D-fructofuranoside fructohydrolase, E.C. 3.2.1.26) or the formation of UDP-glucose or ADP-glucose and fructose by the reverse reaction of sucrose synthetase. Several workers have suggested that *in vivo* sucrose synthetase is responsible for sucrose breakdown and sucrose phosphate synthetase for sucrose synthesis. The suggestion has been based mainly on a comparison of the properties of the two enzymes since both enzymes are present in most plant tissues in which sucrose breakdown and synthesis occur.

However, seeds contain easily-separable organs in which either sucrose breakdown or synthesis is the predominant reaction depending on the stage of development or germination.

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[4] F. L. MILTHORPE and J. MOORBY, *Annu. Rev. Plant Physiol. 20,* 117 (1969).
[5] J. PREISS and T. KOSUGE, *Annu. Rev. Plant Physiol. 21, 433 (1970).*

During the development of cereal seeds sucrose is converted to starch in the endosperm[6,7] and during germination the starch is hydrolysed to glucose which is converted to sucrose by the scutellum.[8–10] Cotyledons of most leguminous seeds store starch which is converted to sucrose during germination.[2,3] Sucrose is converted to fat in the endosperm of developing castor beans, while during germination fat is converted to sucrose, which is absorbed by the cotyledons and translocated to the embryonic axis.[11,12]

Sucrose synthetase and sucrose phosphate synthetase were first detected in wheat germ.[13,14] In seeds sucrose synthetase has been detected in developing maize and rice endosperm[6,7] in the endosperm of germinating castor bean seeds,[15] in scutella of germinating rice seeds[10] and in germinating broad bean seeds.[16] Sucrose phosphate synthetase has been found in scutella of germinating wheat[8] and rice seeds.[10] Fekete[2,3] determined the activities of both enzymes in the cotyledons of developing and germinating broad bean seeds.

This paper reports a comparative study of activities of enzymes concerned with sucrose metabolism in seeds of maize, broad bean and castor bean to investigate the roles of the enzymes *in vivo.* Sucrose phosphatase, one of the enzymes studied, occurs in many plant tissues[17] but its activity has not previously been determined in seeds.

RESULTS

Activities of Enzymes from Broad Beans

The enzyme activities generally agreed with those found by Fekete[2,3] except that phosphatase activity at pH 5·0 was at least 10 times higher than she reported (Table 1). Sucrose phosphatase activity increased about IO-fold on a fresh weight basis during development of the broad bean cotyledons (Table 1). Fekete[3] reported 10 per cent inhibition of sucrose phosphate synthetase from broad bean cotyledons by 10 mM-sucrose. However, sucrose at concentrations of 38, 76, 95 and 190 mM had no significant effect on the synthesis of sucrose phosphate by extracts from soaked broad beans in the present work.

Activities of Enzymes from Maize and Rice

The results in Figs. 1-3 show that invertase activity at pH 5 was low in both endosperm and scutella of maize seeds at all stages examined. At pH 7·5, lower values were obtained. In the endosperm sucrose synthetase activity was greatest during development of the seeds, whereas in the scutella the activity of this enzyme did not change markedly. Sucrose phosphate synthetase activity was low in the endosperm but in scutella it increased to a maximum at a stage when movement of sugar from endosperm to the developing root and shoot was rapid. High activity of the enzyme was also observed in a preliminary

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[7] T. MURATA, T. MINAMIKAWA and T. AKAZAWA, *Arch. Biochem. Biophys. 113, 34 (1966).*
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[15] C. R. BENEDICT and H. BEEVERS, *Plant Physiol.* 37, 176 (1962).
[16] J. B. PRIDHAM, M. W. WALTER and H. G. J. WORTH, *J. Exptl. Bot. 20, 317 (1969).*
[17] J. S. HAWKER and M. D. HATCH, *Biochem. J. 99, 102* (1966).

TABLE 1. ACTIVITIES OF ENZYMES FROM COTYLEDONS OF BROAD BEAN SEEDS

Enzyme source	Enzyme activity* (Units/g fr. wt.) Cotyledons† Young	Mature	Soaked
Enzyme			
Sucrose synthetase	0.26	0·20	0·06
Sucrose phosphate synthetase	0·15	0·14	0·31
Sucrose phosphatase	2.20	28·20	23·30
Invertase (at pH 5·0)	0003	0005	0004
Invertase (at pH 7.5)	0·07	—	—
Phosphatase (with Fru 6-P at pH 5)	0.22	0·12	0·09
Phosphatase (with UDP at pH 8.1)	0.49	0·20	0.16
Phosphatase (with Fru 6-P at pH 8.1)	0.02	0·18	0.10
Protein (mg/g fr. wt.)	14	37	67

* Assay procedures and enzyme units are described in the Experimental section. All values are means of determinations on three samples.

