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Communication

Fructokinases from Developing Maize Kernels Differ in Their Specificity for Nucleoside Triphosphates

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ABSTRACT

A new form of fructokinase has been identified from developing maize (*Zea mays* L.) kernels that utilizes CTP, UTP, and GTP from four to eight times more effectively than ATP at nonlimiting concentrations. Ten millimolar dithiothreitol was necessary to stabilize activity. A second form of fructokinase was nonspecific for nucleoside triphosphate whereas a third form was fairly specific for ATP.

Sucrose degradation in developing maize kernels generates large amounts of free fructose. This fructose must be phosphorylated by FK[1] before it can be further metabolized, although fructose may be converted into sorbitol by ketose reductase present in maize endosperm (1). Earlier studies from this laboratory indicated that UTP-dependent as well as ATP-dependent FK activities were present in comparable activities in developing maize kernels (1, 2). FKs subsequently isolated from developing maize kernels utilized ATP more effectively than other NTPs (3) and could not account for the observed UTP-dependent FK activity found in crude extracts. UTP-dependent FK activity has also been reported in sycamore cell cultures (4) and lima bean seeds (6). Nonspecific NTP-dependent FK activity was reported in potato tubers and other tissues (7). UTP-dependent FK activity is of particular interest because of the role it may play in cycling UTP during sucrose degradation by sucrose synthase (4). In the presence of sucrose and UDP, sucrose synthase produces fructose and UDP-Glc. In the presence of PPi, UDP-Glc will be converted into UTP and Glc-1-P by UDP-Glc pyrophosphorylase. Utilization of UTP as a phosphoryl-donor to fructose would regenerate UDP, allowing further sucrose degradation by sucrose synthase.

In this study, FK activities of differing NTP specificities were isolated from developing maize kernels by modified methods developed in an earlier study in which ATP-dependent FKs were isolated. Stability of UTP-dependent FK activity was found to be dependent on high levels of sulfhydryl reducing power.

[1] Abbreviations: FK, fructokinase; NTP, nucleoside triphosphate.

MATERIALS AND METHODS

FKs were extracted from *Zea mays* L. kernels, purified, and assayed by the same methods described earlier (3) with the exception of 10 mM DTT being included in all extraction, purification, and assay solutions. Purification of FKs included collection and resuspension of a 40 to 80% ammonium sulfate precipitate, Ultrogel[2] AcA 44 gel permeation chromatography, chromatography with Matrix Blue-A (Amicon Corp.) to which glucokinases and ketose reductase bound but FKs did not, and, finally, separation of FK isoforms on Mono-Q ion exchange chromatography (Pharmacia). FK activity was measured by coupling Fru-6-P formation with phosphoglucoisomerase, Glc-6-P dehydrogenase, and $NADP^+$ reduction as described earlier (3). Free Mg^{2+} concentration in assays was maintained at 1 mM over MgNTP concentration according to the formulations described by Morrison (5). A unit of activity is defined as the activity required to produce one μmol of product in one min at 30°C.

RESULTS

When FKs were isolated by the methods described earlier (3), UTP-dependent FK activity was labile and often undetectable after chromatography. The addition of 10 mM DTT was found to stabilize UTP-dependent FK so that its isolation could be achieved (data not shown).

Mono-Q anion exchange chromatography of crude whole OH43 kernel extracts performed and assayed in the presence of 10 mM DTT resulted in recovery of ATP-dependent FK activity in a pattern similar to that reported earlier (3). Two major forms, designated FK-1 and FK-2 in an earlier study, were consistently present along with a less active, earlier eluting form, now designated FK-0. When assayed for UTP-dependent FK activity, FK-0 was found to contain more activity than FK-1 or FK-2 (Fig. 1). Thus, FK-0 appeared to represent much of the UTP-dependent FK activity observed in previous studies (1, 2). FK-1, previously found to be specific for ATP, was found to contain considerable UTP-dependent FK activity when assayed in the presence of 10 mM DTT. The characteristics of FK-2 were unchanged by chromatog-

[2] Mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

raphy with 10 mM DTT, and the properties of this enzyme were not investigated further.

