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INFLUENCE OF BORON ON ENZYMATIC REACTIONS ASSOCIATED WITH BIOSYNTHESIS OF SUCROSE [1, 2]

W. M. DUGGER, JR [3], AND T. E. HUMPHREYS

It has been proposed that boron enhances the uptake and transport of sugars in plants (9, 10, 24). The hypothesis that this effect was caused by the formation of a borate-sugar complex which facilitates the absorption and transport of added sugar or photosynthate, however, has not been accepted by all investigators (17, 22).

In addition to the proposed effect of boron on uptake of sugar in plants it has been suggested that the element may influence transport of sugars by inhibiting starch phosphorylase (7). This observation has prompted a more comprehensive investigation of the carbohydrate metabolism of plants as influenced by boron. The phase of this work reported here is concerned with the enzymatic reactions of UDPG and sucrose biosynthesis as recently developed by Leloir and coworkers (4, 14, 20) and others (1, 8, 11, 21, 26, 27, 28), and the effect of boron on these reactions. These reactions are:

I. Uridine triphosphate (UTP) + Glucose-1-phosphate (G-1-P) $\rightleftharpoons$ Uridine diphosphate glucose (UDPG) + pyrophosphate (PP)

II. A. UDPG + fructose $\rightleftharpoons$ Sucrose + Uridine diphosphate (UDP)

B. UDPG + Fructose-6-phosphate (F-6-P) $\rightleftharpoons$ Sucrose-phosphate + UDP

III. UDP + Adenosine triphosphate (ATP) $\rightleftharpoons$ UTP + Adenosine diphosphate (ADP)

MATERIALS AND METHODS

Seven day-old pea seedlings (*Pisum sativum* L., var. Emerald), grown in the dark at 23° C, were harvested and immediately frozen in liquid air. While frozen the seedlings were ground to a powder (hereafter referred to as homogenate), quickly separated into 1 gram samples, and transferred to reaction flasks containing 0.5 ml of 2 M Tris (hyroxymethyl)-aminomethane buffer pH 7.2, 0.4 ml of 0.1 M G-1-P, 0.2 ml of 0.1 M $MgCl_2$, 0.4 ml of 0.02 M UTP, 0.2 ml of 0.1 M ATP, 0.4 ml of 0.1 M fructose-C^{14} (specific activity of 1 μc/mg), 0.1 ml of yeast inorganic pyrophosphatase, and sufficient water or 0.1 M boric acid to make a total volume of 4.2 ml. In the center well of the flask was placed 0.2 ml 20 % KOH. Immediately after adding the ground plant material, the flask was stoppered and placed in a water bath adjusted to 35° C. After an incubation period of 4 hours, 20 ml of boiling 90 % ethanol was added to stop the reaction and the mixture was allowed to simmer on the hotplate for 5 minutes. The suspension was filtered and the residue washed with hot 83 % ethanol. The filtrate was diluted to 50 ml before 10 ml aliquots were removed and concentrated to 3 ml. After clearing the filtrate with the $Ba(OH)_2$ and $ZnSO_4$ method of Nelson (18), it was assayed for sucrose using chemical and chromatographic techniques. The chemical procedures were; A. Resorcinal method of Roe (23) as modified by Cardini, et al (4) to measure sucrose and, B. Specific glucose oxidase method (7, 29) to measure glucose before and after inverting the sucrose. Chromatograms were developed in *n*-propanol-water-NH_4OH (16:3:1) (27) and the

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sugars were located by spraying with *p*-anisidine. The areas on the chromatograms containing the sugars were cut into 2 cm strips and the sugars eluted with water. The sugars were assayed chemically as just described and the products of hydrolysis were chromatographed on paper and the R_f's obtained compared to known glucose and fructose. Radioactivity was determined in a windowless flow counter. Other chromatograms were placed in contact with Eastman no-screen X-ray film for radioautographic detection. In experiments where glucose-C^{14} was used instead of fructose-C^{14}, hexokinase and phosphoglucomutase were also added to the reaction mixture.

Uncleared aliquots of the alcohol solution were concentrated and chromatogramed in ethanol-molar-ammonium acetate (7:3) pH 7.5 (20) to separate nucleotides. These compounds were detected by UV light. Areas showing fluorescence were cut out, eluted, and the eluate assayed for radioactivity. UDPG content was also determined enzymatically with UDPG dehydrogenase.

