

## Effects of Univalent Cations on the Activity of Particulate Starch Synthetase<sup>1</sup>

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**Abstract.** An investigation was made to determine the univalent cation requirements of starch synthetase from a variety of plant species of economic importance. The particulate enzyme from sweet corn was shown to have an absolute requirement for potassium, with the optimum activation occurring at 0.05 M KCl. Rubidium, cesium, and ammonium were 80% as effective as potassium while sodium and lithium were respectively 21% and 8% as effective as potassium. The  $K_A$  for potassium was determined to be 6 mM. In the case of the particulate starch synthetase from wheat, bush beans, field corn, soybeans, peas, or potatoes, considerable stimulation of enzyme activity was obtained by the addition of potassium to the reaction mixture. In these studies, low enzyme activity was observed in the absence of added potassium, but the content of endogenous univalent cations in the reactions may be sufficient to account for the activities observed. Anions of various types had no effect on starch synthetase activity. Divalent cations produced slight activation in the presence or absence of potassium. All efforts to show a potassium requirement for glycogen synthetase from rat liver have been negative.

A consistent consequence of potassium deficiency in plants is the inhibition of starch synthesis and the accumulation of soluble carbohydrates and reducing sugars (4). In higher plants, carbohydrate and reducing sugar utilization might be impaired as a result of the loss of activity of one or more of several enzymes known to require potassium or other univalent cations (4, 12). In the case of starch synthesis, a dual role of potassium has only recently been suggested:

In 1966 Akatsuka and Nelson (1) demonstrated that potassium stimulated the activities of both the embryonic and endospermic derived particulate starch synthetase systems from immature maize seeds. They reported that potassium stimulated both systems when adenosine diphosphate glucose (ADPG) was the substrate, but inhibited at the same level when uridine diphosphate glucose (UDPG) was the substrate. EDTA in the presence of potassium stimulated enzyme activity but was inhibitory when

potassium was omitted from the reaction mixture containing the embryonic starch synthetase. Saturation curves for potassium activation were presented although no attempt was made to eliminate potassium from the enzyme components or the reagents. In fact, dipotassium salts of the substrates were utilized in their investigation. Potassium also was shown to have a protective effect against thermal inactivation of the enzymes at 60°.

In an investigation of the starch synthesizing system from *Chlorella pyrenoidosa*, Preiss and Greenberg (16) showed that the partially purified soluble starch synthetase was stimulated 15 to 30% by the addition of either KCl, GSH, or bovine plasma albumin. If all 3 of these components were omitted from the reaction mixture, activity was reduced 60%. Similar results also were obtained in an investigation of the synthesis of bacterial glycogen (6). These authors did not indicate whether or not potassium was removed from the components of the assay mixture.

In a preliminary report in 1968 (15), Nitsos and Evans presented evidence that partially purified particulate starch synthetase from sweet corn was completely dependent upon univalent cations for activity. Potassium was the most effective activator, but rubidium, cesium, and ammonium also were effective. Sodium, lithium, and tris were ineffective in the assay system.

More recently, Murata and Akazawa (13) examined the role of potassium in the starch synthetase

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from sweet potato roots and certain other sources. In their report, a 7-fold stimulation of the activity of the enzyme was observed by the addition of 0.1 M KCl. From kinetic studies, the Michaelis-Menten constant for activation,  $K_A$ , for KCl was determined to be 13 mM. The enzyme was saturated at 0.05 M KCl, and no effect of KCl on the  $K_m$  for ADPG could be demonstrated. A protective effect of potassium against thermal inactivation of the enzyme also was shown.

Since a univalent cation requirement for the starch synthesizing systems of various sources has been indicated, it seemed that a study of the detailed alkali metal requirements of the starch synthetase systems would lead to a better understanding of the factors controlling carbohydrate metabolism. This report represents an elaboration of a preliminary report (15) of the univalent cation requirements of starch synthetase from a variety of plant species of economic importance.

