Plant Physiol. (1973) 51, 960-966

Hexokinase from Maize Endosperm and Scutellum[1]

Received for publication December 29, 1972

EDWARD L. COX AND DAVID B. DICKINSON
Department of Horticulture, University of Illinois, Urbana, Illinois 61801

ABSTRACT

Hexokinase (EC 2.7.1.1) was isolated from endosperm and scutellum of developing and germinating maize (*Zea mays*) seeds. With fructose as the variable substate, Michaelis constant values for the scutellum enzyme were about one-third those of the endosperm enzyme (0.05 *versus* 0.15 mM), and no developmental differences were observed. With glucose as the variable substrate, Michaelis constant values were all in the range 0.1 to 0.2 mM. The enzyme preparation from germinating scutellum was studied further; when glucose was varied over a wide range, a Michaelis constant of 3.4 mM was observed in addition to the much lower Michaelis constant noted above. This low affinity binding of glucose may have regulatory significance and may indicate the presence of a glucokinase in addition to hexokinase.

The hexokinase reaction represents the first step in the incorporation of hexoses into respiratory and biosynthetic pathways of plants and animals. Mammalian hexokinase (EC 2.7.1.1) has been studied intensively and is particularly complex, having regulatory properties (22) and tissue specific isozymes (13, 27). Most noteworthy of the isozymes is the liver glucokinase of nonruminant animals which is thought to regulate blood glucose levels (2, 5). Its relatively high Michaelis constant (Km = 10–40 mM glucose) is related to the high levels of glucose found in liver and in the blood. There is also information concerning hexokinase from yeast (7, 23) and various tissues of higher plants (17, 25).

Hexokinase in seeds of cereal grains is of particular interest. In developing seeds, this enzyme is the first step in starch biosynthesis because translocated sucrose is hydrolyzed to its constituent hexoses prior to absorption by endosperm tissue (28). In germinating seeds, starch hydrolysis produces glucose which is converted to sucrose in the scutellum (9). Hence, scutellum hexokinase is important during germination as the first step in conversion of glucose to sucrose, and fructose would not be a major substrate during germination.

The relatively few studies of hexokinase from seeds of cereal grains include reports on properties of the wheat germ enzyme (18, 25) and a Ph.D. thesis concerned with the maize scutellum enzyme (12). Maize endosperm hexokinase increases rapidly at the time of starch accumulation (3, 30) and is present at a relatively low level during germination (3). However, there seems to be no published information on kinetic properties of the endosperm enzyme, and hence no knowledge whether the endosperm and scutellum enzymes differ kinetically because of metabolic differences within the tissues. Some kinetic properties of hexokinase from maize endosperm and scutellum are presented in this paper.

MATERIALS AND METHODS

Chemicals. The chemicals used were of reagent grade and were dissolved in glass distilled water. Chemicals purchased from Sigma Chemical Company were N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES buffer), glucose-6-P, glucose-1-P, fructose-6-P, phosphoenolpyruvate, NADP, ATP, ADP, glucose-6-P dehydrogenase (type VII from baker's yeast), lactic dehydrogenase (type III from beef heart), phosphoglucoisomerase (grade III from yeast), and pyruvate kinase (type II from rabbit skeletal muscle). The enzymes were all crystalline suspensions in ammonium sulfate. DEAE[2]-cellulose (DE-52, microgranular, preswollen) and DEAE-cellulose paper discs (DE-81, 2.5 cm diameter) were Whatman products purchased from Reeve Angel (Clifton, N.J.). The DE-52 resin was prepared for use according to the manufacturer's directions and was equilibrated with CO_2-free 5 mM potassium phosphate, pH 8.0, containing 0.1 mM EDTA. Other reagents included dithiothreitol and Aquacide from Calbiochem. and Glucostat from Worthington Biochemical Corp. Uniformly labeled D-(^{14}C) glucose, 210 mc/mmole and D-(^{14}C) fructose, 210 mc/mmole were obtained from International Chemical and Nuclear Corp.

Plant Materials. The tissues used in these experiments were from developing and germinating seeds of *Zea mays* L. Hybrid seeds were produced from inbred lines by hand pollinating the silks of line WF9 with pollen from line M14 to ensure that the tissues were of known genotype. Developing seeds were harvested 25 days after pollination. Individual kernels were removed at random from the cob, rinsed in cold distilled water, blotted dry, frozen in liquid nitrogen, and stored at −20 C. The seeds were dissected by allowing them to partially thaw until the seed coats and scutellum could easily be separated from the endosperm. Mature seeds were surface sterilized with 2% NaOCl and germinated with 0.1 mM $CaCl_2$ in the dark at 28 ± 2 C (10). After 5 days in darkness the seeds were dissected into shoot, scutellum, and endosperm (including seed coats). Scutellum and endosperm portions were used for these experiments.

Preparation of Cell-free Extracts. The tissue was ground into a fine powder under liquid nitrogen with a mortar and pestle. Grinding time was 2 to 4 min. The frozen powder was then extracted by stirring for 30 min at 0 C with ice-cold extraction medium (2 ml medium/g powder). The medium contained 50 mM potassium phosphate (pH 7.5), 5 mM $MgCl_2$, 1 mM EDTA,

[1] This work was supported by National Science Foundation Grant GB8764 and the Illinois Agriculture Experiment Station.

