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PYROPHOSPHATE FUNCTIONS AS PHOSPHORYL DONOR WITH UDP-GLUCOSE-TREATED MAMMALIAN PHOSPHOFRUCTOKINASE

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Phosphofructokinase of rabbit muscle, which is specific for nucleoside triphosphates such as ATP, dissociated and gained the capability to utilize pyrophosphate as phosphoryl donor following incubation with UDP-glucose. The pyrophosphate- and ATP-linked activities of UDP-glucose-treated muscle phosphofructokinase were promoted by a protein species that showed a molecular weight of 80 kDa (vs. 320 kDa for the untreated enzyme). In the presence of citrate, a known inhibitor of PFK, the pyrophosphate-dependent activity elicited by UDP-glucose treatment was activated by fructose-2,6-bisphosphate. On removal of the UDP-glucose by either dialysis or dilution, the treated enzyme reassociated and became ATP-specific. ATP, dithiothreitol, and fructose-2,6-bisphosphate stimulated reassociation. The results suggest that metabolite-mediated catalyst conversion, yielding an enzyme form capable of utilizing both ATP and pyrophosphate, takes place with the phosphofructokinases of animal tissues.

We recently reported evidence for a new type of enzyme regulation in which metabolites promote the conversion of an enzyme from one catalytic type to another ("metabolite-mediated catalyst conversion") (1). Partially purified preparations of spinach leaf cytosolic phosphofructokinase (PFK) (Eq. 1) and its pyrophosphate-linked counterpart [inorganic pyrophosphate-D-fructose-6-phosphate 1-phosphotransferase (PFP)] (Eq. 2) were interconvertible when incubated with appropriate metabolites. That is, UDP-glucose (UDPG) and pyrophosphate (PP_i) converted PFK to PFP, and fructose-2,6-bisphosphate (Fru-2,6-P_2) plus either ATP or fructose-6-phosphate promoted the reverse reaction -- i.e., conversion of PFP to PFK. These conversions were accompanied by changes in charge and, in some cases, in molecular weight.

Abbreviations: PFK, phosphofructokinase (EC 2.7.1.11); PFP, inorganic pyrophosphate-D-fructose-6-phosphate 1-phosphotransferase (EC 2.7.1.90); UDPG, UDP-glucose; PP_i, pyrophosphate; Fru-2,6-P_2, fructose-2,6-bisphosphate.

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$$(1)\quad \text{Fructose-6-phosphate} + \text{ATP} \xrightarrow{\text{PFK}} \text{Fructose-1,6-bisphosphate} + \text{ADP}$$

$$(2)\quad \text{Fructose-6-phosphate} + PP_i \underset{}{\overset{\text{PFP}}{\rightleftharpoons}} \text{Fructose-1,6-bisphosphate} + P_i$$

The question now arises as to whether a similar conversion takes place with PFK from animal cells (2,3). We have therefore examined mammalian PFK and now report positive evidence for this effect. Incubation with UDPG was found to promote conversion of muscle PFK from a nucleoside triphosphate-specific 320 kDa form to an 80 kDa protomer using both ATP and PP_i as phosphoryl donor. In the presence of citrate, the protomer utilized only pyrophosphate and, under these conditions, it was activated by Fru-2,6-P_2. Following removal of the UDPG, incubation with ATP, dithiothreitol, and Fru-2,6-P_2 induced association and yielded an ATP-specific enzyme showing the original molecular weight of 320 kDa. The results suggest that metabolite-mediated catalyst conversion occurs in mammalian tissues.

MATERIALS AND METHODS

Biochemicals and Enzymes. Purified rabbit muscle PFK, (Types I and III), and lyophilized coupling enzymes, aldolase, triosephosphate isomerase, and α-glycerophosphate dehydrogenase, were obtained from Sigma Chemical Co., St. Louis, MO. Other reagents were purchased from commercial sources and were of the highest quality available.

Enzyme assays. Aliquots of enzyme preparations were treated for 1 min at 20°C with UDPG as indicated. Reactions were initiated by addition of preincubated or non-preincubated PFK or, when indicated, by substrate. PFP and PFK were routinely assayed as described previously (4,5) except that the buffer, containing 1 mM EDTA, was 50 mM Tris-HCl, pH 7.0 for PFK, and pH 7.9 for PFP.