### Materials and Methods

**Preparation of Reagents.** Glassware utilized for the preparation and storage of reagents was acid washed and thoroughly rinsed with doubly distilled, deionized water. Reagents were prepared with deionized water. Reagent grade sucrose, EDTA, cysteine, and glycine were recrystallized before use. All other chemicals also were reagent grade and utilized without further purification. The buffers used throughout this investigation were either tris (hydroxymethyl)-aminomethane (tris) or *N*-tris (hydroxymethyl)methyl glycine (tricine). The pH of the tris solutions were adjusted with HCl, and that of the tricine solutions with tetramethyl-ammonium hydroxide. Potassium concentrations of the various reagents and solutions were determined by flame photometry (7). Maximum concentration of endogenous potassium in all assays was less than 0.01 mM.

The dipotassium and disodium salts of both ADPG and UDPG, and the barium salt of glucose 6-P were purchased from Sigma Chemical Company (St. Louis, Missouri). The nucleoside diphosphate sugars were converted to their respective tris salts by passing them through a cation exchange column (Amberlite CG-50) which had been equilibrated with 0.01 M tris, pH 8.0. Concentrations of the substrates were determined on the basis of their molar extinction coefficients at pH 8.0 ( $\epsilon_{259\text{m}\mu} = 15.4 \times 10^3$  for ADPG, and  $\epsilon_{260\text{m}\mu} = 1.0 \times 10^4$  for UDPG). The barium salt of glucose 6-P was converted to the tris salt by precipitation of the barium with a slight excess of tris-sulfate. These reagents were stored at  $-15^\circ$  until used.

Adenosine diphosphate glucose- $^{14}\text{C}$  (glucose- $^{14}\text{C}$  U.L.) was purchased from New England Nuclear Corporation (Boston, Massachusetts). The ethanolic solution of ADPG- $^{14}\text{C}$  was evaporated in the cold

under reduced pressure, and the substrate dissolved in 0.5 ml of 0.05 M tricine, pH 8.0. Specific radioactivity of this compound was determined to be 225 mC/mmole.

**Preparation of Enzymes.** Starch granules were prepared from freshly harvested immature seeds of sweet corn (*Zea mays* L.), peas (*Pisum sativum* L.), bush beans (*Phaseolus vulgaris* L.), field corn (*Zea mays* L.), wheat (*Triticum aestivum* Linn.), soybeans (*Glycine max.* L.), and from potato tubers (*Solanum tuberosum* L.) utilizing a modified method of Leloir, de Fekete, and Cardini (10). The seeds or potato tubers were macerated in an Omnimixer for 2 min in 4 volumes of cold ( $4^\circ$ ) deionized water. The homogenate was filtered through cheesecloth and centrifuged for 5 min at 1000g in a refrigerated centrifuge ( $0-4^\circ$ ). The white pellet was washed 4 times with cold, deionized water and suspended in 4 volumes of redistilled acetone at  $-15^\circ$ . The starch preparation was collected by filtration on a Buchner funnel at  $-15^\circ$ , and was washed 4 additional times with 4 volumes of cold acetone. The final enzyme preparation (starch granules) was dried under reduced pressure at  $0^\circ$  and stored at  $-20^\circ$  until utilized. Prior to assaying for starch synthetase activity, the granules (240 mg) were washed at least 3 times with 6 ml portions of 0.1 M tricine, pH 8.0. The final pellet was resuspended in 3 ml of 0.05 M tricine, pH 8.0.

Glycogen synthetase was obtained from freshly excised rat livers by a modified method of Leloir and Goldemberg (8). Rats were fed 30% sucrose *ad libitum* for 12 hr. killed by  $\text{CO}_2$  suffocation, the livers immediately removed and placed on ice. The livers were macerated in a Ten Broeck homogenizer in 3 volumes of cold 0.25 M sucrose containing 0.001 M EDTA. The resulting homogenate was centrifuged at 2000g for 10 min, and the supernatant (crude enzyme) stored at  $-15^\circ$  until assayed. In this form, the glycogen synthetase is reported to be stable for at least 1 month (8).