FKs were partially purified from whole maize kernel extracts by a previously described procedure (3). The UTP-dependent FK activity present in FK-0 was purified 73-fold with an overall yield of 6% of the activity in crude extracts (data not shown). The UTP-dependent FK activity present in FK-1 was purified 23-fold with a yield of 3% of that present in crude extracts (data not shown). No changes in nucleotide specificity for the FK preparations were observed during purification until the final ion-exchange step where the three forms were separated (Fig. 1).

The NTP specificity of FK-0 and FK-1 was tested (Table I). FK-0 showed the highest activity with CTP as the phosphoryl donor, but UTP and GTP were both more effective substrates than ATP. In contrast, ATP was the most effective substrate for FK-1, although 45 to 78% of the ATP-dependent activity was detected by using GTP, CTP, or UTP as substrates.

Because these enzymes are of interest for the role they may play in UTP cycling, the concentration kinetics of FK-0 and FK-1 for ATP and UTP were investigated. FK-1 had a lower apparent K_m (ATP) than did FK-0, and FK-1 had a higher V_{max} with ATP than FK-0 (Table II). In contrast, FK-0 and FK-1 had about the same apparent K_m (UTP), but FK-0 had a much higher V_{max} with UTP than did FK-1 (Table II). Thus, it appeared that FK-0 utilized UTP more effectively than FK-1, whereas FK-1 utilized ATP more effectively than FK-0.

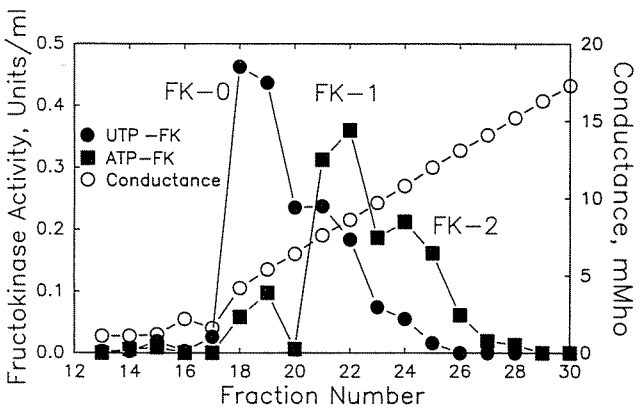

Figure 1. Separation of ATP- and UTP-dependent FK activities from crude extracts of developing maize kernels by Mono-Q anion exchange chromatography.

Table I. *NTP Specificity of Two Fructokinases from Developing Maize Kernels*

Assays contained 2.5 mM MgNTP and 1 mM fructose. Activity was detected spectrophotometrically by coupling Fru-6-P formation with phosphoglucoisomerase, Glc-6-P dehydrogenase, and NADP reduction. Values are the mean ± SE of three replications, each replication derived from a separate purification.

MgNTP	FK-0	FK-1
	relative activity	
MgATP	1.0[a]	1.00[b]
MgCTP	7.4 ± 1.1	0.78 ± 0.06
MgGTP	3.1 ± 0.7	0.45 ± 0.03
MgUTP	6.0 ± 1.2	0.55 ± 0.05

[a] Mean specific activity, 1.8 units (mg protein)$^{-1}$. [b] Mean specific activity, 4.4 units (mg protein)$^{-1}$.

Table II. *Kinetic Constants of FK-0 and FK-1 for MgATP and MgUTP*

Kinetic constants were determined by linear replots of the MgATP and MgUTP substrate saturation response of FK-0 and FK-1, respectively, with 1 mM fructose. Data represents the mean from two determinations.

Enzyme (MgNTP)	Apparent K_m (MgNTP)	V_{max}
	mM	*units (mg protein)$^{-1}$*
FK-0 (MgATP)	0.46	2.9
FK-1 (MgATP)	0.18	7.0
FK-0 (MgUTP)	1.01	14.8
FK-1 (MgUTP)	0.92	4.1

Fructose concentration kinetics of the FKs studied here were about the same as those reported previously (3) where fructose concentrations over 1 mM caused substrate inhibition (data not shown).