[2] Abbreviations: DEAE: diethylaminoethyl; DTT-buffer: dithiothreitol buffer.

10 mM KCl, and 1 mM dithiothreitol (3). All subsequent steps were done at 0 to 4 C. The extract from developing endosperm was immediately centrifuged (40,000*g*, 20 min); the brei from germinating endosperm was squeezed through eight layers of cheesecloth prior to centrifuging. The scutellum extracts were centrifuged at 2,000*g* for 1 hr to consolidate the lipid into films which were removed by pouring the supernatant fluids through pads of glass wool. The filtrates were then centrifuged (40,000*g*, 20 min). The supernatant fluids were assayed for hexokinase and were the starting point in studies of enzyme purification. The possibility of particulate hexokinase in scutellum was tested by suspending the 2,000*g* pellets in small volumes of extraction medium, centrifuging at low speed (395*g*, 10 min), and assaying the supernatant fluid.

Spectrophotometric Enzyme Assays. Maize hexokinase was assayed spectrophotometrically at 340 nm by adding purified glucose-6-P dehydrogenase to link the production of glucose-6-P to reduction of NADP (29). The reactions were run at 30 C in microcells (1-cm light path) containing 0.2 ml of reaction mixture. The contents of this reaction mixture included maize extract, 150 milliunits of glucose-6-P dehydrogenase and, in μmoles, HEPES buffer (pH 8.0), 10; $MgCl_2$, 0.5; ATP, 0.5; glucose, 1; NADP, 0.2. This assay was used for hexokinase purification and for glucose saturation curves at high levels of glucose.

ADP inhibits maize scutellum hexokinase (12). The possibility that inhibitory amounts of this compound were produced during the spectrophotometric assay was checked by including the ADP-trapping reactions (pyruvate kinase, lactic dehydrogenase) used in studies of yeast hexokinase (15). In the present study, no stimulation of hexokinase occurred when the trapping reaction was included at pH 8.1 or 6.6 so the ADP-trapping enzymes were omitted in subsequent studies. Phosphorylation of fructose was measured by substituting fructose and 200 milliunits of phosphoglucoisomerase for glucose in the standard reaction mixture.

Several maize enzymes were assayed at the same temperature and pH as hexokinase to obtain an estimate of their activity in the hexokinase assays. Phosphatase (EC 3.1.3.1) activity was measured by incubating an aliquot of maize enzyme with 10 μmoles of HEPES buffer, 0.5 μmole of $MgCl_2$, and 1 μmole of glucose-6-P for 30 min in a 0.2-ml reaction mixture. The reaction was terminated by heating for 1 min at 100 C. Glucose released during incubation was measured with the Glucostat reagent. Glucose-6-P dehydrogenase (EC 1.1.1.49) was assayed at 340 nm by adding 0.1 μmole of NADP to the phosphatase reaction mixture. Phosphoglucomutase (EC 2.7.5.1) and glucosephosphate isomerase (EC 5.3.1.9) were estimated with the same reaction mixture by adding 200 milliunits of glucose-6-P dehydrogenase and substituting 1 μmole of glucose-1-P or fructose-6-P for glucose-6-P.

Isotopic Assays. The standard isotopic (21) reaction mixture contained the following in μmoles: HEPES, (pH 8.0), 5; $MgCl_2$, 0.25; ATP, 0.25; and ^{14}C-glucose, 0.1. Total volume was 0.1 ml in 10- × 75-mm test tubes. ^{14}C-Glucose was varied or ^{14}C-fructose was substituted for glucose in some experiments. ATP saturation curves employed the standard reaction mixture with 5 mM $MgCl_2$. Reactions were initiated with enzyme, incubated 10 min at 30 C, and terminated by heating 30 sec at 100 C. Labeled hexose phosphate was separated from unreacted substrate with anion exchange paper. Twenty μl of each reaction mixture was put in the center of a DEAE-cellulose paper disc and placed, with two additional discs underneath, in a washing apparatus (8). Each sample was washed with 400 ml of glass distilled H_2O, pH 7.1, at a flow rate of 15 to 20 ml/min. The discs were dried 1.5 hr at 60 C in a forced air oven, and radioactivity was measured in a scintillation counter at about 64% counting efficiency as described earlier (8). Recovery of labeled product was checked by adding known amounts of authentic ^{14}C-glucose-6-P to standard reaction mixtures containing ^{12}C-glucose instead of ^{14}C-glucose. Comparison of washed *versus* unwashed DEAE-discs showed that 88 to 91% of applied radioactivity was retained during the standard washing procedure. Results of isotopic assays were not corrected for this small loss. The amount of phosphorylated product was calculated from the specific radioactivity of the ^{14}C-glucose (7468 cpm/nmole) or ^{14}C-fructose (2850 cpm/nmole) in the reaction mixture. The radioactivity of complete reaction mixtures was corrected by subtraction of radioactivity of controls which lacked enzyme or ATP. This assay was extremely sensitive and well suited for the present study in which limited amounts of purified enzyme were available.