Further purification of the glycogen synthetase was accomplished by centrifugation of 30 ml of the crude preparation at 25,000g for 10 min at 0 to  $4^\circ$  and the transparent, yellow pellet (particulate glycogen) was resuspended in 3 ml of 0.05 M tricine, pH 8.5, containing 0.05 M glucose 6-P and 50 mg/ml soluble starch. The suspension was centrifuged at 25,000g and the washing procedure repeated 3 times. The final pellet was resuspended in 3 ml of 0.05 M tricine, pH 8.5, containing 0.05 M glucose 6-P, and assayed immediately. A 300-fold purification of the enzyme resulted from the above procedure.

**Enzyme Assays.** The reaction mixture for the assay of starch synthetase contained the following reagents in a total volume of 0.2 ml: tricine buffer pH 8.0, 10  $\mu\text{moles}$ ; ADPG, 1.0  $\mu\text{mole}$ ; 0.05 ml of the enzyme preparation (4 mg granules containing 20  $\mu\text{g}$  protein); and the appropriate cation concentration. The reaction was initiated by the addition

of the substrate, incubated on a shaker for 1 hr at 37°, and terminated by placing the tubes in a boiling water bath for 1 min. In many of the previous reports involving starch synthesis, the specific activities presented were based on starch rather than protein content. This basis fails to take into account the age, size, and enzyme content of the starch granules. Using protein content as a basis for starch synthetase activity eliminates these variations.

Glycogen synthetase was assayed using a reaction mixture of the following composition in a total volume of 0.2 ml: 2.0  $\mu$ moles glucose 6-P; 0.6  $\mu$ mole cysteine; 1.6 mg glycogen; 10.0  $\mu$ moles tricine, pH 8.5; 1.0  $\mu$ mole UDPG; the appropriate univalent cation concentration; and 0.02 ml of the enzyme preparation containing approximately 1.5  $\mu$ g protein. The reaction was initiated by addition of the substrate, incubated for 10 min at 37°, and terminated by boiling for 1 min.

Nucleoside diphosphates released in the synthetase reactions were determined by means of a pyruvic kinase assay (12) using excess amounts of pyruvic kinase. After boiling the starch granules for 10 min in 0.1 ml of 0.1 N KOH, protein was determined by Folin's Method (11). Protein content per mg starch was approximately 4  $\mu$ g. In the case of glycogen synthetase, the protein was precipitated by 5% (w/v) trichloroacetic acid prior to protein determination. All results presented are the mean values of triplicate experiments.

**Radioisotope Experiments.** Assay conditions involving the labeled substrate were the same as those previously described for starch synthetase with the exception that  $^{14}\text{C}$  labeled substrates were utilized. The reaction was stopped by the addition of 2.0 ml of 75% (v/v) methanol and the precipitated starch washed 5 times with 2.0 ml aliquots of 75% methanol. The final pellet was hydrolyzed with 2.0 ml of 0.7 N HCl for 2 hr in a boiling water bath, and the final volume after hydrolysis reconstituted to 2.0 ml with water. A sample of 0.5 ml was added to 10 ml of Bray's solution (3), and the radioactivity determined with a model 3375 Packard Tri-Carb Liquid Scintillation Spectrometer. The counting efficiency of this system was approximately 65%.