Molecular Weight Determinations. Molecular weights were determined at 4°C by gel filtration with a Sephacryl S-300 column (1.6 x 90 cm) developed with buffer A (50 mM Tris-HCl, pH 7.9, containing (v/v) 10°/o glycerol and 0.1°/o 2-mercaptoethanol). The column was calibrated with known molecular weight markers, kDa: PFK (rabbit muscle), 320; catalase (bovine liver), 210; aldolase (rabbit muscle), 158; bovine serum albumin, 68.

Analytical Procedure. The concentration of PFK was determined spectrophotometrically (6). An absorption of 1.07 ml at 280 nm was equivalent to 1 mg/ml.

RESULTS AND DISCUSSION

Conversion of PFK to PFP. Our previous work showed that cytosolic PFK of spinach leaves is converted to PFP by incubation with UDPG and PP_i (1). In the current study, UDPG produced a similar change with PFK from mammalian sources. Table I shows that muscle PFK, which is specific for nucleoside triphosphates such as ATP, was converted to a form utilizing PP_i as phosphoryl donor following incubation or treatment with UDPG. Dithiothreitol enhanced the effectiveness of UDPG. Linear PFP activity appeared 2 to 5 min after adding UDPG and dithiothreitol. Accompanying this change in catalytic capability was a dissociation of the enzyme from its original 320 kDa form (Fig. 1A) to an 80 kDa protomer (Fig. 1B). The new catalytic species that was obtained by subjecting UDPG-treated PFK to Sephacryl S-300 gel filtration retained the capability of utilizing ATP while gaining the capability of utilizing PP_i (Fig. 1B). The PFK activity of the 80 kDa protomer obtained by UDPG treatment was enhanced by Fru-2,6-P_2 to about 10°/o the extent of the initial 320 kDa oligomer (data not shown). By contrast, the PFP activity

Table I. Generation of PFP Activity from Rabbit Muscle PFK

Treatment	°/o of Total PFK + PFP Activity[1)]	
	PFK	PFP
a) Control	100	0
b) Dithiothreitol	100	0
c) UDPG	67	33
d) Dithiothreitol plus UDPG	49	51

1) The actual total activities, based on steady state velocities, were (μmol Fru-1,6-P_2 formed/min.mg protein): a) 9.32; b) 12.54; c) 22.58; d) 32.11.

The concentrations of added metabolites were (mM): dithiothreitol, 100; UDPG, 0.5. Reactions were initiated by addition of 5 μl (2.25 μg) rabbit muscle PFK (Sigma Type I) which was appropriately diluted with water. Similar results were obtained with Sigma Type III PFK.