Hexokinase Purification. After isolation as described above, each maize hexokinase preparation was immediately chromatographed on DEAE-cellulose (Table I). Sufficient DEAE-cellulose was packed into 8-mm diameter glass columns to give 2 ml of resin/ml of tissue extract—a maximum of 4 mg of protein/ml resin. The columns were equilibrated with 5 mM potassium phosphate, pH 8.0, containing 0.1 mM EDTA and 1 mM dithiothreitol. Enzyme was loaded onto the column and washed with DTT-buffer until absorbance approached zero. No

Table I. *Partial Purification of Hexokinase from Maize Endosperm and Scutellum*

Fraction	Parameter	Endosperm		Scutellum	
		Developing	Germinating	Developing	Germinating
40,000*g* supernatant fluid	Volume (ml)	8.8	10.0	3.1	5.1
	Hexokinase (milliunits)[1]	1716.	323.	2838.	3679.
	Protein (mg)[2]	30.0	22.3	23.0	28.8
	Specific activity (milliunits/mg protein)	57.	22.3	123.	128.
DEAE-cellulose	Volume (ml)	19.4	15.6	19.2	19.2
	Hexokinase (milliunits)	407.	70.7	185.	686.
	Protein (mg)	1.58	0.61	1.36	2.91
	Specific activity (milliunits/mg protein)	258.	116.	136.	236.
	Purification (-fold)	4.5	5.2	1.1	1.8
	Yield (%)	23.7	21.9	6.5	18.6

[1] One milliunit = 1 nmole product formed/min.

[2] Lowry protein was determined as described earlier (3).

hexokinase was detected in these fractions. Adsorbed hexokinase was eluted with a linear gradient of KCl in DTT-buffer, and each tissue gave a single peak of enzyme activity at 120 to 165 mM KCl. The eluted enzyme fractions were pooled and concentrated by dialysis against Aquacide III or ultrafiltration in an apparatus sold by Biomed Instruments, Inc. (Chicago, Ill.). Hexokinase from germinating scutellum was not affected by the concentration step, but a loss of 56 to 80% occurred with enzyme from endosperm and developing scutellum. The concentrated preparations were stored in liquid nitrogen and remained stable for several months.

Substrate Saturation Curves. Two levels of enzyme were included for each substrate concentration when the isotopic assay was used, so each point in the substrate saturation curves is an average of two values. Two levels of enzyme were assayed at selected substrate concentrations in the spectrophotometric assays to make sure that velocity was proportional to enzyme concentration. Kinetic constants were calculated from plots of S/v *versus* S in which the x-intercept equals $-Km$, and V_{max} is the reciprocal of the slope (6). The best fitting straight lines were calculated by the least squares method. The curves in plots of v *versus* S were derived by substituting numerical values for Km and V_{max} in the hyperbolic form of the Michaelis-Menten equation and then solving for v at various values of S.

RESULTS

Intracellular Distribution of Hexokinase. Only 20% of the enzyme from developing scutellum sedimented between 395 and 40,000*g*, and a similar particulate fraction from germinated scutellum contained no activity besides that due to occluded supernatant fluid. Earlier studies with maize endosperm (4) showed that less than 10% of the hexokinase was particulate, in contrast with studies of the lentil root enzyme (19). Hexokinase from several plant tissues is associated with mitochondria when an osmoticum is included in the isolation medium (20, 25). An osmoticum was not used in the present study because the goal was a kinetic characterization of the soluble enzyme.

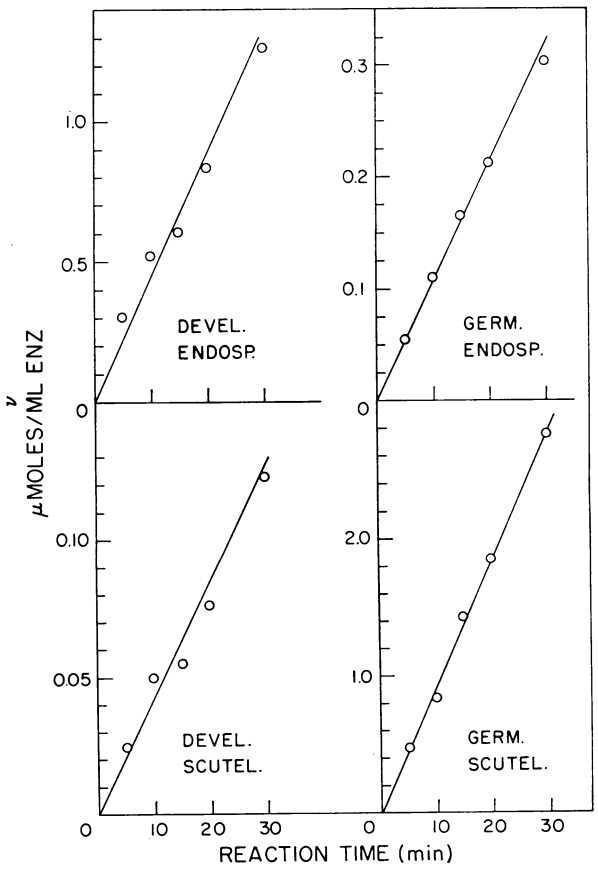


FIG. 1. Hexokinase reaction progress curves. The standard isotopic reaction mixture was used. Each point is the mean of two assays containing 10 and 20 μl of enzyme preparation and normalized to product formed/ml enzyme.

