SUMMARY

1. Metabolic processes associated with free sugars, organic acids and amino acids in higher plants subjected to fluoride fumigation were studied quantitatively.

2. Fluoride-fumigated leaves contained more reducing sugars and less sucrose than the normal leaves. This result suggested inhibition of sucrose synthesis by fluoride.

3. Necrotic leaves contained increased total concentrations of organic acids, which were mostly attributable to malic acid, malonic acid and citric acid. The greater increase in malic acid relative to that of citric acid was the reverse of results observed in chlorotic tissue.

4. Necrotic leaves contained enhanced amounts of free amino acids. The greatest increase occurred in the concentration of asparagine and might be related to the increased respiratory rate of necrotic leaves. Pipecolic acid accumulated in large quantities in necrotic tissue and was not detected in normal leaves.

5. The accumulation of organic acids and amino acids in leaves during fluoride fumigation was evidenced by a lowered respiratory quotient.

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Biochemical Studies on the Effect of Fluoride on Higher Plants

2. THE EFFECT OF FLUORIDE ON SUCROSE-SYNTHESIZING ENZYMES FROM HIGHER PLANTS

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Leaves of plants that were fumigated with hydrogen fluoride contained markedly decreased concentrations of sucrose and increased concentrations of reducing sugars compared with those in leaves of control plants (Yang & Miller, 1963). Presumably, sucrose synthesis was retarded in the

fluoride-fumigated leaves. Several authors (Cardini, Leloir & Chiriboga, 1955; Leloir & Cardini, 1955; Burma & Mortimer, 1956) have shown that UDP-glucose is involved in the biosynthesis of sucrose in higher plants. In green leaves sucrose is synthesized by reactions shown in Scheme 1.

Scheme 1

Phosphoglucumutase (Cardini, 1951), uridine diphosphate glucose pyrophosphorylase (Turner & Turner, 1958), uridine diphosphate glucose-fructose transglucosylase (Cardini *et al.* 1955; Burma & Mortimer, 1956; Nakamura, 1959) have been extracted from higher plants. However, the effect of fluoride on these enzymes has not been completely elucidated. The present study was initiated to characterize the properties of partially purified phosphoglucumutase, uridine diphosphate glucose pyrophosphorylase and uridine diphosphate glucose-fructose transglucosylase, from various plant sources, with respect to activation by metal ions and inhibition by fluoride.

EXPERIMENTAL

Preparations of phosphoglucumutase. Jack-bean meal, obtained from Fisher Scientific Co., New York 14, N.Y., U.S.A., was blended with twice its weight of distilled water. This mixture was strained through four layers of cheese-cloth and fractionated with ammonium sulphate. The fraction precipitating between 50 and 65% saturation with respect to ammonium sulphate was dissolved in water and dialysed twice against water. A considerable quantity of protein was precipitated during dialysis. After centrifuging, the solution was further purified by rapid heating to 45° and was maintained at this temperature for 2 min. Through these procedures about two-thirds of the protein was removed with little loss in enzyme activity. The enzyme solution was stored at -20° and remained active for several months. Attempts to purify the enzyme further by using techniques developed for yeast phosphoglucumutase (McCoy & Najjar, 1959) were unsuccessful.

Measurement of phosphoglucumutase activity. Glucose 1-phosphate (purchased from Schwarz Laboratories, Mount Vernon, N.Y., U.S.A., and containing as an impurity the coenzyme glucose 1,6-diphosphate) was used as substrate. Reaction mixtures contained: 20 μ moles of tris-HCl buffer, pH 7.5, 10 μ moles of cysteine, various concentrations of glucose 1-phosphate, KF, and MnCl_2 or MgCl_2 (as indicated in Tables or Figures), and enzyme solution in a total volume of 0.5 ml. The reaction was started by adding the enzyme solution and incubating at 37°. The reaction was stopped by adding 2 ml. of 1.25N-perchloric acid. The remaining glucose 1-phosphate was hydrolysed by heating in a boiling-water bath for 7 min.

The hydrolysed solution was made up to 15 ml. with water, and inorganic phosphate was determined by the method of Chen, Toribara & Warner (1956). The glucose 6-phosphate formed was calculated from the decrease of acid-labile phosphate. Values were confirmed by reaction with glucose 6-phosphate dehydrogenase and NADP as followed by the increase in extinction at 340 m μ .