† Enzymes were extracted from cotyledons of young developing seeds (1·4 cm long), nearly-mature seeds (2.5 cm long) and seeds soaked overnight (2.8 cm long).

experiment at this stage of germination. Peaks of activity of enzymes of the glyoxylate cycle have recently been observed in scutella of germinating maize seeds[18] and a peak of activity of sucrose phosphate synthetase was found in scutella of rice seeds during germination.[10] The activity of sucrose phosphatase compared to other enzymes was high in all

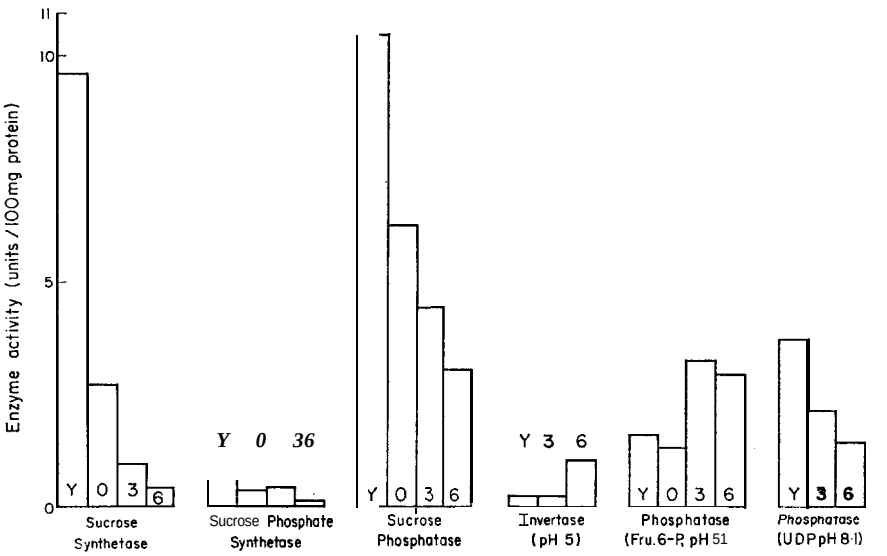

FIG. 1. ACTIVITIES OF ENZYMES EXTRACTED FROM ENDOSPERM OF DEVELOPING MAIZE SEEDS AT THE MID-MILKY STAGE (Y), FROM MATURE DRY SEEDS (0), AND FROM MATURE SEEDS GERMINATED AT 30 FOR 3 AND 6 DAYS (3, 6).

The protein content of the extracts was equivalent to 7, 8·5, 6·3 and 6·3 mg/g fr. wt. respectively for Y, 0, 3 and 6. Values are means of determinations on 2 or 3 samples.

[18] C. P. LONGO and G. P. LONW, *Plant Physiol. 45,249* (1970).