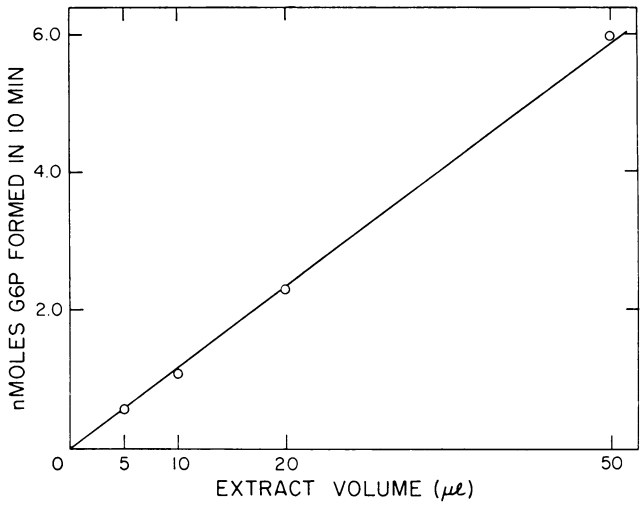
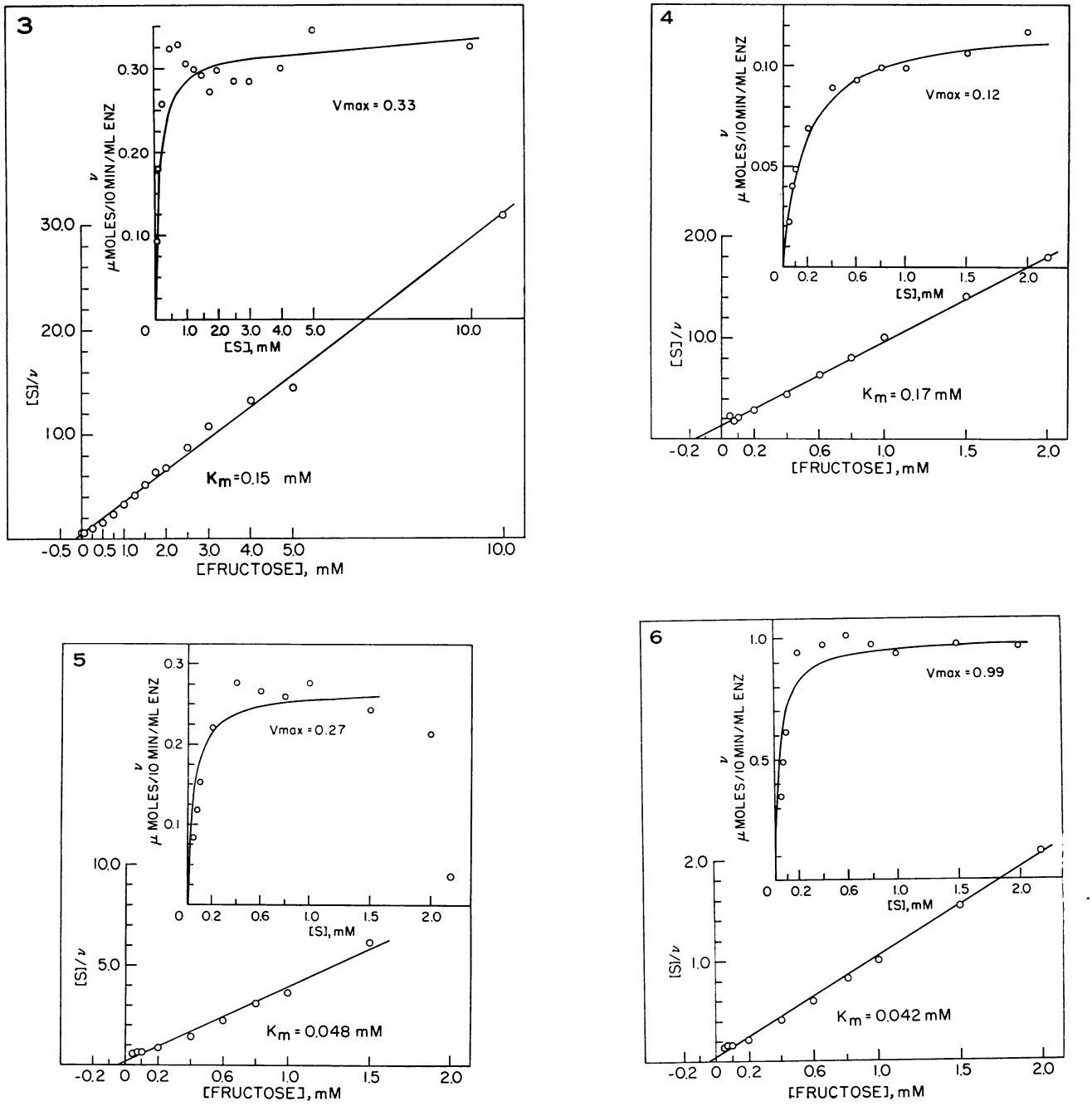


FIG. 2. Proportionality of product formation with volume of hexokinase preparation. The standard isotopic reaction mixture was used.