Preparation of uridine diphosphate glucose pyrophosphorylase and uridine diphosphate glucose-fructose transglucosylase. Acetone-dried pea-seed powder, prepared as described by Evans (1955), was homogenized in four times its weight of 0.05M-phosphate buffer, pH 7.0, and fractionated with ammonium sulphate. The fraction that was precipitated between 15 and 40% saturation with respect to ammonium sulphate was dissolved in phosphate buffer and dialysed against 0.01M-tris-HCl buffer, pH 7.0, with several changes of the buffer solution.

Measurement of uridine diphosphate glucose-pyrophosphorylase activity. The reaction mixture contained: 4 μ moles of glucose 1-phosphate, 1 μ mole of UTP, 20 μ moles of tris-HCl buffer, pH 7.4, 0.1 ml. of enzyme solution containing 40 μ g. of protein, and various concentrations of MgCl_2 and KF in a total volume of 0.5 ml. After incubation for 20 min. at 37°, the reaction was stopped by heating for 3 min. in a boiling-water bath. The UDP-glucose formed was determined as the increase in extinction of NADH_2 at 340 m μ by the action of uridine diphosphate glucose dehydrogenase (Strominger, Maxwell, Axelrod & Kalckar, 1957), which was found to be insensitive to fluoride. The reaction mixture containing the same components but not incubated was used as control. Glucose 1-phosphate, UTP, UDP-glucose and uridine diphosphate glucose dehydrogenase were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A.

Measurement of uridine diphosphate glucose-fructose-transglucosylase activity. The assay system contained: 50 μ moles of tris-HCl buffer, pH 8.0, 4 μ moles of fructose, 1 μ mole of UDP-glucose, 0.6 mg. of enzyme protein and various concentrations of MgCl_2 and KF in a total volume of 0.5 ml. The system was incubated at 37° for 1 hr. The reaction was stopped by adding 0.5 ml. of 0.4N-NaOH and heating in a boiling-water bath for 10 min. to decompose any remaining free fructose. The sucrose content was determined by the method of Roe (1949). A reaction mixture containing the same components but not incubated was used as control.

Determination of protein. The protein contents of the enzyme solutions were determined with Folin's phenol reagent (Lowry, Rosebrough, Farr & Randall, 1951).

RESULTS

Activation of phosphoglucomutase by bivalent cations. Under the conditions of the assay the rate of formation of glucose 6-phosphate remained linear with time until about 80 % of the substrate had been consumed (Fig. 1). This point was not exceeded in the various experiments reported in the present study.

In the absence of added bivalent cations, enzyme activity was about 13 and 25 % of that observed in the presence of an optimum concentration of Mn^{2+} and Mg^{2+} ions respectively (Fig. 2). The

Fig. 1. Time-activity curve of phosphoglucomutase at 37°. The reaction mixture contained: 40 mM-tris-HCl buffer, pH 7.5, 20 mM-cysteine, 1.1 mg. of enzyme protein and glucose 1-phosphate as indicated, in a total volume of 0.5 ml. Activity is expressed as the disappearance of acid-labile phosphate. ●, 2 mM-Glucose 1-phosphate; ▲, 8 mM-glucose 1-phosphate. (a) In the presence of 1 mM-MgCl₂; (b) in the presence of 1 mM-MnCl₂.

maximum rate with manganese chloride at a concentration of 0.5 mM was more than twice that obtained with optimum concentrations of magnesium chloride (1 mM). The results shown in Fig. 2 that are free of apparent inhibition yield linear Lineweaver-Burk plots, and K_m values determined were 0.24 mM for Mg^{2+} ions and 0.009 mM for Mn^{2+} ions, as compared with 0.05 mM for Mg^{2+} ions for phosphoglucomutase from muscle (Milstein, 1961). Different experimental conditions (buffer, temperature etc.) between this experiment and that of Milstein (1961) could conceivably have caused differences in Mg^{2+} ion-enzyme binding, thus affecting the value of K_m . Various bivalent cations activated the phosphoglucomutase reaction. Threefold activation was found with Mg^{2+} ions, sixfold with Mn^{2+} ions and twofold with Co^{2+} ions; Ca^{2+} , Ni^{2+} and Zn^{2+} ions were inhibitory.