Pea seeds were ground and extracted with acetone and ether as described by Turner (27). The dried powder was extracted with 0.2 M $NaHCO_3$ for 3 hours at room temperature, and the larger suspended particles were removed by low speed centrifugation. The supernatant was centrifuged at 20,000 G and the precipitate discarded. Cold, neutralized, saturated $(NH_4)_2SO_4$ was added to the supernatant until the mixture was 80 % saturated and the precipitate collected by centrifugation (20 min at 20,000 G). The precipitate was dissolved in 10 ml of water and dialyzed against 0.025 M phosphate buffer, pH 7, for 24 hours. This preparation will be referred to as A.S. preparation. Contrary to the results of Turner (27) activity of the enzymes was not lost by dialyzing longer than 6 hours. This preparation from pea seeds contained UDPG pyrophosphorylase, UDPG transglycosylase (saccharese), UDP kinase, myokinase, and nucleoside monophosphate kinase.

Young sugar cane seedlings, (*Saccharose officinarium* var. CP50–28), grown in soil under greenhouse conditions, were harvested 3 to 4 weeks after the stem bud germinated. The above ground parts were removed from the seed piece, cut into small sections, and immediately frozen in liquid air. The frozen material was ground with a large mortar and pestle and transferred to the reaction flasks for incubation with labeled sugars plus the same addenda that was used with the pea plant homogenate.

When A. S. preparations were to be made, the frozen material was transferred to a square of nylon cloth placed in a funnel. As the material thawed, water was added (1 ml/gm tissue) prior to expressing the fluid from the tissue. The filtrate was centrifuged at 20,000 G and solid $(NH_4)_2SO_4$ slowly added with stirring to 80 % saturation. The pH was maintained at 7 to 7.2 by adding 14 % NH_4OH. The precipitate was separated by centrifugation and dissolved in water. After dialyzing against 0.025 M phosphate buffer, pH 7, the preparation was stored in a deep freeze. UDPG transglycosylase activity, but not UDPG pyrophosphorylase activity was destroyed by freezing and thawing repeatedly.

The reaction mixture used to determine UDPG pyrophosphorylase activity consisted of 0.1 ml of 2 M Tris buffer, pH 7.2, 0.1 ml of 0.1 M G-1-P, 4.5 μmoles of UTP, 0.05 ml of inorganic pyrophosphatase, and 0.05 ml of 0.1 M $MgCl_2$. One-tenth ml of A. S. preparation (0.8–1.6 μg N) was added to start the reaction after the reaction mixture was diluted to 0.9 ml with water or 0.1 M borate, pH 7.2.

UDPG transglycosylase was assayed by adding 0.1 ml of the A. S. preparation (0.2–0.5 mg N) to 0.1 ml of 2 M Tris buffer pH 7.2, 0.1 ml of 0.1 M fructose or F-6-P, 4.5 μmole UDPG, 0.05 ml of 0.1 M $MgCl_2$ and sufficient water or borate solution to make a total volume of 1.0 ml.

UDP kinase activity was determined by adding the A. S. preparation to the reaction mixture containing 0.1 ml of 2 M Tris buffer, pH 7.2, 1 μmole UDP, 0.05 ml of 0.1 M $MgCl_2$, 0.05 ml of 0.1 M ATP, and sufficient water or borate solution to make a total volume of 1.0 ml. Where this reaction was coupled to the UDPG pyrophosphorylase reaction and assayed by the UDPG dehydrogenase method, 0.1 ml of 0.1 M G-1-P was added. If the method of Berg and Joklik (2) was used, G-1-P was not added.

In the coupled reaction of sucrose synthesis from UTP, G-1-P and fructose, these materials were added at the same levels as in the previous assay methods. $MgCl_2$, ATP, and A. S. preparation were also added at the same level as in the kinase studies.

UDP, UDPG, UMP, UTP, Zwischenferment, hexokinase, and glucose-1-phosphate were supplied by Sigma Chemical Co.; ATP was obtained from Schwarz Laboratories. UDPG dehydrogenase was initially purchased from Sigma Chemical Co., but a more purified preparation was later made from fresh calf liver (25). Inorganic pyrophosphatase was prepared from bakers yeast by a modification of the method used by Heppel and Hilmoe (13). Adenylate kinase, 5′-adenylic acid deaminase, and phosphoglucomutase were prepared from rabbit muscle (5, 16, 19).

UDPG pyrophosphorylase assays were conducted in two ways; the first involved reacting UDPG and PP with the A. S. preparation. The G-1-P formed by the reaction could be measured by coupling with the phosphoglucomutase, glucose-6-phosphate dehydrogenase reactions and observing the change in OD_{340} as TPN was reduced (15). The second method involved the oxidation of the produced UDPG to UDP-glucuronic acid by the enzyme UDPG dehydrogenase when the A. S. preparation was incubated with G-1-P and UTP. The change in optical density as DPN was reduced was followed at 340 mμ (25).