Hexokinase Purification. Kinetic studies of mammalian hexokinase have been done with crude extracts prepared by homogenization followed by centrifugation (1, 14, 32). This is not feasible with the maize enzyme because of phosphatase activity in the crude extract. Maize hexokinase proved difficult to purify because of low enzyme activity (endosperm), limited tissue (scutellum), and instability of the isolated enzyme. Ammonium sulfate precipitation caused extensive inactivation; protamine fractionation was unsatisfactory because the protamine precipitated ATP in the reaction mixture; the enzyme was not heat-stable even when substrates were added. Finally, the crude extracts were chromatographed directly on DEAE-cellulose, and the eluted enzyme stored in liquid nitrogen (see "Materials and Methods"). The four partially purified preparations were used in subsequent studies. In all preparations the activity of phosphatase was reduced to less than 5% of hexokinase activity, and NADP reductase was absent. The reaction rate was constant for at least 30 min with all four hexokinase preparations (Fig. 1). Activity was also proportional to amount of enzyme for germinated endosperm (Fig. 2) and the other three preparations (data not shown).

Kinetics of Low Hexose Concentrations. Initial kinetic studies were conducted to learn whether scutellum and endosperm hexokinases differ in their affinities for glucose or fructose and whether developmental differences exist. Preliminary experiments indicated that the preparations possessed Km values of approximately several tenths millimolar or less so the range of hexose concentrations for saturation curves was chosen accordingly. The upper limit was several millimolar ^{14}C-hexose because the blank values increased in proportion to the hexose concentration, progressively decreasing the sensitivity of the isotopic assay.

The dependence of velocity on concentration of fructose is shown in Figures 3 through 6. For each tissue the fructose



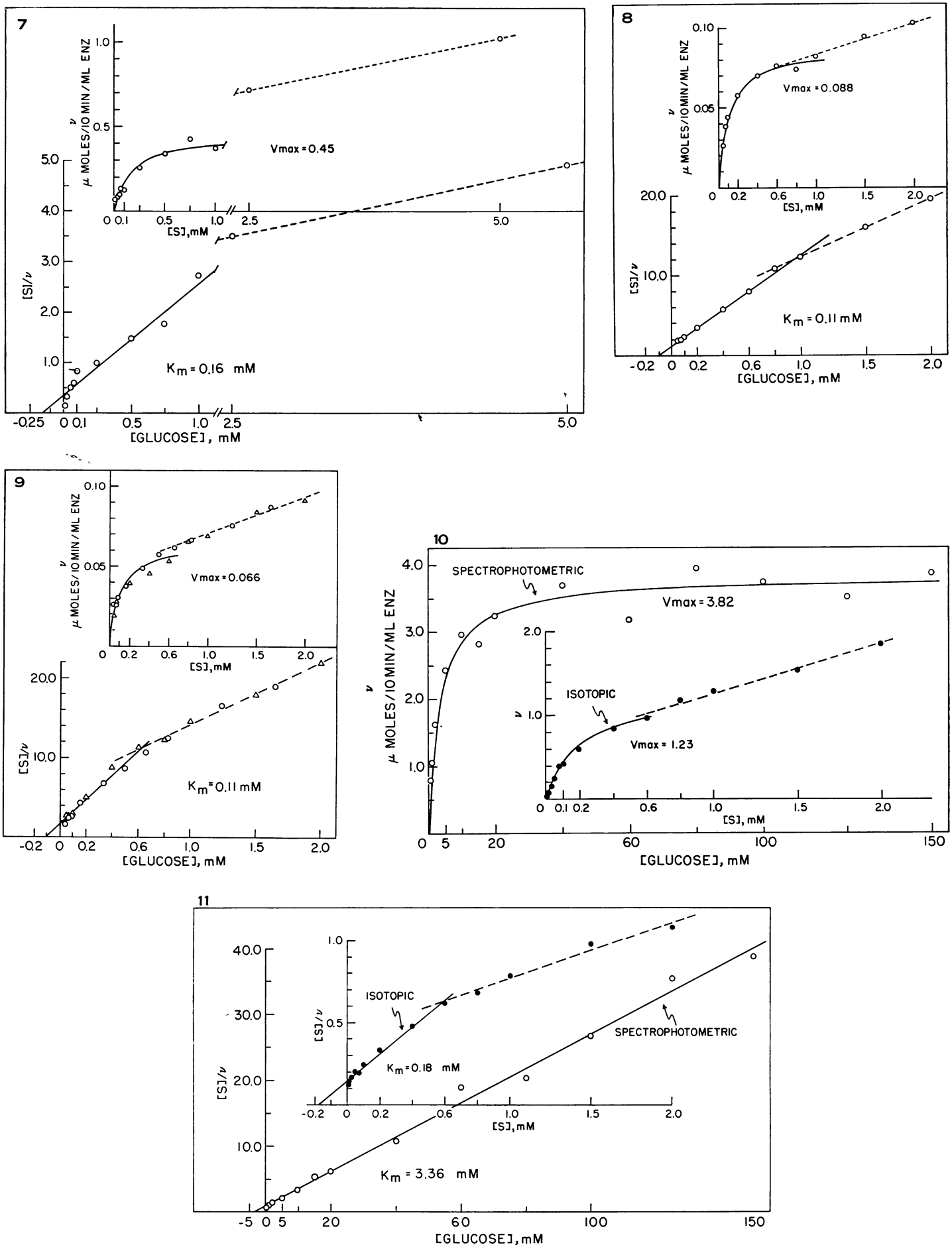
FIGS. 3 to 6. Fructose saturation curves and reciprocal plots for hexokinase from endosperm and scutellum. The standard isotopic assay was used. Each point is the mean of two assays containing different amounts of enzyme. *3*, Developing endosperm; *4*, germinated endosperm; *5*, developing scutellum; *6*, germinated scutellum.