## Results

**Validity of the Starch Synthetase Assays.** To establish the validity of the starch synthetase assay systems, experiments were conducted dealing with the effects of incubation time, pH, enzyme and substrate concentrations on the activity of the enzyme. The experimental data presented in Fig. 1 represent the incorporation of  $^{14}\text{C}$ -glucose into starch as a function of time. The rate of incorporation is linear for 90 min. At this time, approximately 4% of the added label had been incorporated into the starch. Assays utilizing the pyruvic kinase method for determining the rate of liberation of ADP from ADPG

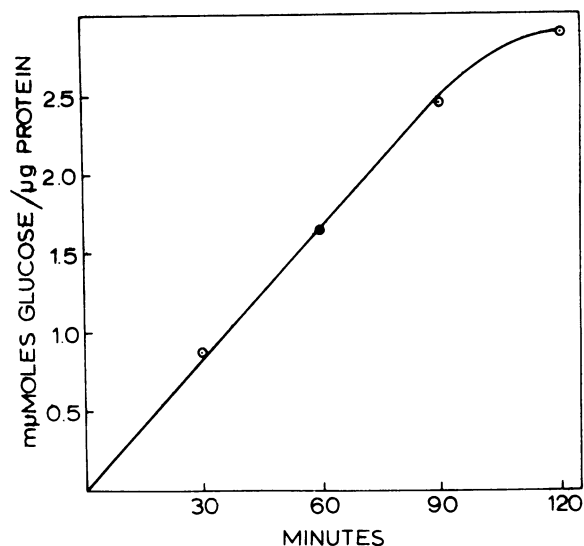


FIG. 1. The incorporation of  $^{14}\text{C}$ -glucose as a function of time by starch synthetase from sweet corn. The reaction mixture contained the following components in a total volume of 0.2 ml: tricine buffer, pH 8.0, 10  $\mu$ moles; KCl, 20  $\mu$ moles; ADPG, 1.0  $\mu$ mole ( $2.6 \times 10^6$  dpm); and 4 mg starch granules (20  $\mu$ g protein). Assays were conducted by the procedure described in the Materials and Methods.

also yielded comparable results. The pH optimum for the sweet corn preparation in the presence of potassium was found to approximate 8.0. This pH value is similar to that reported for the activity of the maize enzyme in glycylglycine buffer but is higher than the optimum pH (7.5) reported for tris buffer (1). Tris buffer was not used in this investigation because of the reported (17) inhibitory effects of this cation on certain univalent cation activated enzymes.

Fig. 2 represents the effect of both  $\text{tris}_2\text{ADPG}$  and  $\text{K}_2\text{ADPG}$  on the activity of particulate starch synthetase assayed in the presence of excess KCl (0.125 M). The 2 curves are quite similar indicating that the tris salt of the substrate had no appreciable inhibitory effects on the activity of the enzyme. Concentrations of  $\text{tris}_2\text{ADPG}$  used in all subsequent reaction mixtures were greater than 3.5 mM so that any interference by the tris cation was considered to be of little consequence. As shown by the reciprocal plots (inset of Fig. 2), the apparent  $K_m$  values for  $\text{tris}_2\text{ADPG}$  and  $\text{K}_2\text{ADPG}$  were 1.1 and 1.0 mM respectively.

**Effect of Univalent Cations.** The activation of starch synthetase by a series of univalent cation salts is shown in Fig. 3. Potassium shows the greatest activation, and appears to saturate at approximately 0.05 M. The additions of either rubidium, cesium or ammonium cations produced similar levels of activation, but the maximum velocities observed are