UDPG transglycosylase was assayed by measuring the formation of sucrose when UDPG and fructose or fructose-6-phosphate were incubated with the A. S. preparation. UTP and G-1-P were sometimes used as sources of UDPG if the UDPG pyrophosphorylase reaction was coupled to the UDPG transglycosylase

reaction. Sucrose was determined by the method of Roe (23) after fructose was destroyed by boiling 10 minutes in 0.2 *N* NaOH (4). In later experiments sucrose was hydrolyzed with invertase at pH 4.9 and the glucose determined by the specific glucose oxidase method previously reported (7). This method took longer than the resorcinol method but did avoid the disadvantage of color formations from the reactants. Blanks with boiled A. S. preparation or without UDPG gave readings of 0 to 10 on the Klett colorimeter after 24 hours incubation as compared to readings of 50 to 70 for the reagent blanks by the method of Roe. In the results given in the tables of this report sucrose was estimated by this latter method.

UDP kinase was assayed by reacting UDP and ATP and measuring the formation of UDPG in the presence of excess glucose-1-phosphate and inorganic pyrophosphatase. The method of Berg and Joklik (2) was also used to show the presence of UDP kinase. This involved the reactions:

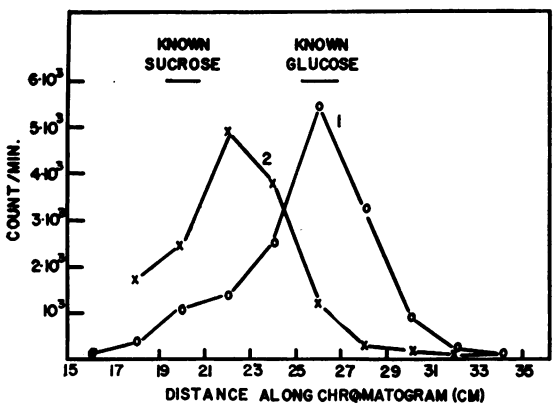
$$UDP + ATP \rightleftharpoons UTP + ADP \text{ (UDP kinase)}$$

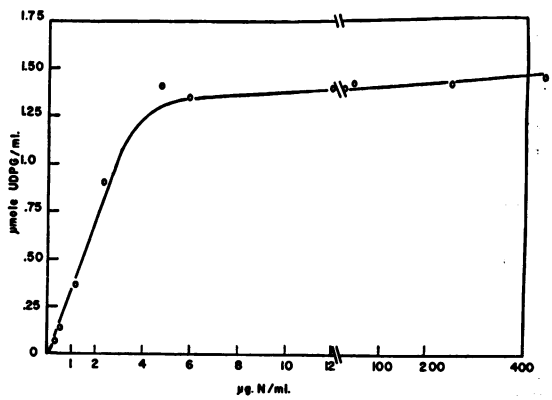
$$2\ ADP \rightleftharpoons ATP + AMP \text{ (adenylate kinase)}$$

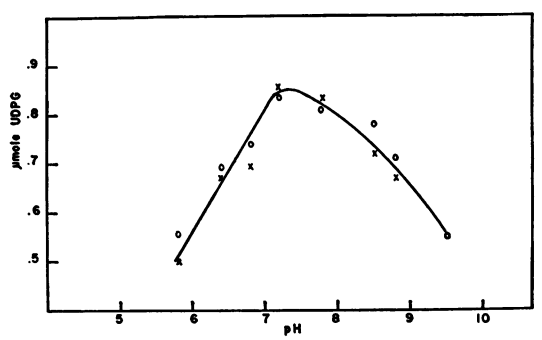
$$AMP \rightleftharpoons IMP \text{ (inosine monophosphate)} + NH_3 \text{ (adenylic acid deaminase)}$$

The reaction was followed by measuring the decrease in absorption at 265 mμ caused by the deamination of AMP.

Phosphorus was determined by the method of Dryer, et al (6), and nitrogen by Nesslerization.