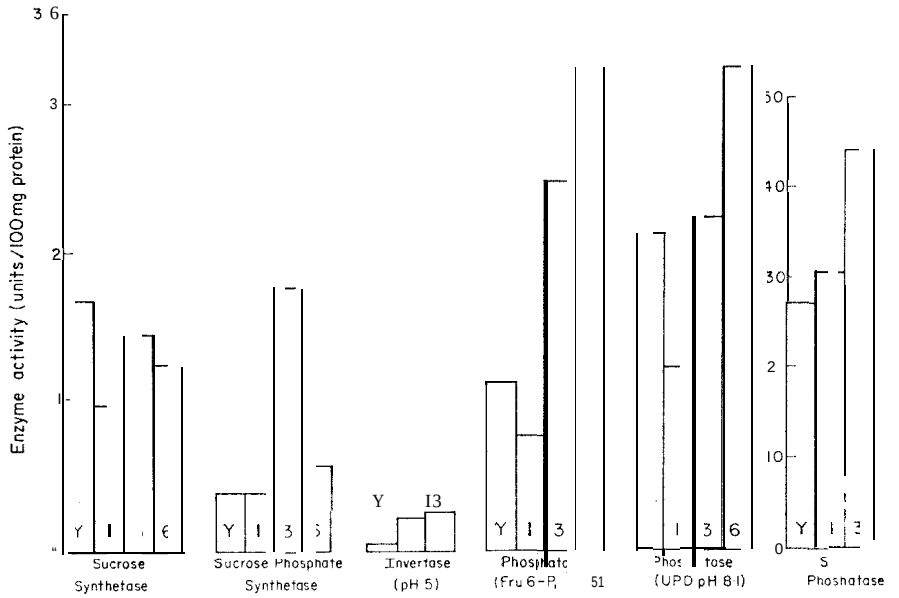

FIG. 2. ACTIVITIES OF ENZYMES EXTRACTED FROM SCUTELLA OF DEVELOPING MAIZE SEEDS AT THE MID-MILKY STAGE (Y), AND FROM MATURE SEEDS GERMINATED AT 30" FOR 1, 3 AND 6 DAYS (1, 3, 6).

The protein content of the extracts was equivalent to 28.6, 39, 27 and 18.7 mg/g fr. wt. respectively for Y, 1, 3 and 6. Values are means of determinations on 2 or 3 samples.

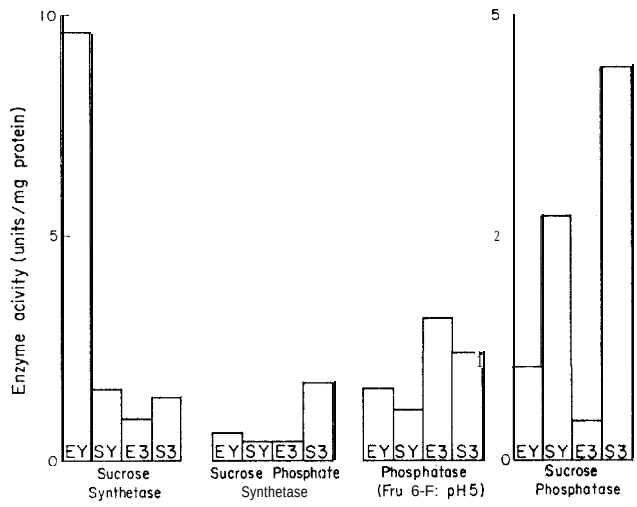

FIG. 3. COMPARISON OF ACTIVITIES OF ENZYMES (FROM FIGS. 1 AND 2) IN ENDOSPERM (E) AND SCUTELLA (S) OF MAIZE SEEDS AT MID-MILKY STAGE (Y) AND ON THE 3rd DAY OF GERMINATION (3).

tissues. However, the activity was 4-10 times higher in the scutella than in the endosperm, while in contrast phosphatase activity (with fructose 6-phosphate as substrate) was higher in the endosperm than in the scutella (Fig. 3).

In dry mature endosperm of rice seeds, sucrose synthetase activity was comparable with that found in maize endosperm (Table 2). The activities of invertase, sucrose phosphate synthetase and sucrose phosphatase were very low (Table 2).

TABLE 2. ACTIVITY OF ENZYMES FROM RICE ENDOSPERM AND ENDOSPERM AND COTYLEDONS OF CASTORBEANSEEDS

Enzyme	Enzyme activity* (Units/100 mg protein) Enzyme source Rice	Castor bean		
		Germinating		Young
	Endosperm	Endosperm	Cotyledon	whole seed
Sucrose synthetase	2·0	1.1	0·8	4 7
Sucrose phosphate synthetase	0·1	1·0	0·6	1·2
Sucrose phosphatase	0·8	9.8	5·4	21·1
Invertase (pH5)	0·2	0·5	2·8	4.7
Phosphatase (with Fru 6-P at pH 5)	4·0	1.9	1.6	2.2
Phosphatase (with UDP at pH 8.1)	4.9	2 4	3·3	4 4
Phosphatase (with Fru 6-P at pH 8.1)	0·9	0·4	0·6	0·7
Protein (mg/g fr. wt.)	8.9	28.1	120	5.2

* Enzymes were extracted from the endosperm of mature dry rice seeds, from young developing castor bean seeds and from castor bean seeds on the 4th day of germination and assayed as described in the Experimental section. The mean fr. wt. of the young castor bean seeds was 0·1 g. Values are means of determinations on two or three samples.