Inhibition of phosphoglucomutase by fluoride. Tables 1 and 2 show the effect of different concentrations of fluoride on phosphoglucomutase activity at various concentrations of Mg^{2+} and Mn^{2+} ions. By denoting the activity without inhibitor (fluoride) as V , the activity with inhibitor as v , and the fractional inhibition as $(V-v)/v$, the consistent constant obtained by dividing the fluoride concentration by the fractional inhibition, throughout the range of fluoride concentrations used in this experiment, shows a linear relation between the concentration of fluoride and the fractional inhibition. The Mg^{2+} ion-activated system was about sevenfold more sensitive to fluoride than was the Mn^{2+} ion-activated system, as estimated from the value of $[\text{F}^-] \times v/(V-v)$ in Tables 1 and 2. Although inhibition of phosphoglucomutase by fluoride was little dependent on the concentration of Mg^{2+} or Mn^{2+}

Fig. 2. Activation of phosphoglucomutase by Mg^{2+} and Mn^{2+} ions. The reaction mixture contained: 40 mM-tris-HCl buffer, pH 7.5, 6.6 mM-glucose 1-phosphate, 20 mM-cysteine and 1.1 mg. of enzyme protein in a total volume of 0.5 ml. Incubation was at 37° for 25 min. ●, In the presence of MgCl_2 ; ▲, in the presence of MnCl_2 .

Table 1. *Inhibition of phosphoglucomutase by fluoride in the presence of Mg^{2+} ions*

The results of Fig. 3 were used for the calculation of fractional inhibition. V , Activity without inhibitor (fluoride); v , activity with inhibitor. All concentrations are expressed as mg.ions/l.

$[Mg^{2+}]$	$[F^-]$	Fractional inhibition $\left(\frac{V-v}{v}\right)$	$[F^-] \times \frac{v}{V-v}$
0.1	0	0	—
0.1	0.5	0.14	3.7
0.1	1	0.38	2.6
0.1	5	1.47	3.4
0.1	10	2.48	4.0
0.5	0	0	—
0.5	0.5	0.16	3.1
0.5	1	0.30	3.3
0.5	2.5	0.67	3.8
0.5	5	1.30	3.8
0.5	10	2.78	3.6
1	0	0	—
1	0.5	0.15	3.3
1	1	0.30	3.3
1	2.5	1.06	2.4
1	5	1.85	2.7
1	10	3.32	3.0
5	0	0	—
5	0.5	0.23	2.1
5	1	0.39	2.6
5	2.5	1.00	2.5
5	5	2.00	2.5
5	10	2.70	3.7

Table 2. *Inhibition of phosphoglucomutase by fluoride in the presence of Mn^{2+} ions*

The results of Fig. 4 were used for the calculation of fractional inhibition. V , Activity without inhibitor (fluoride); v , activity with inhibitor. All concentrations are expressed as mg.ions/l.

$[Mn^{2+}]$	$[F^-]$	Fractional inhibition $\left(\frac{V-v}{v}\right)$	$[F^-] \times \frac{v}{V-v}$
0.01	0	0	—
0.01	2.5	0.19	13
0.01	10	0.66	15
0.01	25	2.05	12
0.05	0	0	—
0.05	2.5	0.10	25
0.05	10	0.52	19
0.05	25	1.47	17
0.1	0	0	—
0.1	2.5	0.10	25
0.1	10	0.54	19
0.1	25	1.42	18
0.5	0	0	—
0.5	2.5	0.10	25
0.5	10	0.46	22
0.5	25	1.33	19
1	0	0	—
1	2.5	0.12	21
1	10	0.63	16
1	25	1.42	18

Fig. 3. Effect of different concentrations of fluoride on phosphoglucomutase activity at various concentrations of Mg^{2+} ions. The reaction mixture contained: 40 mm-tris-HCl buffer, pH 7.5, 6.6 mm-glucose 1-phosphate, 20 mm-cysteine, 1.1 mg. of enzyme protein and various concentrations of $MgCl_2$ and KF as indicated, in a total volume of 0.5 ml. Incubation was at 37° for 50 min. Concn. of KF: ○, 0; □, 0.5 mm; △, 1 mm; ●, 2.5 mm; ■, 5 mm; ▲, 10 mm.