DISCUSSION

The finding of a FK specific for NTPs other than ATP (FK-0) is apparently novel to plant or animal studies. This enzyme may provide a unique function by allowing UTP cycling during sucrose degradation as suggested by Huber and Akazawa (4). The high activity obtained with CTP as a substrate suggests that this enzyme may also function in CTP metabolism. The relationship between either CTP or GTP and fructose metabolism in developing maize kernels is not understood, but phosphofructokinase activities from lima bean seeds (6) and loblolly pine roots (7) have also been reported to be nonspecific for NTPs.

Stability of UTP-dependent FK activity was dependent on 10 mM DTT. The physiological significance of this observation is not clear, although the possibility exists that this may represent a regulatory mechanism. The cell may be able to regulate the NTP specificity of its fructose phosphorylating capacity by changing the reducing power of the cellular environment. However, we were not always able to regenerate activity lost in the absence of 10 mM DTT by adding DTT, nor were we able to regenerate UTP-dependent fructokinase activity from samples treated chemically to oxidize sulfhydryl groups (data not shown).

An earlier study from this laboratory (3) characterized FK-1 and FK-2 in the presence of 1 mM DTT. In that study, ATP was found to be the most effective phosphoryl donor while CTP, GTP, and UTP resulted in less than 20% of the activity with ATP. FK-0 was detected, but in such low activities that it was not characterized. In this study, FK-1 assayed in the presence of 10 mM DTT exhibited 50 to 75% of its ATP-dependent activity with CTP, UTP, or GTP as substrates (Table I). The apparent K_m (ATP) is also lower than that previously reported, and the specific activity of the purified preparation is higher than that reported in the presence of 1 mM DTT. Although the separation of FK-0 and FK-1 was sufficient to ensure that only a small amount of cross-contam-

ination could occur, it is not clear if the UTP-dependent activity observed in FK-1 was due to the modified properties of the previously characterized ATP-dependent FK-1, or due to the recovery of another new form of FK, stabilized by DTT and cochromatographing with FK-1. Further attempts to separate UTP-dependent FK activity from ATP-dependent FK activity in FK-1 by variations in Mono-Q chromatography, hydroxylapatite chromatography, or by ammonium sulfate gradient solubilization failed to separate these activities (data not shown).

The three FK preparations isolated from maize kernels have a wide range of NTP specificity. FK-0 utilized CTP, UTP, and GTP effectively, but not ATP; FK-1 utilized ATP about as well as other NTPs (Table I); and FK-2 utilized ATP more effectively than other NTPs (3). The differences in FK specificity for different NTPs indicates a specialization of enzyme forms for different functions and would allow for the phosphorylation of fructose with any of the four NTPs. Whereas the significance of UTP metabolism to fructose metabolism is clear, the relevance of GTP and CTP to fructose metabolism is not understood. The presence of diverse and distinct functional forms of FK indicate that fructose phosphorylating activity in developing maize kernels is more efficient than previously suspected.

LITERATURE CITED

1. **Doehlert DC** (1987) Ketose reductase activity in developing maize endosperm. Plant Physiol **84:** 830–834
2. **Doehlert DC, Kuo TM, Felker FC** (1988) Enzymes of sucrose and hexose metabolism in developing maize kernels. Plant Physiol **86:** 1013–1019
3. **Doehlert DC** (1989) Separation and characterization of four hexose kinases from developing maize kernels. Plant Physiol **89:** 1042–1048
4. **Huber SC, Akazawa T** (1986) A novel sucrose synthase pathway for sucrose degradation in cultured sycamore cells. Plant Physiol **81:** 1008–1013
5. **Morrison JF** (1979) Approaches to kinetic studies on metal-activated enzymes. Methods Enzymol **63:** 257–294
6. **Xu DP, Sung SJS, Black CC** (1989) Sucrose metabolism in lima bean seeds. Plant Physiol **89:** 1106–1116
7. **Xu DP, Sung SJS, Lobata T, Kormanik PP, Black CC** (1989) Characterization of sucrolysis via the uridine diphosphate and pyrophosphate-dependent sucrose synthase pathway. Plant Physiol **90:** 635–642