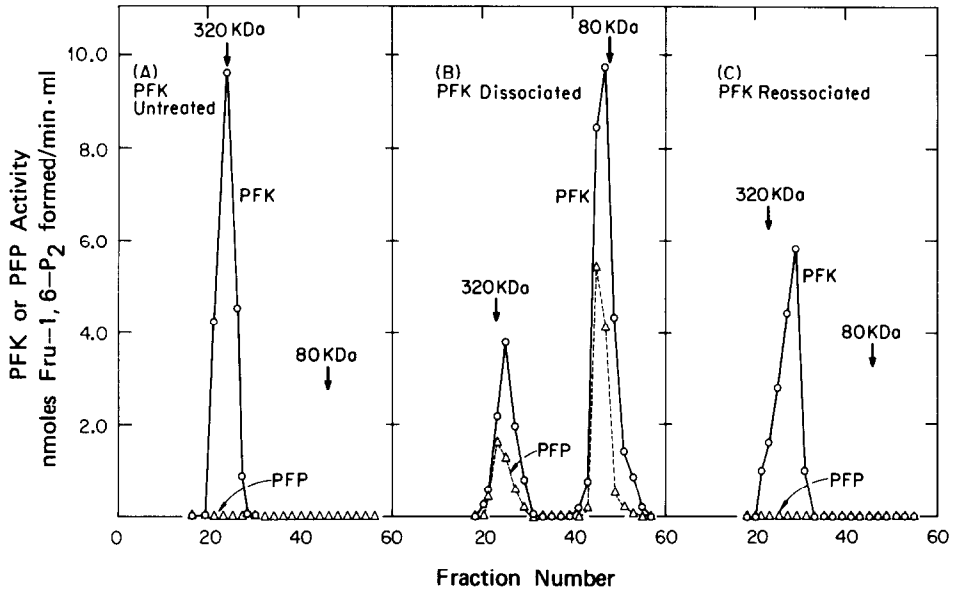

Fig. 1. Activity profiles of (A) untreated, (B) UDPG-dissociated, and (C) ATP- and Fru-2,6-P_2-reassociated PFK of rabbit muscle on Sephacryl S-300 chromatography. In (A) untreated PFK, 2.0 mg (0.5 mg/ml) was applied to the column and eluted in buffer A. In (B), 8.0 mg (2.0 mg/ml) PFK was preincubated with 1 mM UDPG for 30 min at 20°C, applied, and eluted from the column with buffer A containing 1 mM UDPG, the column was pre-equilibrated with this same buffer. The activities of PFK and PFP in (1B) were those shown in the ordinate multiplied by 5. In (C), 0.01% BSA was added to 4.0 mg (1.0 mg/ml) of the PFK protomer isolated from (B), which was then concentrated by dialysis for 8 hours against buffer A containing 2 mM ATP, 5 μM Fru-2,6-P_2 and 50 % glycerol; the preparation was then applied to the column which was equilibrated and eluted with this same buffer without the 50% glycerol.

associated with the UDPG-treated preparation showed a low response to Fru-2,6-P_2 unless citrate, a known PFK inhibitor (2,7), was present (Table II). In the presence of citrate, PFP activity of the UDPG-treated preparation was also greatly enhanced by dithiothreitol (cf. 8). In contrast to the leaf cytosolic PFK (1), the PFK and PFP activities associated with the new catalytic (80 kDa) form were not separated by fast protein liquid chromatography (FPLC) (data not shown). It is also worth noting that the conversion promoted by UDPG was not accompanied by transglucosylation as determined by experiments in which muscle PFK was incubated with UDP-^{14}C-glucose and the two components were separated on a Sephacryl S-300 column and counted.

Table II. Stimulation of PFP Activity Associated with UDPG-treated Rabbit Muscle PFK by Fru-2,6-P_2 in the Presence of Citrate

Treatment	PFP Activity (μmole Fru-1,6-P_2 formed/min.mg protein)	
	Control	+ Fru-2,6-P_2
a) None	0.98	1.15
b) Citrate	0.54	1.34
c) Citrate plus dithiothreitol	0.89	2.51

Reactions were initiated by addition of 10 μl of UDPG-treated PFK (4.5 μg). As indicated, 1 μM of Fru-2,6-P_2 was added after the rate became linear (about 5 min). Citrate (5 mM) and dithiothreitol (10 mM) were present initially.

Association of Protomer to PFK. Once UDPG was removed by dialysis, the protomer of muscle PFK was found to assume its original specificity for ATP. This reversion was enhanced by the inclusion of ATP, 2 mM, and Fru-2,6-P_2, 5 μM, in the dialysis buffer and by the presence of dithiothreitol, 100 mM, in the assay mixture (data not shown). Accompanying this change in catalytic capability was a return to a molecular weight approximating that of the original enzyme, 320 kDa, and a return to its specificity for ATP (Fig. 1C). It should be noted that ATP was found earlier to enhance the reassociation of mammalian PFK protomer (9). Under our conditions, fructose-6-phosphate had no effect on reassociation.

CONCLUDING REMARKS

The present results demonstrate that, on dissociation by UDPG, mammalian PFK changes from a 320 kDa nucleoside triphosphate-specific oligomer to an 80 kDa protomer that also utilizes PP_i as donor for the phosphorylation of fructose-6-phosphate. In the presence of citrate, the PFK activity associated with the protomer is suppressed and the newly formed enzyme becomes specific for PP_i. Under these conditions, the PFP reac-

tion is markedly stimulated by Fru-2,6-P_2. When UDPG is removed, the protomer reassociates to yield an oligomeric nucleoside triphosphate-specific enzyme seemingly similar to the native one. ATP, Fru-2,6-P_2 and dithiothreitol enhance this reassociation.

The question now arises as to whether the PFP activity associated with mammalian PFK is significant physiologically. Although there are indirect indications to this effect (10), additional work will be needed to determine if indeed this is the case.

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