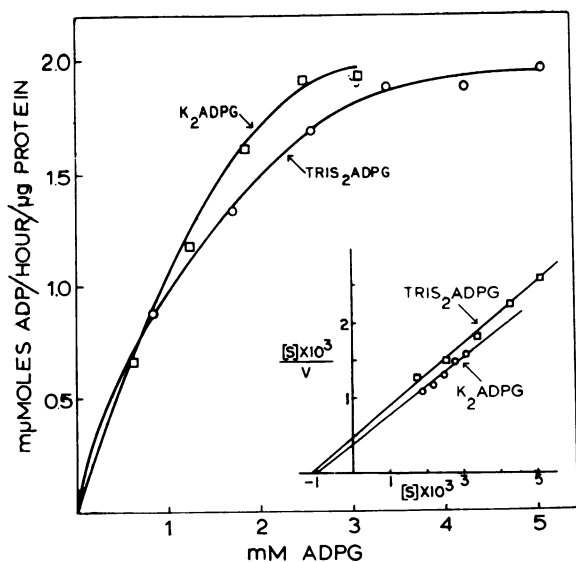


FIG. 2. The effects of the potassium or tris salt of ADPG on the activity of starch synthetase from sweet corn. Reaction mixtures contained 10  $\mu$ moles tricine buffer, pH 8.0; 20  $\mu$ moles KCl; 4 mg starch granules (20  $\mu$ g protein); and the indicated substrate concentration in a total volume of 0.2 ml. Assay conditions are described in Materials and Methods.

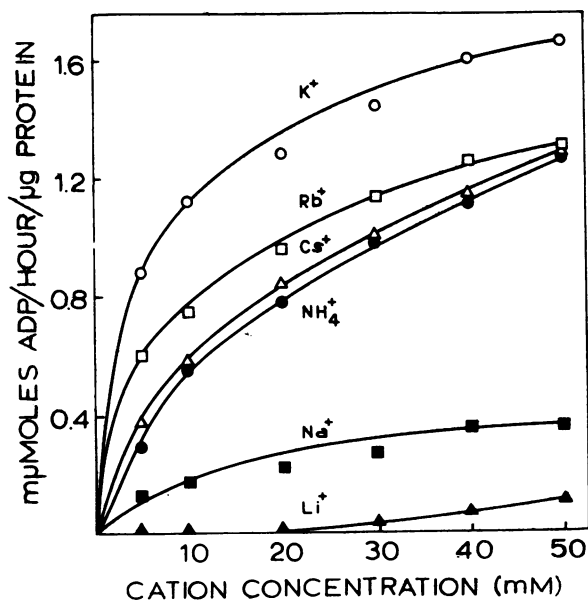


FIG. 3. The effects of univalent cation chlorides on the activity of starch synthetase from sweet corn. The reaction mixtures contained 10  $\mu$ moles tricine buffer, pH 8.0; 1.0  $\mu$ mole tris<sub>2</sub>ADPG; 4 mg starch granules (20  $\mu$ g protein); and the indicated univalent cation concentration in a total volume of 0.2 ml. Assay conditions are described in the text.

less than that obtained with potassium (table I). The addition of sodium or lithium resulted in only slight activation. These findings are in agreement with other observations concerning the activation of many different enzymatic reactions by univalent cations (4). The apparent  $K_A$  values and  $V_{max}$  values associated with these cations are given in table I. The values presented for potassium were also determined from an experiment involving <sup>14</sup>C-ADPG and do not differ appreciably from those obtained with the unlabeled substrate.

The effect of potassium on the activities of starch synthetase from different sources are presented in table II. Although the starch granules were washed from 3 to 6 times with 0.1 M tricine, pH 8.0, some activity was observed in most of the preparations in the absence of added potassium. In the absence of added potassium, the starch granules from peas, soybeans, and field corn had the lowest residual activities, ranging from 0.6 to 0.9  $m\mu$ mole ADP liberated per hr per  $\mu$ g protein. Although an absolute potassium requirement of the starch synthetase was demonstrated for the enzyme from sweet corn

Table I. The Apparent  $K_A$  and  $V_{max}$  Values for Different Univalent Cations in the Activation of Starch Synthesis From Sweet Corn

Values were calculated from the data presented in Fig 3.