Activities of Enzymes from Castor Bean

The fresh weight of the endosperm of castor bean seeds more than doubled during germination and the protein concentration (per fresh weight) showed a 7-fold decrease. Enzyme activity is therefore expressed on a per seed basis to show absolute increases and decreases in activity in extracts. All the enzymes studied had maximum activity on the 4th day of germination and subsequently the activity decreased, whereas the protein concentrations in extracts from the endosperm decreased earlier (Figs. 4 and 5). Expressed on a protein basis activities of enzymes in germinating castor bean endosperm have previously been found to increase from 2- to 45-fold, isocitrate lyase and phosphatase increasing 45-fold and 5-fold respectively.[19] The present results show increases in specific activity for isocitrate lyase, phosphatase, sucrose synthetase, sucrose phosphate synthetase and sucrose phosphatase of about 40, 5, 22, 9 and 16-fold, respectively.

The specific activity of sucrose phosphatase in extracts from castor bean cotyledons doubled from day 4 to day 6, that of sucrose phosphate synthetase halved and other enzymes remained nearly constant. Cotyledons were not examined prior to day 3 of germination. Of the enzymes studied only invertase and isocitrate lyase had specific activities which consistently differed greatly between cotyledons and endosperm. Invertase activity was at least 5-fold greater in cotyledons and no isocitrate lyase activity was detected in cotyledons.

[19] P. FILNER, J. L. WRAY and J. E. VARNER, *Science* 165, 358 (1969).

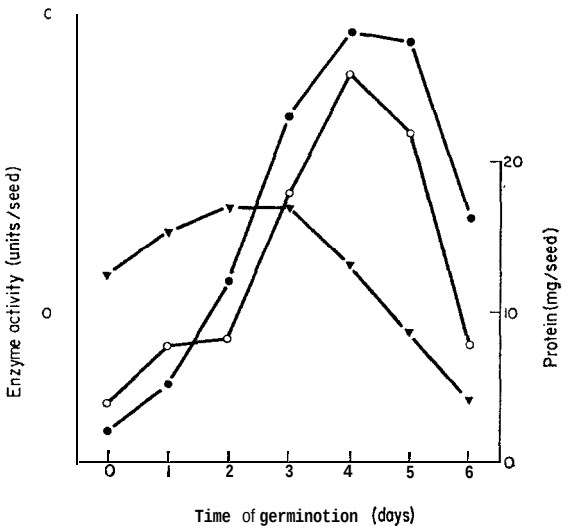

FIG. 4. ACTIVITIES OF SUCROSE SYNTHETASE (●) AND SUCROSE PHOSPHATE SYNTHETASE (0) AND AMOUNT OF PROTEIN (▼) EXTRACTED FROM ENDOSPERM OF GERMINATING CASTOR BEAN SEEDS. ZERO TIME OF GERMINATION REFERS TO SEEDS WHICH HAD BEEN SOAKED OVERNIGHT.

Values are means of determinations on 3 samples. Endosperm fr. wt./seed was 0.19, 0·22, 0·23, 0.31, 0·47, 0·53, 0·43 g for day O-6 respectively.

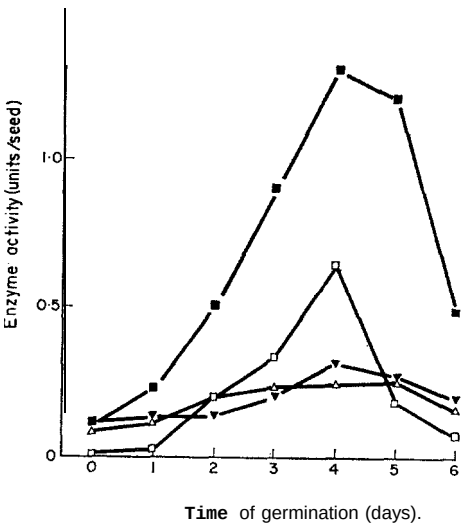

FIG. 5. ACTIVITIES OF SUCROSE PHOSPHATASE (■), ISOCITRATE LYASE (□) PHOSPHATASE WITH FRU 6-P AT pH 5 (△), AND PHOSPHATASE WITH UDP AT pH 8·1 (▲) EXTRACTED FROM ENDOSPERMOF GERMINATING CASTOR BEAN SEEDS.

A comparison of enzyme activities in extracts from endosperm and cotyledons on day 4 of germination is shown in Table 2.