Fig. 4. Effect of different concentrations of fluoride on phosphoglucomutase activity at various concentrations of Mn^{2+} ions. The reaction mixture contained: 40 mm-tris-HCl buffer, pH 7.5, 6.6 mm-glucose 1-phosphate, 20 mm-cysteine, 1.1 mg. of enzyme protein and various concentrations of $MnCl_2$ and KF as indicated, in a total volume of 0.5 ml. Incubation was at 37° for 25 min. Concn. of KF: ○, 0; □, 2.5 mm; △, 10 mm; ●, 25 mm.

Table 3. *Effect of glucose 1-phosphate on the inhibition of phosphoglucumutase by fluoride*

The system contained: 40 mM-tris-HCl buffer, pH 7.5, 20 mM-cysteine, 1.1 mg. of enzyme protein and other components as indicated, in a total volume of 0.5 ml. Concentrations of ions are expressed in mg./ions/l. and concentrations of glucose 1-phosphate in m-moles/l. Incubation was at 37° for 30 min. for systems containing 1 mM-glucose 1-phosphate and 15 min. for systems containing 2 mM-glucose 1-phosphate. *V*, Activity without inhibitor (fluoride); *v*, activity with inhibitor.

[Mg ²⁺]	[Mn ²⁺]	[F ⁻]	[Glucose 1-phosphate]	Glucose 6-phosphate formed (μmoles)	Fractional inhibition $\left(\frac{V-v}{v}\right)$
1	0	0	8	1.64	0
1	0	1.7	8	1.05	0.56
1	0	0	2	0.50	0
1	0	1.7	2	0.40	0.25
0	1	0	8	3.18	0
0	1	11	8	2.16	0.47
0	1	0	2	0.80	0
0	1	11	2	0.66	0.21

ions, as evidenced from the fairly constant values of $[F^-] \times v/(V-v)$ throughout the range of Mg²⁺ ion or Mn²⁺ ion concentrations shown in Tables 1 and 2, the concentration of Mg²⁺ ions for maximum activation was dependent on fluoride concentration. In the absence of fluoride or with low fluoride concentration, maximum activity was observed with 1 mM-magnesium chloride, but, in the presence of higher fluoride concentration, maximum activity was found at lower Mg²⁺ ion concentrations (Fig. 3). However, the concentration of Mn²⁺ ions for maximum activation was found to be independent of fluoride concentration (Fig. 4). This shift of the concentration of Mg²⁺ ions for maximum activation to the lower Mg²⁺ ion concentration in the presence of higher fluoride concentrations indicated that Mg²⁺ ions affected the inhibition of phosphoglucumutase by fluoride more than did Mn²⁺ ions, and the inhibition was slightly enhanced as the Mg²⁺ ion concentration increased.

In contrast with the general type of inhibition in which the inhibition is alleviated (competitive inhibition) or not affected (non-competitive inhibition) by increasing the concentration of substrate, the inhibition of phosphoglucumutase by fluoride was enhanced by increasing the concentration of substrate, glucose 1-phosphate (Table 3). However, the fractional inhibition $(V-v)/v$ was not proportional to the concentration of substrate, as reported by Najjar (1948) for muscle phosphoglucumutase.

Uridine diphosphate glucose pyrophosphorylase. The effect of Mg²⁺ ions and fluoride on uridine diphosphate glucose pyrophosphorylase is illustrated in Fig. 5. The prepared enzyme from pea seed was active without added Mg²⁺ ions. It was not possible to ascertain whether the enzyme could act in the complete absence of Mg²⁺ ions, however, inasmuch as traces of the ion present in the preparation could have been responsible for the observed

Fig. 5. Effects of Mg²⁺ ions and fluoride on uridine diphosphate glucose pyrophosphorylase. The reaction mixture contained: 8 mM-glucose 1-phosphate, 2 mM-UTP, 80 mM-tris-HCl buffer, pH 7.4, 40 μg. of enzyme protein and various concentrations of MgCl₂ as indicated, in a total volume of 0.5 ml. Incubation was at 37° for 20 min. ○, In the absence of KF; △, in the presence of 10 mM-KF.

activity. Although maximum enzyme activation was found with 10 mM-magnesium chloride, the enzyme was completely insensitive to fluoride at concentrations as high as 10 mM.