Cation	$K_A$	$V_{max}$
	mM	$m\mu$ moles ADP/hr $\times$ $\mu$ g protein
K <sup>+</sup>	6	1.67
Rb <sup>+</sup>	11	1.49
Cs <sup>+</sup>	14	1.44
NH <sub>4</sub> <sup>+</sup>	19	1.54
Na <sup>+</sup>	3	0.25

Table II. The Effects of Potassium on Starch Synthetase Activity From Different Plant Sources

The reaction mixture contained the following components in a total volume of 0.2 ml: 10  $\mu$ moles tricine buffer, pH 8.0; 1.0  $\mu$ mole ADPG, either 4 mg starch granules (bush bean, potato, wheat, field corn, peas, sweet corn) or 2 mg starch granules (soybeans); and the indicated univalent cation concentration. Assay conditions are described in the text.

Source of enzyme	KCl Concentration (mM)						
	0	5	10	25	50	75	100
Sweet corn	0	3.8	4.6	5.4	6.5	6.9	7.1
Peas	0.6	2.9	3.5	4.8	5.6	6.8	7.3
Soybeans	0.7	3.6	5.7	9.0	10.0	12.4	13.1
Field corn	0.9	5.5	6.8	7.6	9.0	9.4	9.8
Wheat	2.0	4.5	6.0	6.8	7.2	7.4	7.5
Bush beans	2.1	5.0	7.2	7.4	7.9	8.3	8.3
Potato	2.8	6.1	9.8	12.6	15.2	17.4	17.4

only, the activities of the enzymes from other sources were strikingly stimulated by KCl. Perhaps these preparations contain sufficient endogenous potassium to account for the activities observed in the absence of added univalent cation. The soybean enzyme does not appear to be completely saturated at 0.1 M KCl although all of the other enzyme preparations were saturated in the range of 0.05 M to 0.075 M KCl.

*Effect of Substrate on Univalent Cation Response.*

The effects of ADPG and UDPG as substrates on the response of starch synthetase to potassium and sodium were determined (table III). With ADPG as the substrate, enzyme activity was stimulated considerably when potassium was present in the reaction mixture, but only slightly stimulated in the presence of sodium. With UDPG as the substrate, appreciable activity was observed in both the presence and absence of either sodium or potassium. A small degree of stimulation was observed with either potassium or sodium added to the assay.

Table III. *The Effects of Univalent Cations on the Utilization of ADPG or UDPG as Substrates for Starch Synthetase Activity From Sweet Corn*

The reaction mixtures contained the following components in a total volume of 0.2 ml: 10  $\mu$ moles tricine buffer, pH 8.0; 1.0  $\mu$ mole ADPG or UDPG, 4 mg starch granules (20  $\mu$ g protein); and 20  $\mu$ moles KCl or NaCl. Assay conditions as described in the text.

Substrate	Enzyme activity		
	No salt	0.1 M NaCl	0.1 M KCl
	<i>m</i> $\mu$ moles ADP or UDP/hr $\times$ $\mu$ g protein		
ADPG	0.0	0.79	4.46
UDPG	2.11	2.63	3.68

*Anion Effects.* The results of the effects of different anionic salts of potassium are presented in table IV. In the experiments where chloride, bromide, nitrate, or phosphate salts of potassium were utilized, no appreciable effects on activity were observed. When sulfate and iodide salts of potassium were added, a slight inhibition in activity was observed, but this amounts to only 15% at concentrations of 0.1 M.

Since anions have been reported (12) to affect the activity of pyruvic kinase, experiments were conducted to ascertain if these anions were affecting the pyruvic kinase portion of the assay. In these assays, the potassium and magnesium concentrations of the reaction mixtures were optimum for the activity of pyruvic kinase. The results obtained indicated that the activity of pyruvic kinase was not appreciably affected by the presence of any of these anions.

Table IV. *The Effect of Anions on the Activity of Starch Synthetase From Sweet Corn*

The reaction mixture contained 10  $\mu$ moles tricine buffer, pH 8.0; 1.0  $\mu$ mole ADPG, 4 mg starch granules (20  $\mu$ g protein), and the indicated salt concentration in a total volume of 0.2 ml. Assay conditions are described in the text.