Developing castor bean seeds were not readily available and at the one stage obtained endosperm and embryo were not separated. In these seeds which had accumulated little

protein or fat the ratio of the activity of sucrose synthetase to both sucrose phosphate synthetase and sucrose phosphatase was higher than in germinating seeds (Table 2). Invertase activity was relatively high probably partly due to the presence of cotyledons (Table 2). Expressed on a fresh weight basis the activities of sucrose phosphate synthetase and sucrose phosphatase were 2–4-fold lower in the young seeds than in the endosperm of germinating seeds.

Some Properties of Sucrose Phosphatase from Seeds

The apparent Michaelis constant for sucrose phosphate determined by the procedure of Lineweaver and Burk[20] gave values between $4{\cdot}5$ and $6{\cdot}5 \times 10^{-5}$ M with enzymes extracted from broad bean, maize and castor bean seeds.

Sucrose phosphatases from plants require Mg^{2+} ions for activity. They are inhibited by some di- and tri-saccharides and the amount of inhibition by the different sugars varies depending on the species from which the enzyme is extracted.[21] EDTA completely inhibited the hydrolysis of sucrose phosphate by enzyme preparations from broad bean, maize and castor bean (Table 3). Inhibition by both sucrose and maltose was greater than

TABLE 3. EFFECT OF EDTA AND SUGARS ON THE HYDROLYSIS OF SUCROSE PHOSPHATE BY ENZYMES FROM PLANTS

	Source of enzyme				
Compound	Grape.	Maize	Broad bean	Castor bean	sugar cane
	Inhibitions (%)*				
EDTA	97	100	100	100	100
sucrose	22	73	82	74	74
Maltose	79	85	89	90	45
Melezitose	22	48	69	51	86
Turanose	15	26	50	40	7
Glucose		0	0	0	0
Fructose	8	0	0	0	0

* Values are percentage inhibition by EDTA (30 mM) and sugars (100 mM) of the hydrolysis of 71 μM-(Fructosyl-^{14}C) sucrose phosphate. The values for grape and sugarcane are included for comparison and are taken from Hawker.[21,33]

the inhibition by melezitose, a pattern different to that observed from sucrose phosphatase from either grape berries or sugar-cane (Table 3). Sucrose had no effect on the hydrolysis by enzyme preparations of fructose 6-phosphate at pH 5. The above results indicate the presence of specific sucrose phosphatases in the seeds for the reasons given previously[22,23] and allow the quantitative estimation of the activity of sucrose phosphatase in crude seed extracts.

DISCUSSION

Changes in enzyme activities ***in vitro*** do not necessarily indicate change of rates of reactions ***in vivo*** unless the reaction is a rate-limiting step in a metabolic pathway and

[20] H. LINEWEAVER and D. BURK, *J. Am. Chem. Soc.* 56, 658 (1934).
[21] J. S. HAWKER, *Biochem. J. 102,401* (1967).
[22] J. S. HAWKER, *Phytochem. 5,* 1191 (1966).
[23] J. S. HAWKER, *Biochem. J.* 105, 943 *(1967).*

conditions at the site of action of the enzyme remain constant. However, it is generally accepted that when enzyme activities ***in vitro*** approximate rates of reactions measured ***in vivo*** and when these activities are amongst the lowest of enzymes in a metabolic pathway, large changes in enzyme activities are probably indicative of changing rates of reactions ***in vivo.*** The activities of sucrose synthetase and sucrose phosphate synthetase in extracts of the tissues of the seeds examined compare well with rates of sucrose metabolism measured by other workers. With fructose as substrate it was found that sucrose was synthesized at rates between 0·3 and 0·4 μmol/min/g fresh weight by slices of maize scutellum,[24] while the present results show that the activities of sucrose phosphate synthetase varied between 0·1 and 0·5 μmol/min/g and of sucrose synthetase between 0·2 and 0·5 μmol/min/g fresh weight. From the results of Kriedemann and Beevers[12] it can be calculated that the maximum rate of sucrose synthesis in the endosperm of germinating castor bean seeds was about 0·1 μmol/min/seed and in the present work activities between 0·01 and 0·14 for sucrose synthetase and 0·02 and 0·13 μmol/min/seed for sucrose phosphate synthetase were found in extracts of endosperm. Fekete[2,3] and Edelman *et al.*[8] showed that the activities of the two enzymes were lower than many other enzymes concerned with carbohydrate metabolism in broad bean cotyledons and wheat scutella. The above facts suggest that the rates of conversion and synthesis of sucrose, at least in some seed tissues, are dependent on the activities of the sucrose and sucrose phosphate synthetases and therefore changes in activities of these enzymes are relevant when discussing the roles of the enzymes in sucrose metabolism.