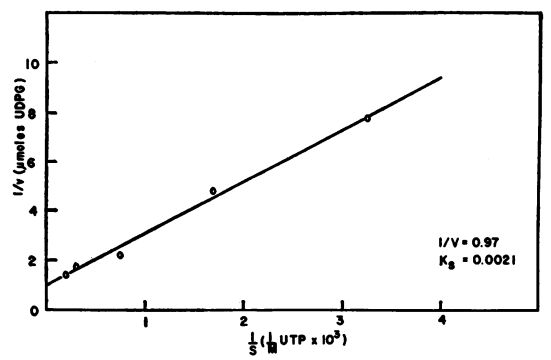


FIG. 1. Radioactive assay of 2 cm strips cut from chromatograms of a reaction mixture in which sugar cane seedling homogenates were incubated with C^{14} glucose and other constituents. Known glucose and sucrose areas on the chromatograms are given for reference. 1. Reaction mixture contained no boron; 2. Reaction mixtures contained 10 μmoles boron/ml.

FIG. 2. Relationship of protein nitrogen of the pea enzyme preparation on the synthesis of UDPG by UDPG pyrophosphorylase. UTP concentration, 4.5 μmole/ml.

FIG. 3. Effect of pH of the reaction mixture on the synthesis of UDPG by UDPG pyrophosphorylase from pea seed preparation. Tris buffer adjusted to indicated pH by HCl.

FIG. 4. Effect of UTP concentration on the rate of UDPG synthesis. The reaction mixtures were as described under Materials and Methods.

Results and Discussion

The incorporation of glucose-C^{14} into sucrose by sugar cane seedling homogenate was qualitatively indicated by chromatographic and radioautographic methods. The radioactivity of sucrose eluted from chromatograms indicated quantitatively the influence of boron on sucrose synthesis. Boron more than doubled the synthesis of sucrose from glucose-C^{14}, UTP, and fructose (fig 1). Hexokinase and phosphoglucomutase in the reaction mixture enhanced the formation of sucrose from glucose-C^{14}. Previous studies have shown that the activity of hexokinase from yeast or pea seedlings is unaffected by levels of borate used in these experiments (7), nor does boron affect the enzymatic activity of rabbit phosphoglucomutase that was added to these systems to facilitate the synthesis of C^{14} glucose-1-phosphate. In other experiments the spots corresponding to the 19 to 21 cm portion of the chromatograms (fig 1) were eluted and sucrose identified colorimetrically and chromatographically as described in Materials and Methods. Table I shows the results of another experiment in which the influence of specific co-factors on the synthesis of sucrose was studied. The phosphorylation and transformation of glucose to glucose-1-phosphate by added enzymes and ATP resulted in increased labeling of sucrose by the sugar cane material. Borate also increased the synthesis of sucrose in this experiment as shown in the last row of the table.

Fructose-C^{14} was incorporated into sucrose by ground sugar cane and pea seedling homogenates as well as glucose-C^{14}. As glucose-1-phosphate was included in the reaction mixture, it was unnecessary to add hexokinase and phosphoglucomutase to obtain maximum labeling of sucrose from labeled fructose. Table II gives the results of an experiment in which ground pea seedling homogenate was incubated with fructose-C^{14} and other necessary ingredients for sucrose synthesis. Chemical and radioactive assay of the sucrose formed in 4 hours indicated that borate increased the synthesis. The decrease in sucrose concentration from the initial value may possibly be accounted for by the respiration of the plant material during the 4 hours of incubation at 35° C, or by invertase action. However, it was found that boron had no effect upon the activity of commercial preparations of invertase. This was thoroughly checked prior to using the invertase and glucose oxidase assay method for determining sucrose synthesis.

It was also found that boron had no effect on hexokinase or on phosphoglucomutase. These enzymes along with invertase would be implicated in the initial reactions leading to sucrose degradation. Thus, it appears that boron affects the synthesis of sucrose. The appearance of C^{14} in sucrose is no indication of net synthesis as sucrose utilizing and sucrose synthetic reactions are occurring. The data of table II show that there was no correlation between total sucrose and C^{14} content of the sucrose. The chemical methods of sucrose estimation are more reliable than the chromatographic method.

Results with the homogenates pointed to a possible relationship between boron and enzyme systems associated with sucrose synthesis. As mentioned in the previous section, A. S. preparations from sugar cane seedlings and pea seeds contained both UDPG pyrophosphorylase and UDPG transglycosylase. The A. S. preparation from sugar cane was less active than that from pea seeds, and on storage at −16° C the activity decreased at a greater rate than with the pea seed A. S. preparation. Therefore, the results reported in this phase of the work will be from experi-

Table I

Effect of Cofactors on Synthesis of Sucrose by Ground Sugarcane Leaves

Experiment	Relative radioactivity	
	Sucrose* %	UDPG** %
1. Complete	100	0
-UTP	11	0
-fructose	11	61
-hexokinase and phosphoglucomutase	5	0
2. Complete	100	
-ATP	46	
-UTP	57	
-fructose	56	
10 μmole B/ml	176	

* Chromatographed in propanol-water-NH_4OH (16:3:1)

** Chromatographed in ethanol-ammonium acetate (7:3) pH 7.5

Sucrose and UDPG spots eluted from chromatograms prior to radioactive assay.