saturation curves were hyperbolic because points in the reciprocal plots fit a straight line. No developmental differences were observed, but there were differences between tissues. K*m* values for the scutellum enzyme were about 3-fold lower than the values for the endosperm enzyme (0.04–0.05 mM *versus* 0.15–0.17 mM).

There are apparently no other reports of the affinity of maize endosperm hexokinase for fructose, but there is information concerning the scutellum enzyme. Jones (12) reported a fructose K*m* of 1.6 mM for the enzyme isolated from scutellum of germinating seeds (variety Funk's G-76). This value is considerably higher than was obtained in the present study and may be a varietal difference.

The dependence of velocity on concentration of ^{14}C-glucose is shown in Figures 7 through 9 and the inset of Figure 10. The four reciprocal plots of the same data are given in Figures 7 through 9 and in Figure 11. At lower glucose concentrations —0.6 mM or less for scutellum preparations and 1 mM or less for endosperm preparations—the reciprocal plots gave good fits to straight lines. The apparent K*m* values showed no consistent developmental or tissue-specific differences and were all in the range 0.11 to 0.18 mM. These values are somewhat lower than the K*m* values 0.3 to 0.6 mM for glucose exhibited by the various isozymes of yeast hexokinase (23) and the value of 0.44 mM reported for wheat germ hexokinase (25).

The stability of the maize enzyme during frozen storage is



FIGS. 7 to 11. Glucose saturation curves and reciprocal plots for hexokinase from endosperm and scutellum. *7*, Developing endosperm, isotopic assay; *8*, germinated endosperm, isotopic assay; *9*, developing scutellum, isotopic assay; *10*, germinated scutellum, dependence of velocity on glucose: *11*, germinated scutellum, reciprocal plots were calculated from data in Fig. 10.

demonstrated in Figure 9 where one saturation curve (○) is superimposed on a second saturation curve (△) done 2 months later.

Kinetics of High Glucose Concentrations. In the isotopic assays (Figs. 7–10), the highest levels of glucose gave points on reciprocal plots which deviated from the line fitting the lower points. The deviant values are connected by a dotted line. The hexokinase from scutellum of germinating seeds was used in a study of this departure from first order kinetics.

The spectrophotometric hexokinase assay was used to study the dependence of velocity on high glucose concentrations (Fig. 10; reciprocal plot, Fig. 11). Velocities measured by this assay averaged 15% lower than those obtained with the isotopic assay at equivalent glucose concentrations (0.5, 1.0, 2.0 mM). The data reveal a second class of glucose-binding sites having an apparent K*m* of about 3.4 mM. This value is 19-fold higher than the apparent K*m* calculated for the same enzyme preparation assayed at low glucose concentrations. At 1 to 2 mM glucose, the low affinity binding site becomes sufficiently active to cause the deviation from first order kinetics noted in the isotopic assays.

Hexokinase from the scutellum of developing seeds also possessed a low affinity site for glucose, and maximal velocity was reached at 80 to 100 mM glucose. However, this enzyme was not stable at 0 C during the time required for spectrophotometric assays at various glucose concentrations. Enzyme activity decreased with time when assayed at 40 mM glucose or less, but *increased* with time when assayed at 60 mM glucose or higher.

Biphasic glucose saturation curves were also observed in studies of hexokinase from brain (1) and liver (32). The complex kinetic pattern of liver preparations is due to a glucokinase with a K*m* of 10 to 30 mM and three hexokinases with glucose K*m* values of 0.2 mM or less (13, 32). Such complex kinetics seem not to have been reported for higher plant hexokinase with the possible exception of the enzyme from *Hevea* latex (11). The 6-point saturation curve for the *Hevea* enzyme seemed to reach a plateau at 0.3 to 3 mM glucose, yet the velocity increased about 4-fold when glucose increased from 3 to 67 or 267 mM.