Uridine diphosphate glucose-fructose transglucosylase. The effect of Mg²⁺ ions and fluoride on uridine

Fig. 6. Effects of Mg^{2+} ions and fluoride on uridine diphosphate glucose-fructose transglucosylase. The reaction mixture contained: 100 mM-tris-HCl buffer, pH 8.0, 8 mM-fructose, 2 mM-UDP-glucose, 0.6 mg. of enzyme protein, and various concentrations of $MgCl_2$ and fluoride, in a total volume of 0.5 ml. Incubation was at 37° for 1 hr. Concn. of KF: $\circ$, 0; $\square$, 1 mM; $\bullet$, 10 mM.

diphosphate glucose-fructose transglucosylase is illustrated in Fig. 6. The prepared enzyme from pea seed was active in the absence of added Mg^{2+} ions, and the presence of magnesium chloride at concentrations of 10–100 mM increased the activity by only 45%. The degree of activation by Mg^{2+} ions was not large, but no inhibiting effect was exerted by magnesium chloride even at a concentration of 100 mM. Since the presence of K^+ ions at concentrations of up to 100 mg.ions/l. did not activate the enzyme activity in any degree, the activation effect of Mg^{2+} ions was not due to an ionic strength effect.

The enzyme was noticeably inhibited by fluoride at concentrations of 1 or 10 mM, but the enhancing effect of Mg^{2+} ions was not completely suppressed by fluoride. With the enzyme preparation used in this experiment the activity of uridine diphosphate glucose-fructose transglucosylase was 20-fold greater than that of uridine diphosphate glucose-fructose 6-phosphate transglucosylase. Free fructose might be liberated from fructose 6-phosphate by the action of contaminating phosphatase, and could form sucrose by the action of uridine diphosphate glucose-fructose transglucosylase. Therefore the true uridine diphosphate glucose-fructose 6-phosphate-transglucosylase activity could have been less than the low value already observed.

DISCUSSION

All the three enzymes, phosphoglucomutase, uridine diphosphate glucose pyrophosphorylase and uridine diphosphate glucose-fructose transglucosylase, were activated by Mg^{2+} ions; however, their responses to fluoride inhibition were different. Phosphoglucomutase was very sensitive to fluoride inhibition; uridine diphosphate glucose-fructose transglucosylase showed some fluoride inhibition; uridine diphosphate glucose pyrophosphorylase

manifested an almost complete requirement for Mg^{2+} ions, but no fluoride inhibition was exhibited.

In contrast with the finding by Cardini *et al.* (1955) that Mg^{2+} ions were without effect on uridine diphosphate glucose-fructose transglucosylase, our pea-seed enzyme preparation showed a 45% activation by 10 mM-magnesium chloride (Fig. 6). Bean & Hassid (1955) also found that Mg^{2+} ions (1 mg.ion/l.) stimulated sucrose synthesis from UDP-glucose and fructose by 30% in their partially purified pea-enzyme extract, but they attributed the effect of Mg^{2+} ions to the formation from fructose of fructose 6-phosphate, which led to the formation of sucrose phosphate by the action of uridine diphosphate glucose-fructose 6-phosphate transglucosylase. This reasoning would be invalid, however, in interpreting our results, which showed little, if any, uridine diphosphate glucose-fructose 6-phosphate transglucosylase activity that was too low to contribute to the 45% increase of sucrose synthesis exhibited on the addition of Mg^{2+} ions. The conclusion thus is made that uridine diphosphate glucose-fructose transglucosylase itself was activated by Mg^{2+} ions and inhibited to a slight degree by fluoride.

Of the three enzymes studied, only phosphoglucomutase was very sensitive to fluoride. It is likely that the inhibition of sucrose synthesis in fluoride-fumigated plants might be due to the inhibition of phosphoglucomutase, which plays an important role in carbohydrate metabolism. However, at present, there is insufficient evidence to show the inhibition of phosphoglucomutase *in vivo* by fumigation with hydrogen fluoride.