Complete reaction mixture. See Materials and Methods.

Table II

Influence of Boron on Synthesis of Sucrose-C^{14} from Fructose-C^{14} by Pea Seedlings Homogenate

Treatment	Sucrose (μmole/gm)	Sucrose chromatographed (c/m × 10^3)*
Pea seedlings (before incubation)	8.5 ± 0	
Homogenate without boron (after incubation)	5.5 ± 0.5	6.6
5 μmole B/ml (after incubation)	9.5 ± 0.5	15.2
10 μmole B/ml (after incubation)	10.5 ± 1.2	11.0
15 μmole B/ml (after incubation)	10.0 ± 0.3	15.8

* Chromatographed in propanol-water-NH_4OH (16:3:1). Sucrose area eluted and counted in windowless flow counter.

TABLE III

INFLUENCE OF BORON ON SYNTHESIS OF SUCROSE BY ENZYME PREPARATIONS FROM PEA SEEDS*

BORON CONC (μMOLE/ML OF DIGEST)**	ENZYME PREPARATION	
	1	2
	SUCROSE (μMOLE/MGN)	
0	0.60	0.72
5	0.45	0.69
10	0.52	0.56
20	0.32	0.52

* Incubation time, 30 minutes, temperature, 35° C.
** Reaction mixture. See Materials and Methods section.

ments using A. S. preparation from pea seeds. These results will also be limited to those experiments in which fructose was included as the acceptor of the glucose from UDPG in the transglycosylase reaction due to the observation that sucrose-phosphate synthesis from both pea seed and sugar cane seedling A. S. preparation was much less active than sucrose synthesis. This is in agreement with the results of Bean and Hassid (1) and Leloir and Cardini (14). Only after repeated extraction with 0.05 M ammonium sulfate were these latter investigators able to obtain an enzyme from wheat germ more active with fructose-6-phosphate as the acceptor of glucose than with fructose. The additional chemical steps necessary to estimate sucrose-phosphate synthesis in these experiments introduced errors often as large as the quantity of sucrose-phosphate determined.

With boron included in the reaction mixture, sucrose synthesis was inhibited (table III). In contrast to the results obtained using homogenates, boron caused a decrease in the amount of sucrose synthesized by the A. S. preparation. If the digests were incubated for 24 hours under a layer of toluene, or if antibiotics were included to prevent microorganism growth, there was little if any effect of boron on sucrose synthesis.

Contrary to the findings by Turner (27) for a similar preparation from pea seeds, it was found that DPN was not necessary for maximum sucrose synthesis, and further that ATP enhanced the synthesis in the coupled reaction (UTP + G-1-P + fructose $\rightleftharpoons$ UDP + PP + sucrose). In the experiments reported by Turner (27), 0.2 mg DPN and 34 μmoles ATP were included per 0.9 ml digest. The enzyme preparation was dialyzed for 2 hours in 25 mM phosphate buffer (pH 8) and the digest time was 19 hours. In experiments reported in this paper, where ATP inhibited the individual reactions and enhanced the coupled reaction, 0.25 mg DPN and 10 μmoles ATP were included per ml of digest. The A. S. preparation was dialyzed 24 hours in 25 mM phosphate buffer and the digest time was for shorter periods. In reaction periods longer than 12 hours it was found necessary to add a layer of toluene to the reaction mixture or include antibiotics to prevent the metabolic utilization of sucrose by microorganisms. Non-dialyzed or 2,4, and 6 hour dialyzed A. S. preparations from pea seeds reduced DPN; this was enhanced by adding cysteine. In a later paper by Hatch and Turner (12) it was shown that the 80 % ammonium sulfate precipitate from pea seeds contained all the enzymes necessary for oxidation of hexose to CO_2 and ethanol. The enhanced production of CO_2 by added DPN is associated with glycolysis and, in some way, as reported by Hatch and Turner (12), influenced sucrose synthesis.

Because of the inconsistent results between the experiments using homogenates and those using A. S. preparations, a more thorough investigation of the individual reactions concerned with sucrose synthesis was attempted.