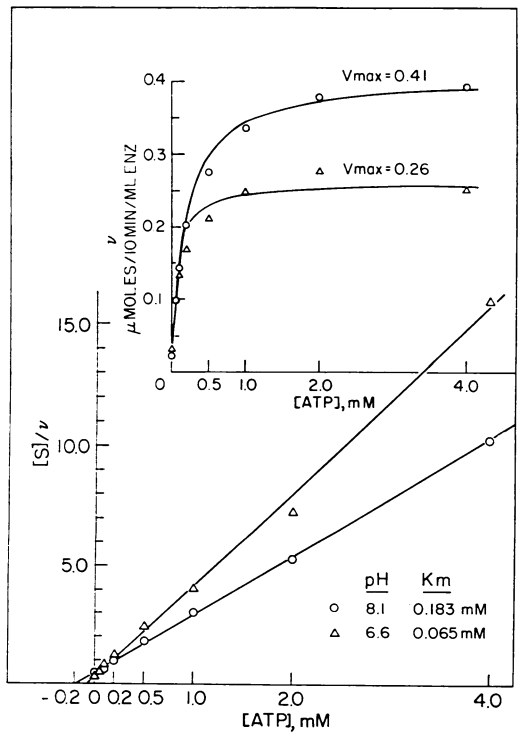


FIG. 12. Hexokinase from developing endosperm, dependence of velocity on ATP. The isotopic assay was used (see "Materials and Methods"), and each point represents a single assay with 20 μl enzyme.

Dependence of Velocity on ATP. Hexokinase from developing endosperm gave ATP saturation curves which were hyperbolic (Fig. 12). The K*m* at pH 8.1, 0.18 mM, was somewhat higher than the value of 0.08 mM reported by Jones (12) for maize scutellum. Reducing the pH of the reaction by 1.5 units reduced V_{max} by 36% and decreased K*m* some 3-fold. Decreased pH also caused reductions in these kinetic constants when the P_{II} isozyme of yeast hexokinase was assayed at low ATP concentrations (16). Addition of 1 mM citrate had no effect on maize hexokinase in contrast to the yeast enzyme which is activated by citrate when the pH is less than optimal (16).

DISCUSSION

The presence of a low and a high affinity glucose-binding site may indicate that maize seeds contain a glucokinase in addition to hexokinase. Liver glucokinase has been separated from hexokinase (13, 24, 31), and the two occur in different kinds of liver cells (26). Alternatively, a single maize enzyme might be present which exhibits a complex kinetic pattern for glucose but first order kinetics for the substrates fructose and ATP. The glucose saturation curve at high glucose concentrations gives a maximal velocity about three times higher than that obtained at low glucose concentrations (Figs. 10 and 11), possibly indicating a predominance of glucokinase over hexokinase in the scutellum preparation. Whether this ratio changes during purification has not been established.

Maize scutellum slices are capable of rapidly absorbing and metabolizing externally supplied glucose with only a modest increase in endogenous glucose which ranges from about 2 to 4 μmoles/g fresh weight (9). These values may represent glucose concentrations near the K*m* of 3.4 mM glucose calculated for the kinase in the present study (Fig. 11). This suggests that glucose concentration in the maize scutellum, and perhaps in other parts of the seed, is regulated by the kinase. The kinase would become more active as glucose content increased due to absorption of external glucose; the result would be increased flux through the pathway and a dampening effect on the increase in glucose.

Mammalian glucokinase possesses a K*m* for glucose at least 200-fold higher than that of hexokinase from the same tissue (1, 32), whereas only a 19-fold difference is reported here. Hence, the simplifying assumptions made in calculating kinetic constants of the mammalian enzymes (32) may not be valid for maize, so kinetic constants reported here must be regarded as approximations.

LITERATURE CITED

1. BACHELARD, H. S. 1967. Glucose-phosphorylating enzyme with high Km in human brain. Nature 215: 959–960.
2. BALLARD, F. J. 1965. Glucose utilization in mammalian liver. Comp. Biochem. Physiol. 14: 437–443.
3. COX, E. L. AND D. B. DICKINSON. 1971. Hexokinase from maize endosperm. Phytochemistry 10: 1771–1775.
4. COX, E. L. AND D. B. DICKINSON. 1972. Increased hexokinase levels in endosperm of mutant maize. Phytochemistry 12: 291–297.
5. DIPIETRO, D. L., C. SHARMA, AND S. WEINHOUSE. 1962. Studies on glucose phosphorylation in rat liver. Biochemistry 1: 455–462.
6. DOWD, J. E. AND D. S. RIGGS. 1965. A comparison of estimates of Michaelis-Menten kinetic constants from various linear transformations. J. Biol. Chem. 240: 863–869.
7. GAZITH, J., I. T. SCHULZE, R. H. GOODING, F. C. WOMACK, AND S. P. COLOWICK. 1968. Multiple forms and subunits of yeast hexokinase. Ann. N.Y. Acad. Sci. 151(1): 307–331.