During the development of seeds, sucrose which is translocated to the endosperm or storage cotyledons must be converted to glucose and fructose or nucleotide sugars for use in respiration or synthetic processes and at this stage synthesis of sucrose is quantitatively unimportant.[2] The activity of sucrose synthetase was relatively high in such tissues examined in the present work (young developing broad bean cotyledons and castor bean seeds, and maize endosperm) while invertase activity was low, except in young castor bean seeds which was probably due to the presence of cotyledons. In the same tissues the activities of sucrose phosphate synthetase and sucrose phosphatase were relatively low compared to their activities in other tissues. These results support the hypothesis put forward by several workers[1,2,25–27] that sucrose synthetase plays a vital role in converting sucrose to nucleotide sugars and fructose in some plant tissues.

During germination of seeds synthesis of sucrose becomes quantitatively important in broad bean cotyledons, in maize scutella but not in the endosperm, and in castor bean endosperm but probably not in the cotyledons. In broad bean cotyledons sucrose phosphate synthetase activity reached a maximum during germination while sucrose synthetase activity decreased (Table 1 and Fekete[3]). Sucrose phosphatase activity was also high (Table 1). Fekete[3] used the absence of phosphatase and the consequent lack of fructose in broad bean cotyledons during starch breakdown by phosphorylase to support the claim that sucrose synthesis was not catalysed by sucrose synthetase. In contrast, extracts in the present work showed considerable activity with fructose 6-phosphate as substrate (Table 1).

Sucrose phosphate synthetase activity in scutella of maize seeds increased during germination, sucrose phosphatase activity was high and sucrose synthetase activity was com-

[24] T. E. HUMPHREYS and L. A. GARRARD, ***Phytochem. 5, 653 (1966).***
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[26] D. P. DELMER and P. ALBERSHEIM, ***Plant Physiol. 45, 782 (1970).***
[27] ***G.*** A. MACLACHLAN, A. H. DATKO, J. ROLLIT and E. STOKES, ***Phytochem. 9, 1023 (1970).***

parable to the highest activity of sucrose phosphate synthetase (Fig. 2). In castor bean endosperm the activity of all three enzymes increased during germination (Figs. 4 and 5). Thus it can be seen that tissues of seeds which are rapidly synthesizing sucrose have comparatively high activities of both sucrose phosphate synthetase and sucrose phosphatase but only some have high activities of sucrose synthetase. Tissues in which sucrose synthesis is not rapid (young broad bean cotyledons, young castor bean seeds and maize and rice endosperm) have only low activities of either sucrose phosphate synthetase or sucrose phosphatase or both. It seems clear that at least part of the sucrose synthesized involves sucrose phosphate as an intermediate. Whether or not the synthesis of sucrose catalysed by sucrose synthetase is important *in vivo* remains uncertain.

Sucrose synthetase is located in the vascular strands of sugarcane stems[28,29] and it is possibly involved in the loading of sucrose into the phloem or in translocation of sucrose. The presence of conducting tissue in broad bean cotyledons, cereal scutella[30] and castor bean cotyledons,[12] could account for the activity of sucrose synthetase observed in these tissues. However, the same argument cannot be used with castor bean endosperm. Cotyledons of castor bean during germination function mainly to absorb and translocate sucrose to the embryonic axis and it was thought that sucrose synthetase activity might be higher than sucrose phosphate synthetase in these tissues. However high activities of both enzymes and also sucrose phosphatase were present and a possible explanation is that the enzymes catalysing sucrose phosphate synthesis and hydrolysis are required during photosynthesis, which normally occurs in cotyledons within a few days of germination.

Hatch[31] described a specific uridine diphosphatase from plant tissues with a pH optimum of 8·1 and suggested that its role may be to prevent reversal of reactions involving nucleotide sugars and UDP (e.g. sucrose synthetase). At pH 8·1 extracts from seeds hydrolysed UDP at a greater rate than fructose 6-phosphate. However, no consistent pattern of activity was observed in the tissues of the seeds examined to suggest a role for UDP hydrolysis and no attempt was made to determine whether the hydrolysis was due to a specific phosphatase.