Salt	Salt concentration (mM)						
	0	5	10	25	50	75	100
	<i>m</i> $\mu$ moles ADP/hr $\times$ $\mu$ g protein						
KCl	0	3.8	4.6	5.4	6.5	6.9	7.1
KBr	0	3.8	4.6	5.5	6.5	7.0	7.4
KI	0	3.6	4.3	5.0	5.6	5.8	5.8
KNO <sub>3</sub>	0	3.8	4.5	5.6	6.1	6.4	6.9
K <sub>2</sub> SO <sub>4</sub>	0	3.3	4.2	5.1	5.3	5.8	5.9
K <sub>2</sub> HPO <sub>4</sub> <sup>-</sup>	0	3.8	4.6	5.4	6.1	6.4	6.8
KH <sub>2</sub> PO <sub>4</sub>							

*Divalent Cations.* Experiments were conducted to determine the effects of divalent cations on the activity of starch synthetase in the presence and in the absence of added potassium (table V). The maximum concentration of divalent cation used in these experiments was 20 mM since higher divalent cation concentrations, especially for cobalt and calcium, are known to affect the activity of pyruvic kinase (12). In the presence of potassium, the divalent cations magnesium, manganese, calcium, and cobalt all showed a stimulatory effect on starch synthetase activity over the entire range of concentrations utilized. Both nickel and copper inhibited activity. In the absence of potassium, all divalent cations

Table V. *The Effects of Divalent Cations on the Starch Synthetase Activity From Sweet Corn in the Presence and Absence of Potassium*

The reaction mixtures contained the following in a total volume of 0.2 ml: 10  $\mu$ moles tricine buffer, pH 8.0, 1.0  $\mu$ mole ADPG, 4 mg starch granules (20  $\mu$ g protein), 20  $\mu$ moles KCl (when added), and the indicated divalent cation concentrations. Assays are described in the text.

Divalent cation salt	Divalent cation salt concentration (mM)					
	KCl 50 mM	0	2.5	5.0	10.0	20.0
	<i>m</i> $\mu$ moles ADP/hr $\times$ $\mu$ g protein					
MgCl <sub>2</sub>	+	4.1	4.4	5.2	5.4	6.2
MnCl <sub>2</sub>	+	4.1	4.2	4.5	4.9	5.8
CaCl <sub>2</sub>	+	4.0	4.3	4.7	5.0	5.0
CoCl <sub>2</sub>	+	4.1	4.5	4.9	5.4	5.5
NiCl <sub>2</sub>	+	4.1	4.2	4.1	3.9	3.6
CuSO <sub>4</sub>	+	3.9	3.6	3.3	3.1	2.9
MgCl <sub>2</sub>	—	0.0	0.5	0.5	0.8	0.9
MnCl <sub>2</sub>	—	0.0	0.9	1.6	2.1	3.4
CaCl <sub>2</sub>	—	0.0	0.5	1.0	1.2	1.0
CoCl <sub>2</sub>	—	0.0	0.3	0.5	0.8	0.6
NiCl <sub>2</sub>	—	0.0	0.2	0.5	0.7	0.6
CuSO <sub>4</sub>	—	0.0	0.0	0.0	0.0	0.0

Table VI. *The Effects of K<sup>+</sup> and Na<sup>+</sup> on the Activity of Glycogen Synthetase From Rat Livers*

Assay conditions are described in the text.

Salt addition	Salt concentration mM	Glycogen synthetase activity	
		Experiment I	Experiment II
<i>mμmoles UDP/min × mg protein</i>			
KCl	0.0	8.9	8.0
KCl	12.5	10.0	8.4
KCl	25.0	8.9	8.4
KCl	37.5	9.7	8.2
KCl	50.0	9.7	8.2
NaCl	0.0	8.5	8.0
NaCl	12.5	9.2	8.2
NaCl	25.0	8.5	8.2
NaCl	37.5	8.3	8.8
NaCl	50.0	8.5	8.8

except copper resulted in increased starch synthetase activity. The stimulatory effects of the divalent cations, however, are far less than the effects of potassium.

