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A Multiple Role for the Coenzyme in the Mechanism of Action of 6-Phosphogluconate Dehydrogenase

THE OXIDATIVE DECARBOXYLATION OF 2-DEOXY-6-PHOSPHOGLUCONATE*

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SUMMARY

6-Phosphogluconate dehydrogenase from *Candida utilis* catalyzes the oxidative decarboxylation of 2-deoxy-6-phosphogluconate. The 3-keto-2-deoxy-6-phosphogluconate, an intermediate of the reaction, is reduced to 2-deoxy-6-phosphogluconate and decarboxylated to 1-deoxyribulose 5-phosphate when incubated with the enzyme and TPNH. The decarboxylation process does not occur in the absence of the reduced coenzyme, which does not have, in this step, an oxidation-reduction role.

Since TPNH also has a non-redox role in a tritium exchange reaction catalyzed by the enzyme, it appears that the coenzyme has a multiple role in the mechanism of action of 6-phosphogluconate dehydrogenase: a redox role in the dehydrogenation and another (or others) role(s) in the decarboxylation and tritium exchange reactions.

The hydroxyl group present at carbon 2 of 6-phosphogluconate seems to have a dual role in the mechanism of action of the enzyme: one in the binding of the substrate to the enzyme, another in enhancing the decarboxylation of the dehydrogenation product.

These findings are discussed with relations to the mechanism of action of isocitrate dehydrogenase and of the malic enzyme.

The enzymatic oxidative decarboxylation of 2-deoxy-6-phosphogluconate is a new step for the metabolism of the metabolic inhibitor 2-deoxyglucose.

The isocitrate dehydrogenase (L$_s$-isocitrate:NADP oxidoreductase, decarboxylating, EC 1.1.1.42), the malic enzyme (L-malate:NADP oxidoreductase, decarboxylating, EC 1.1.1.40), and the 6-phosphogluconate dehydrogenase (6-phospho-D-gluconate:NADP oxidoreductase, decarboxylating, EC 1.1.1.44), in the presence of TPN, catalyze formally analogous oxidative decarboxylations of isocitrate, malate, and 6-phosphogluconate, respectively. The first two enzymes catalyze also the decarboxylation of, respectively, oxalosuccinate (1, 2) and oxaloacetate (3–7); these two β-keto acids have so far escaped detection as free intermediates of the over-all oxidative decarboxylation of isocitrate and malate. 3-Keto-6-phosphogluconate, the β-keto acid anticipated from 6-phosphogluconate dehydrogenation, was neither isolated nor chemically prepared.

The failure to trap these three β-keto acids has been justified with the hypothesis that they are enzyme-bound and are never released in the medium (8–10). But there is another explanation based on the rates of the partial reactions. In the case of at least isocitrate dehydrogenase (9–12) and 6-phosphogluconate dehydrogenase (13), the dehydrogenation is the rate-limiting step, thus the decarboxylation is faster than the oxidation; in the backward reaction the reduction is faster than the carboxylation. For these reasons there is no possibility to accumulate the intermediate. Furthermore, it is not possible to obtain the carboxylation without reduction, running the reaction backward in the absence of TPNH, since the reduced coenzyme is essential (14, 15) in a step which very likely precedes the carboxylation, *i.e.* the tritium exchange reaction between the products of the enzymatic oxidative decarboxylation and water.

In order to accumulate a β-keto acid intermediate, one could try to decrease the rate of decarboxylation, either by a chemical modification of the enzyme, or by using a substrate analogue which has a lower tendency to decarboxylate.

Choosing this second approach, we have used as substrate of the 6-phosphogluconate dehydrogenase from *Candida utilis* the 2-deoxy-6-phosphogluconate, which, lacking the electron withdrawing effect of the hydroxyl at carbon 2, should be more resistant to decarboxylation than 6-phosphogluconate. The results of this approach are reported in the present paper.

EXPERIMENTAL PROCEDURE

Materials

6-Phosphogluconate dehydrogenase, type I (16), crystalline, was prepared from *C. utilis* as previously described (17). The enzyme used in this work had a specific activity of 34 i.u. Prior to the experiments the crystals were collected by centrifugation and dissolved in quartz-distilled water, and the resulting protein solution was desalted through a Sephadex G-50 column equilibrated with 50 mM Tris-HCl buffer, pH 7.5, containing 1 mM EDTA. 6-Phosphogluconate dehydrogenase, type II from *C.*

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utilis, was prepared as previously reported (16). 6-Phosphogluconate dehydrogenase was purified also from bakers' yeast and hog kidney.

6-Phosphogluconate, 2-deoxyglucose, 2-deoxyglucose 6-phosphate, TPN, TPNH, DPN, DPNH, ATP, 4-aminoantipyrine, hexokinase, and lactate dehydrogenase were purchased from Sigma Chemical Co., St. Louis, Mo.; 2-deoxy[1-^{14}C]glucose (54.6 mCi per mmole) was purchased from New England Nuclear Corp.