In addition to providing evidence on the roles of the two sucrose-synthesizing enzymes in seeds, the present results also demonstrate that the activities of these enzymes can rise and fall markedly in plant tissues within a few days. Studies on the mechanisms controlling activities of the enzymes could lead to a better understanding of control processes in photosynthesis, translocation and storage of carbohydrates.

EXPERIMENTAL

Plant material. Seeds of ***Zea mays*** L., ***Vicia faba*** L., ***Oryzae sativa*** L. and ***Ricinus communis*** L. were used. Developing seeds were obtained from plants grown at ambient temperatures in soil. For germination studies, seeds were soaked in water overnight at room temp. and transferred to moist vermiculite in the dark at 30". At appropriate stages, testas and root and shoot axes were removed and discarded and samples of endosperm, scutella or cotyledons were prepared by dissection.

Preparation of enzyme extracts. Tissue (up to 2·5 g) was ground in a mortar with 5 ml of medium containing Tris-HCl buffer, pH 8·7 (0·5 M), sodium diethyl-dithiocarbamate (20 mM), cysteine-HCl (20 mM) and EDTA (20 mM). All operations were carried out between 0 and 4". The homogenate was centrifuged for 15 min at 20,000 g and 3 ml of the supernatant solution was desalted by passage through a 15 ml column of Sephadex G-25 that had been washed with 5 mM Tris-HCl buffer, pH 7·0. The precipitate in several extractions was shown to contain less than 10% of the total activity of enzymes assayed and subsequently this fraction was not routinely used.

[28] J. S. HAWKER and M. D. HATCH, ***Physiol. Plant.*** 18,444 (1965).
[29] C. R. SLACK, ***Phytochem. 5,397*** (1966).
[30] J. G. SWIFT and T. P. O'BRIEN, ***Austral. J. Bot.*** **18, 45** (1970).
[31] M. D. HATCH, ***Biochem. J. 88,423 (1963).***

Measurement of Enzyme Activities

Enzyme units. All enzymes were assayed at 30". A unit of enzyme is defined as the amount which will break down or synthesize 1 μmol of substrate or product per min at 30". During development seeds accumulate large amounts of storage protein while during germination this protein is mobilized.[32] Specific activities of enzymes under these circumstances often do not give indications of absolute changes in activities. For this reason in the present paper enzyme activities are expressed in terms of fresh weight, number of seeds or protein depending on the tissues being compared. ***Sucrose phosphatase, sucrose synthetase, sucrose phosphate synthetase.*** These enzymes were assayed respectively by measuring the release of labelled sucrose from sucrose phosphate and the incorporation of labelled fructose or fructose 6-phosphate into sucrose and sucrose phosphate.[33] Supernatant (10 μl, after dilution of up to 150-fold) was used for sucrose phosphatase and desalted supernatant was used for the assay of all other enzymes.

Invertase. Reaction mixtures contained enzyme, 5 pmol of (U-^{14}C) sucrose, and 0·5 μmol of sodium acetate buffer, pH 5·0, (or 0·5 μmol sodium phosphate buffer, pH 7.5) in a final volume of 0·11 ml. The reactions were stopped by heating at 100" for 2 min. The percentage hydrolysis of sucrose was calculated after counting the radioactivity in the sucrose, glucose and fructose spots of chromatograms developed in ethyl acetate–pyridine–H_2O (8:2:1 by vol.).

Phosphatuse. Reaction mixtures at pH 5·0 contained enzyme, 6 μmol of fructose 6-phosphate and 50 μmol of sodium acetate buffer, pH 5·0, in a final volume of 0·6 ml. The reactions were stopped by adding 1 ml of 10% $HClO_4$. P_i was determined after removal of precipitated protein.[34] Reaction mixtures at pH 8·1 contained enzyme, 0·5 μmol of UDP or 1 μmol of fructose 6-phosphate, 0·4 μmol of $MgCl_2$, 6 μmol of Tris-HCl buffer, pH 8.1, in a final volume of 0·1 ml. The reactions were stopped by adding 1·2 ml of 8% perchloric acid and P_i was determined as above.

Isocitrate lyase (E.C. 4.1.3.1.). The method of Olson[35] was used.

Protein assay. The method of Lowry ***et al.***[36] ***was*** used, with bovine serum albumin as standard.

Acknowledgement-Technical assistance by Mr. B. J. Michael is gratefully acknowledged.

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