The A. S. preparation catalyzed the reversible reaction UTP + G-1-P $\rightleftharpoons$ UDPG + PP (15). At the concentration used in the overall reaction where sucrose synthesis from UTP, G-1-P and fructose was measured, 0.27 to 0.47 mg protein N was added with each 0.1 ml of enzyme preparation. However, in order to investigate the UDPG pyrophosphorylase reaction a 1 : 300 dilution of the enzyme was necessary. Figure 2 shows the rate of UDPG formation as a function of A. S. preparation concentration at the level of reactants used in the assay.

The optimum pH for this reaction was 7.2 to 7.4. However, there was an appreciable rate of reaction at lower and higher pH values (fig 3). Other in-

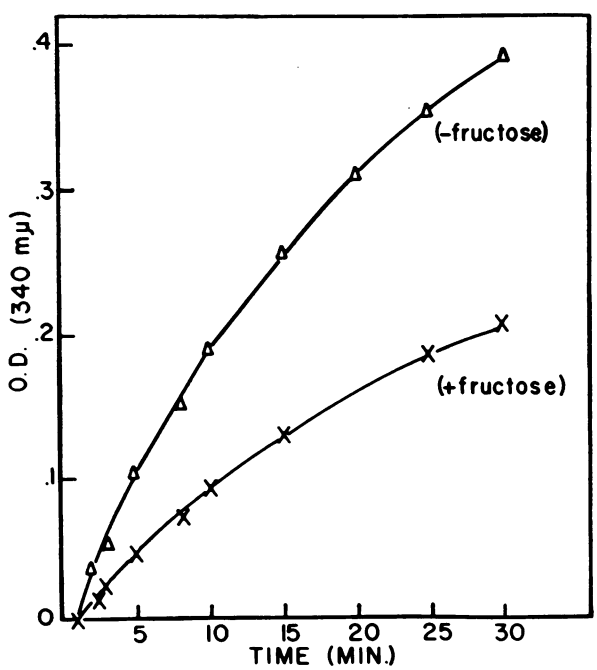


FIG. 5. Effect of fructose in the reaction mixture on the apparent UDPG synthesis. The change in OD_{340} as DPN is reduced by UDPG dehydrogenase is proportional to the concentration of UDPG.

TABLE IV

INFLUENCE OF BORON ON BIOSYNTHESIS OF UDPG BY EXTRACT FROM PEA SEEDS

BORON CONC (μMOLE/DIGEST)*	UDPG CONC μMOLE/10 μG N)	
	1	2
0	2.6	3.0
2	2.5	4.3
5	3.9	4.9
10	5.1	5.9
20	5.1	6.0
40		5.5

* Reaction mixture. See Materials and Methods section.

vestigators have reported a similar pH optimum for this enzyme from plants (8,15). Only by diluting the enzyme 1:300 was it possible to use the UDPG dehydrogenase method of assay (25) without noticeable pyrophosphorylase activity during the 15 to 20 minutes it took to assay for UDPG.

The rate constants for enzyme activity, as dependent upon UTP concentration are given in figure 4. The value of 0.0021 for K_s is approximately ten times the value Ginsburg (11) reported for the reverse reaction catalyzed by a purified enzyme from Mung bean. When fructose was included in the reaction mixture the apparent synthesis of UDPG was less (fig 5). This decrease was caused by the utilization of UDPG in sucrose synthesis. In short time experiments and without ATP in the reaction, this decrease in UDPG when fructose was added to the reaction mixture approximated the quantity of sucrose synthesized by UDPG transglycosylase.

UDPG synthesis in the presence of boron was enhanced. Table IV shows two experiments where approximately 100 per cent increase in UDPG formation in 1 hour was obtained by adding 10 and 20 μmole B/ml.