8. HOPPER, J. E. AND D. B. DICKINSON. 1972. Partial purification and sugar nucleotide inhibition of UDP-glucose pyrophosphorylase from *Lilium longiflorum pollen*. Arch. Biochem. Biophys. 148: 523-535.
9. HUMPHREYS, T. E. AND L. A. GARRARD. 1964. Glucose uptake by the corn scutellum. Phytochemistry 3: 647-656.
10. INGLE, J., L. BEEVERS, AND R. H. HAGEMAN. 1964. Metabolic changes associated with the germination of corn. I. Changes in weight and metabolites and their redistribution in the embryo axis, scutellum and endosperm. Plant Physiol. 39: 735-740.
11. JACOB, J. AND J. D'AUZAC. 1967. Sur l'existence conjointe d'une hexokinase et d'une fructokinase au sein du latex d'*Hevea brasiliensis*. C. R. Acad. Sci., Paris D 265: 260-263.
12. JONES, H. C., III. 1965. The purification and properties of a hexokinase from the corn scutellum. Ph.D. thesis. University of Florida, Gainesville.
13. KATZEN, H. M. 1967. The multiple forms of mammalian hexokinase and their significance to the action of insulin. *In:* G .Weber, ed., Advances in Enzyme Regulation, Vol. 5, pp. 335-356. Pergamon Press, New York.
14. KATZEN, H. M., D. D. SODERMAN, AND H. M. NITOWSKY. 1965. Kinetic and electrophoretic evidence for multiple forms of glucose-ATP phosphotransferase activity from human cell cultures and rat liver. Biochem. Biophys. Res. Commun. 19: 377-382.
15. KOSOW, D. P. AND I. A. ROSE. 1970. Product inhibition of the hexokinases. J. Biol. Chem. 245: 198-204.
16. KOSOW, D. P. AND I. A. ROSE. 1971. Activators of yeast hexokinase. J. Biol. Chem. 246: 2618-2625.
17. MEDINA, A. AND A. SOLS. 1956. A specific fructokinease in peas. Biochim. Biophys. Acta 19: 378-379.
18. MEUNIER, J. C., J. BUC, AND J. RICARD. 1971. Isolation, purification and characterization of wheat germ hexokinases. FEBS Lett. 14: 25-28.
19. MEUNIER, J. C., A. M. MOUSTACAS, AND J. RICARD. 1968. Localisation intracellulaire de l'hexokinase de racines de lentille. C. R. Acad. Sci. Paris, D 267: 1445-1447.
20. MILLERD, A., J. BONNER, B. AXELROD, AND R. BANDURSKI. 1951. Oxidative and phosphorylative activity of plant mitochondria. Proc. Nat. Acad. Sci. U.S.A. 37: 855-862.
21. NEWSHOLME, E. A., J. ROBINSON, AND K. TAYLOR. 1967. A radiochemical enzymatic activity assay for glycerol kinase and hexokinase. Biochim. Biophys. Acta 132: 338-346.
22. PURICH, D. L. AND H. J. FROMM. 1971. The kinetics and regulation of rat brain hexokinase. J. Biol. Chem. 246: 3456-3463.
23. RAMEL, A. H., Y. M. RUSTUM, J. G. JONES, AND E. A. BARNARD. 1971. Yeast hexokinase IV. Multiple forms of hexokinase in the yeast cell. Biochemisty 10: 3499-3508.
24. SALAS, J., M. SALAS, E. VINUELA, AND A. SOLS. 1965. Glucokinase of rabbit liver. Purification and properties. J. Biol. Chem. 240: 1014-1018.
25. SALTMAN, P. 1953. Hexokinase in higher plants, J. Biol. Chem. 200: 145-154.
26. SAPAG-HAGAR, M., R. MARCO, AND A. SOLS. 1969. Distribution of hexokinase and glucokinase between parenchymal and non-parenchymal cells of rat liver. FEBS Lett. 3: 68-71.
27. SCHIMKE, R. T. AND L. GROSSBARD. 1968. Studies on isozymes of hexokinase in animal tissues. Ann. N.Y. Acad. Sci. 151(1): 332-350.
28. SHANNON, J. C. 1968. Carbon-14 distribution in carbohydrates of immature *Zea mays*. kernels following $^{14}CO_2$ treatment of intact plants. Plant Physiol. 43: 1215-1220.
29. SLEIN, M. W., G. T. CORI, AND C. F. CORI. 1950. A comparative study of hexokinase from yeast and animal tissues. J. Biol. Chem. 186: 763-780.
30. TSAI, C. Y., F. SALAMINI, AND O. E. NELSON. 1970. Enzymes of carbohydrate metabolism in the developing endosperm of maize. Plant. Physiol. 46: 299-306.
31. URETA, T., C. GONZALEZ, AND H. NIEMEYER. 1971. Comparative studies on glucose phosphorylating isoenzymes of vertebrates. II. Chromatographic patterns of glucokinase and hexokinases in the liver of rodents. Comp. Biochem. Physiol. 40B: 81-91.
32. WALKER, D. G. 1963. On the presence of two soluble glucose-phosphorylating enzymes in adult liver and development of one of these after birth. Biochim. Biophys. Acta 77: 209-226.