*Univalent Cations and Glycogen Synthesis.* Since starch synthetase activity was strikingly stimulated by univalent cations, the effects of potassium and sodium on the activity of particulate glycogen synthetase from rat liver was investigated. After the pH optimum and proportionality between rate of UDP formation and enzyme concentration were determined, the effects of univalent cations on the activity of the enzyme were examined. The effects of increasing concentrations of potassium or sodium on the activity of glycogen synthetase in 2 different experiments are presented in table VI. These values indicate that neither sodium nor potassium has a stimulatory or inhibitory effect on the activity of this enzyme.

## Discussion

Since potassium deficiency in plants is known to reduce starch accumulation (4), the activation of starch synthetase by potassium would support a regulatory role of this cation in the synthesis of this carbohydrate. The data presented in this investigation establish the absolute requirement for potassium in the particulate starch synthetase system from sweet corn. This enzyme shows optimum activity in the presence of 0.05 M to 0.10 M potassium, and less activity with rubidium, cesium, or ammonium. Sodium or lithium activated only slightly or not at all. These results are consistent with the reported (4) univalent cation activation of many enzymes.

In analyzing the effects of potassium on the activity of starch synthetase from bush beans, peas, soybeans, field corn, wheat, and potatoes, a similar

requirement for potassium was also demonstrated. Murata and Akazawa (14) have shown that potassium stimulates the activity of starch synthetase from roots of sweet potato, taro, white potato, as well as rice grains. The results obtained in this report as well as those by others (1, 6, 13, 15, 16) support the necessity of potassium in the formation of starch, and the regulatory nature of this cation in plants. The optimum concentrations of potassium for starch synthetase activities are equivalent to those concentrations normally found in plant cells (4).

In this investigation, the interaction of potassium and sodium with ADPG or UDPG was also examined. The enzyme utilizes both substrates, although activity is greatest with ADPG (table III). Enzyme activity was stimulated by potassium when either substrate was employed. These results are not consistent with those reported by Akatsuka and Nelson (1), but are in agreement with those of Murata and Akazawa (13).

The mechanism of potassium activation in starch synthesis remains obscure. Murata and Akazawa (13) have shown that potassium does not affect the  $K_m$  for ADPG or the  $K_i$  for ADP, and propose that potassium might influence the binding of the enzyme to the starch granule. Both Akatsuka and Nelson (1) and Murata and Akazawa (13) have reported a protective effect of potassium and of EDTA on the heat inactivation of the particulate enzyme. Frydman and Cardini (5), in examining the effects of mechanical disruption, urea, and  $\alpha$ -amylase treatments on particulate synthetase from maize, have shown that an alteration of the physical status of the enzyme-granule complex results in the complete loss of enzymatic specificity for UDPG. Akazawa and Murata (2) have reported that the soluble starch synthetase of glutinous rice forms a stable amylose-enzyme complex in the presence of amylose. It appears that the action of starch synthetase is influenced by the association of the enzyme with the starch granule and that this interrelationship might very well be affected by potassium. This line of investigation, however, requires further study.

Since the structure and formation of starch and glycogen are similar, it was decided to determine if a potassium requirement existed for the glycogen synthetase system. Nigam and Fridland (14) have reported that potassium affects the incorporation of glucose into glycogen by crude extracts of pigeon livers. The results obtained in this study show that neither potassium nor sodium has an effect on the rate of incorporation of glucose into particulate glycogen when UDPG was used as the substrate. The results of Nigam and Fridland (14), which were obtained by use of a crude homogenate, cannot be interpreted, therefore, on the basis of a direct univalent cation requirement for this enzyme. It is interesting to note that univalent cations rather than organic molecules serve as activators for starch synthesis whereas in glycogen synthesis, glucose 6-P

is an essential cofactor and no cation requirement can be demonstrated.

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