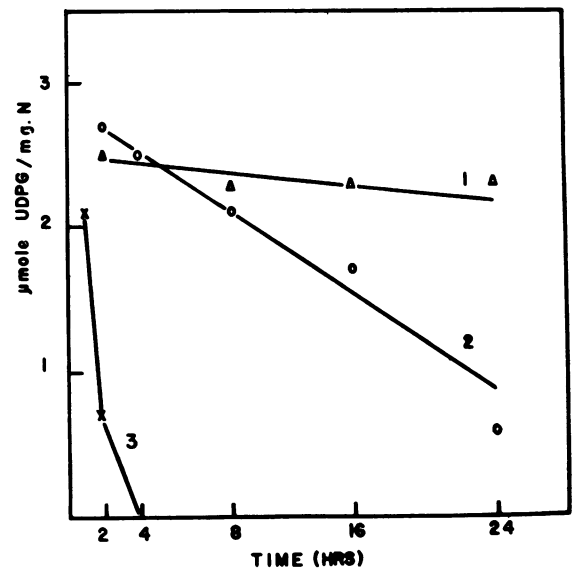
If the UDPG concentration was measured against time in the original reaction mixture where sucrose was synthesized from UTP, G-1-P and fructose in the presence of ATP the influence of boron appears to be that of maintaining UDPG in the mixture (see fig 6). If ATP was not included the UDPG value rose rapidly to a maximum and decreased to zero within 4 hours. This indicates that the A. S. preparation contains UDP kinase and that UDPG is resynthesized from UDP and ATP via UTP. An investigation as to what effect ATP, DPN, and Mg^{++} alone and in combination might have on UDPG synthesis shows

TABLE V

INFLUENCE OF CO-FACTOR ADDITION ON UDPG SYNTHESIS BY UDPG PYROPHOSPHORYLASE FROM PEA SEEDS*

REACTION MIXTURE**	UDPG (μMOLE/10 MG N)
Complete	2.3
+DPN, −Mg^{++}	0.3
+ATP, −Mg^{++}	0.3
+DPN, +Mg^{++}	1.6
+ATP, +Mg^{++}	1.3
+Mg^{++}, +ATP, +DPN	1.5

* Incubation was for one hour at 35° C.
** Reaction mixture. See Materials and Methods section.



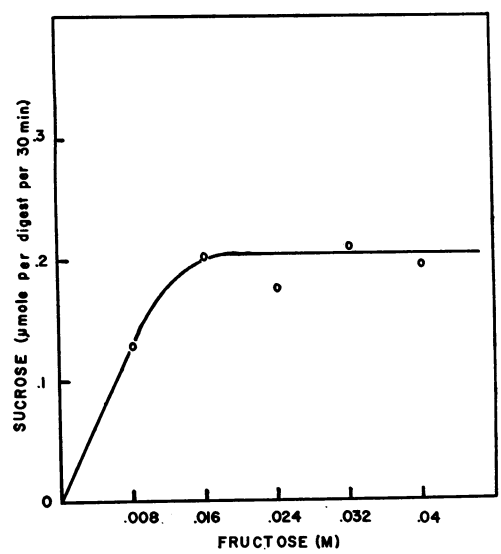


FIG. 6. Effect of ATP and boron on the level of UDPG maintained in the reaction mixture over a 24 hour reaction period. 1. ATP and boron, 10 μmole/ml; 2. ATP; 3. No ATP. The reaction mixtures were as described under Materials and Methods.

FIG. 7. Effect of fructose on the synthesis of sucrose by pea enzyme preparation. The reaction mixtures were as described under Materials and Methods.

TABLE VI

INFLUENCE OF BORON ON SYNTHESIS OF SUCROSE FROM UDPG AND FRUCTOSE BY AN ENZYME PREPARATION FROM PEA SEEDS

REACTION MIXTURE*	BORON CONC (μMOLE/ML)	SUCROSE (μMOLE/MG N)
—fructose	0	0.15 ± 0.2**
complete	0	1.3 ± 0.1
"	5	1.2 ± 0.1
"	10	1.0 ± 0.0
"	20	0.85 ± 0.05

* Reaction mixture. See Materials and Methods section.
**Average of duplicate experiments.

that ATP inhibits the UDPG pyrophosphorylase reaction (see table V). When this reaction is coupled to the transglycosylase and kinase reactions, however, ATP is necessary to maintain UDPG concentration (fig 6) and results in greater sucrose synthesis.

UDPG transglycosylase activity was much less per unit of protein nitrogen than pyrophosphorylase activity. In all experiments where the two reactions were coupled together, UDPG was rapidly synthesized whereas sucrose was synthesized more slowly.

The pH optimum for transglycosylase activity is 7.2 as was also shown for preparations from wheat germ by Cardini et al (4). The effect of fructose concentration on the synthesis of sucrose is shown in figure 7. Boron inhibits this reaction (table VI). The magnitude of inhibition, however, is less than the magnitude of stimulation in UDPG synthesis. Table VII shows the effect of ATP and Mg^{++} concentration on the synthesis of sucrose by UDPG transglycosylase. As shown in this table and table V, ATP inhibits synthesis of UDPG and sucrose in the single reactions, but in the coupled system the addition of ATP facilitates the resynthesis of UDPG and the synthesis of sucrose. When the UTP concentration was reduced to a value less than substrate amounts (0.85 μmole/ml digest) the influence of ATP was more apparent (see table VIII) on UDPG resynthesis and sucrose synthesis. With ATP, the level of UDPG was maintained for 4 hours, whereas with no ATP included in the digest the UDPG was utilized in the transglycosylase reaction and not resynthesized. Sucrose synthesis was approximately doubled when ATP was included in the reaction.