Activation by metal ions and inhibition by fluoride of phosphoglucomutase. The activation-inhibition curves of Mg^{2+} and Mn^{2+} ions (Fig. 2) coincide with eqn. (1), derived by assuming that metal ions interact with two different sets of binding sites on the enzyme, and that the combination at the second set leads to inhibition (Malmström, 1953).

$$V = \frac{kK_2m}{(K_1+m)(K_2+m)} \quad (1)$$

In this equation k is a constant (representing the theoretical value of V_{max} in the absence of inhibition), K_1 and K_2 represent the apparent dissociation constants for two types of complex, and m is the concentration of metal ions. The estimated values of K_1 and K_2 from Fig. 2 were 0.28 and 5.47 mM respectively for Mg^{2+} ions, and 0.009 and 29 mM for Mn^{2+} ions. By applying this equation, the metal ion concentration at maximum activation was estimated to be 1.3 mM for Mg^{2+} ions and 0.54 mM for Mn^{2+} ions.

In our partially purified enzyme from jack bean, Mn^{2+} ion was more than twice as effective as Mg^{2+} ion for enzyme activation; in the crude muscle

enzyme, Mn^{2+} and Mg^{2+} ions were equally effective activators at their optimum concentrations (Cori, Colowick & Cori, 1938); and, with purified yeast enzyme, Mg^{2+} ion was more effective as an activator than was Mn^{2+} ion (McCoy & Najjar, 1959).

The constant value obtained in Tables 2 and 3 for $[F^-] \times v/(V-v)$ at various fluoride concentrations indicates that 1 mol.prop. of fluoride is involved in the inhibition. The results indicate further that inhibition by fluoride is almost independent of the activating metal ions, Mg^{2+} or Mn^{2+} ions. The results in Table 4 show that fractional inhibition was increased by increasing the concentration of substrate, glucose 1-phosphate, but the inhibition was not proportional to the substrate concentration. These results clearly indicate that the inhibition of phosphoglucumutase from jack bean by fluoride was not the type that Najjar (1948) proposed for muscle phosphoglucumutase, in which the inhibition was proportional to $[Mg^{2+}] \times [\text{glucose 1-phosphate}] \times [F^-]^2$, i.e. 1 mol.prop. each of Mg^{2+} ion and glucose 1-phosphate and 2 mol.prop. of fluoride are involved in the inhibition.

Activation by metal ions may be due to a reaction of the metal ion with the enzyme, with the substrate, or with the enzyme-substrate complex (Malmström & Rosenberg, 1959). Based on the kinetic data, possible mechanisms of fluoride inhibition along the alternative mechanisms by metal-ion activation are examined.

Mechanism I. Complex-formation between enzyme and metal ion:

In these equations: E is enzyme, S is substrate, P is product, M is metal ion, and k_1 , k_{-1} , k_2 , k_{-2} and k_3 are rate constants. The suggested inhibition mechanism is:

where F represents inhibitor, fluoride. Solving eqns. (2), (3), (4) and (5) by assuming eqns. (2), (4) and (5) are in equilibrium and eqn. (3) or EMS in steady state, the following equations are obtained for the inhibited rate and fractional inhibition:

$$v = \frac{k_3 K_4 K_5 ems}{K_m (K_1 K_4 K_5 + K_4 K_5 m + K_1 msf) + K_4 K_5 ms} \quad (6)$$

$$\frac{V-v}{v} = \frac{K_1 K_m msf}{K_4 K_5 [ms + K_m (K_1 + m)]} \quad (7)$$

where e is total enzyme concentration, K_m is $(k_{-2} + k_3)/k_2$, K_1 is k_{-1}/k_1 , K_4 is k_{-4}/k_4 , K_5 is k_{-5}/k_5 , v is rate without fluoride, and s , m and f are concentrations of glucose 1-phosphate, metal ion and fluoride respectively. Eqn. (7) coincides with the experimental results in that the fractional inhibition, $(V-v)/v$, is proportional to fluoride concentration, is little dependent on metal ion concentration and increases as substrate concentration increases.