An attempt was made to characterize UDP kinase, the action of which was implicated in the experiments reported above. The synthesis of UDPG was doubled when 10 and 20 μmoles of borate were included in the reaction. This does not conclusively show an effect of boron on the kinase since UDPG synthesis from UTP and G-1-P was increased by boron. The formation of ADP from ATP and UDP was increased when boron was included in the initial kinase reaction mixture. The kinase was assayed by measuring

TABLE VII

INFLUENCE OF ATP AND Mg^{++} ON SYNTHESIS OF SUCROSE FROM UDPG AND FRUCTOSE BY AN ENZYME PREPARATION FROM PEA SEEDS

CONCENTRATION* (μMOLE/ML OF DIGEST)	ATP	Mg^{++}
	SUCROSE	(μMOLE/ML)
0	5.7	3.8
2	5.9	5.5
4	4.2	6.1
6	4.0	6.1
8	4.4	6.3
10	2.3	4.2
20	2.3	4.6

* Reaction mixture. See Materials and Methods section. When ATP was varied, 5 μmoles of Mg^{++} was added. When Mg^{++} was varied, ATP was omitted.

TABLE VIII

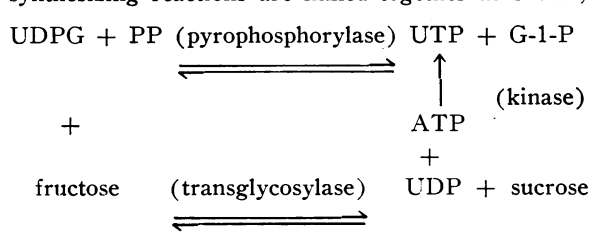
INFLUENCE OF ATP ON UDPG RESYNTHESIS AND SUCROSE SYNTHESIS BY ENZYME PREPARATIONS FROM PEA SEEDS

EXPERIMENT		TIME (HRS) ¼	½	1	2	4	4
		*UDPG (μmoles/ml digest)**					*Sucrose (μmoles/ml digest)*
1	+ATP	...	...	0.32	0.29	0.31	1.5
2	+ATP	0.48	0.41	0.31	0.34	0.45	1.7
1	—ATP	...	...	0	0	0	0.9
2	—ATP	0.25	0.12	0.04	0	0	0.9

* Reaction mixture. See Materials and Methods.

UDPG formed in the presence of G-1-P, UDP, and ATP (reactions III and I) and by measuring ADP formed in the presence of ATP and UDP (reaction III) by the method of Colowick (5). Subsequent work revealed, however, that the A. S. preparation contained myokinase and nucleoside monophosphate kinase which prevented conclusive studies on this phase of the reaction until more purified enzymes are prepared.

The data indicate that the UDPG and the sucrose synthesizing reactions are linked together as shown,



The enhancement by boron of sucrose synthesis as observed in the experiments with pea and sugar

cane seedling homogenates, was related evidently to the influence of the element on UDPG pyrophosphorylase and perhaps on UDP kinase. The observation that the addition of boron to the A. S. preparation does not result in an increased sucrose synthesis from UTP, G-1-P, and fructose is probably related to the difference in the levels of UDPG pyrophosphorylase and UDPG transglycosylase. The former enzyme is present in the A. S. preparation at a level approximately 100 times as great as the latter enzyme. The enzyme levels in the homogenates were not measured. Further understanding of this effect of boron on UDPG pyrophosphorylase must await purification of the enzyme.

Summary

Boron has been observed to enhance the biosynthesis of sucrose by sugar cane and pea seedling homogenates. With an enzyme preparation from pea seeds containing UDPG pyrophosphorylase, UDPG transglycosylase, and UDP kinase boron inhibited the synthesis of sucrose from UTP, G-1-P, and fructose. In this coupled reaction ATP was essential and boron in some way caused the concentration of UDPG to be maintained; however, UDPG pyrophosphorylase and UDPG transglycosylase were inhibited by ATP when assayed individually. UDPG pyrophosphorylase was enhanced by boron while UDPG transglycosylase was inhibited.

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