According to eqn. (7), the larger the value of K_1 relative to the concentration of metal ion, m , the larger the fractional inhibition becomes with increasing m . If K_1 is much smaller than m , the fractional inhibition becomes independent of the metal ion concentration. The estimated dissociation constant of enzyme-metal ion complex, K_1 , from eqn. (1) by using the results of Fig. 2, was 0.28 mM for Mg^{2+} ion and 0.009 mM for Mn^{2+} ion. With activation by Mg^{2+} ions at concentrations comparable with the estimated K_1 value for Mg^{2+} ion (0.28 mM), it can be expected from eqn. (7) that the fractional inhibition by fluoride slightly increases as the Mg^{2+} ion concentration increases. This prediction coincides with the results of Fig. 3, in which the Mg^{2+} ion concentration for maximum activation shifted to the lower Mg^{2+} ion concentration as the fluoride concentration increased. In the Mn^{2+} ion-activated system, where the Mn^{2+} ion concentration used under the present conditions was larger than the estimated K_1 value for Mn^{2+} ion (0.009 mM), it could be expected from eqn. (7) that the fractional inhibition by fluoride is independent of Mn^{2+} ion concentration. This prediction again coincides with the results of Fig. 4, where the Mn^{2+} ion concentration for maximum activation was not shifted as the fluoride concentration increased.

Mechanism II. Complex-formation between enzyme and substrate:

Several alternative mechanisms of fluoride inhibition were examined and the following inhibition mechanism was found to explain the experimental results satisfactorily.

Solving eqns. (8), (9), (10) and (11) by assuming that ES and ESM are in steady state and eqns. (10)

and (11) are in equilibrium, the following inhibited rate and fractional inhibition are obtained:

$$v = \frac{k_3 K_4 K_5 e m s}{f s (K_4 m + K_5 K_m) + g K_4 K_5 m + K_1 K_4 K_5 K_m + K_4 K_5 m s + K_4 K_5 K_m s} \quad (12)$$

where g is k_3/k_1 , and K_4 , K_5 and K_m are as defined above; and

$$\frac{V-v}{v} = \frac{sf(K_4 m + K_5 K_m)}{K_4 K_5 [m(g+s) + K_m(K_1+s)]} \quad (13)$$

Since the Mg^{2+} ion-enzyme system is more sensitive to fluoride than is the Mn^{2+} ion-enzyme system, we may assume that K_5 of the Mg^{2+} ion-enzyme system is smaller than that of the Mn^{2+} ion-enzyme system. If so, it can be further predicted from eqn. (13) that increasing the concentration of the metal ion would prove more inhibitory for the Mg^{2+} ion-enzyme system than for the Mn^{2+} ion-enzyme system. This effect is readily observed in Figs. 3 and 4. Eqn. (13) is consistent with the experimental results in that fractional inhibition is proportional to fluoride concentration, is little dependent on the concentration of metal ions and increases as the substrate concentration increases.

A hybrid mechanism of I and II, i.e. combined reaction paths of eqns. (2), (3), (8), (9), (10) and (11), is kinetically not distinguishable from mechanism II [eqns. (8)–(11)], since the ESM complex which combines with fluoride in eqn. (11) may either be derived through mechanism I [eqns. (2) and (3)] or mechanism II [eqns. (8) and (9)]. From the kinetic study of the dependence of the apparent Michaelis constant for the metal ion on the substrate concentration, Milstein (1961) suggested the hybrid mechanism of I and II, i.e. eqns. (2), (3), (8) and (9), for the activation of muscle phosphoglucomutase by Mg^{2+} ions.

SUMMARY

1. Phosphoglucomutase, uridine diphosphate glucose pyrophosphorylase and uridine diphosphate glucose-fructose transglucosylase, which are involved in sucrose synthesis, were extracted from higher plants, partially purified and characterized with respect to activation by metal ions and inhibition by fluoride.

2. All three enzymes required Mg^{2+} ions for maximum activation but differed in their sensitivity to fluoride. Phosphoglucomutase was

inhibited most markedly by fluoride. Uridine diphosphate glucose-fructose transglucosylase was inhibited slightly at higher fluoride concentration, whereas uridine diphosphate glucose pyrophosphorylase was completely insensitive to fluoride.

3. Mn^{2+} ions gave twice as great an activation of phosphoglucomutase as did Mg^{2+} ions. The inhibition of phosphoglucomutase by fluoride was proportional to the fluoride concentration, was enhanced by increasing the substrate concentration and was almost independent of the concentration of activating metal ions. Phosphoglucomutase was inhibited sevenfold more by fluoride when Mg^{2+} ion served as the activator than when Mn^{2+} ion was activator.

4. Based on the kinetic data, possible mechanisms of the activation by metal ions and the inhibition by fluoride of phosphoglucomutase are examined.

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