Preparation of 2-Deoxy-6-phospho[1-^{14}C]gluconate—2-Deoxy-[1-^{14}C]glucose was diluted with nonradioactive 2-deoxyglucose and phosphorylated to 2-deoxyglucose 6-phosphate with ATP and hexokinase. After barium precipitation of ATP and ADP, the radioactive 2-deoxyglucose 6-phosphate was purified by column chromatography on Dowex 1-Cl. The purified compound was then oxidized with bromine (18) to 2-deoxy-6-phosphogluconate which was purified by a second Dowex 1-Cl column chromatography. The final product had a specific radioactivity of 81,600 cpm per μmole.

Methods

Chemical and Enzymatic Determinations—2-Deoxyglucose 6-phosphate was assayed enzymatically at pH 8.0 using TPN and glucose 6-phosphate dehydrogenase from *C. utilis*. The enzymatic activity of 6-phosphogluconate dehydrogenase on 2-deoxy-6-phosphogluconate was assayed at pH 8.0 in 50 mM Tris-HCl buffer, in the presence of 1.8 mM TPN, taking readings at 340 nm in an ACTA III recording spectrophotometer at 5-s intervals. The concentration of 2-deoxy-6-phosphogluconate was determined as above except that 2 mM $MgCl_2$ was added to the reaction mixture to accelerate the decarboxylation of the 3-keto acid intermediate. 3-Keto-2-deoxy-6-phosphogluconate was determined as a formazan derivative upon coupling with diazotized *p*-nitroaniline (19); freshly prepared oxalacetate was used as standard.

Radioactivity measurements were carried out in a Packard Tri-Carb liquid scintillation counter, using Bray's solution (20).

Determination by Radioactivity Measurements of 2-Deoxy-6-phosphogluconate and 3-Keto-2-deoxy-6-phosphogluconate—These determinations were carried out following the observation that the keto compound was decarboxylated, and thus loosed radioactivity, by boiling or by treatment with 4-aminoantipyrine, while 2-deoxy-6-phosphogluconate was resistant to these treatments. Thus, in order to establish the concentrations of these two radioactive compounds in a solution containing both of them, the following procedure was used. One sample was diluted with acetate buffer (final concentration, 1 mM, pH 3.8), treated for 5 min at room temperature with 4-aminoantipyrine (final concentration, 20 mM) and then kept under vacuum for 5 min. The residual radioactivity was attributed to 2-deoxy-6-phosphogluconate. Another sample was treated as above, except that the treatment with aminoantipyrine was omitted; the residual radioactivity was attributed to the sum of 2-deoxy-6-phosphogluconate and 3-keto-2-deoxy-6-phosphogluconate; then by difference between these two values it was possible to determine the concentration of the radioactive 3-keto-2-deoxy-6-phosphogluconate. The vacuum treatment was required to eliminate the radioactive CO_2 (volatile radioactivity) which was formed by the decarboxylation of the keto acid.

RESULTS

Oxidation of 2-Deoxy-6-phosphogluconate by 6-Phosphogluconate Dehydrogenase—6-Phosphogluconate dehydrogenase from *C. utilis*, in the presence of TPN, catalyzes the oxidative decarboxylation of 2-deoxy-6-phosphogluconate. At pH 8.0 and in the presence of 1.8 mM TPN, the K_m of the enzyme for 2-deoxy-6-phosphogluconate was 0.55 mM as compared with 0.054 mM for 6-phosphogluconate (17). The K_m for TPN was 0.020 mM using either 2-deoxy-6-phosphogluconate or 6-phosphogluconate. When the dehydrogenase activity was tested in presence of a concentration of substrate corresponding to the K_m value, 1 mg of enzyme catalyzed the oxidation of 0.23 and 17.2 μmoles per min of 2-deoxy-6-phosphogluconate and 6-phosphogluconate, respectively. Thus, in these experimental conditions, the dehydrogenation of 2-deoxy-6-phosphogluconate was 75-fold slower than that of 6-phosphogluconate (Table I, Experiments 1 and 2).

TABLE I

Enzymatic activities of 6-phosphogluconate dehydrogenase

Experiment No.	Substrate	Amount	Reaction	I. u.
		mM		
1	6-Phosphogluconate	0.054	Oxidation	17.2
2	2-Deoxy-6-phosphogluconate	0.55	Oxidation	0.23
3	3-Keto-2-deoxy-6-phosphogluconate	0.10	Reduction	0.15
4	3-Keto-2-deoxy-6-phosphogluconate	0.10	Decarboxylation	0.17

The ratio between the dehydrogenase activity on these two substrates was constant in all steps of the 340-fold purification procedure (17) of the enzyme. The same ratio has been found also using the enzyme type II from *C. utilis* and the enzyme purified from bakers' yeast and hog kidney.

TPN was the specific coenzyme for this activity, DPN was inactive.

Evidence for an Intermediate in Oxidative Decarboxylation of 2-Deoxy-6-phosphogluconate—When 2-deoxy-6-phosphogluconate was used as substrate, the reduction of TPN in the first minutes of the reaction was paralleled by a formation of a compound which gave a positive reaction with diazotized *p*-nitroaniline, and thus was a β-keto acid (19). This positive reaction disappeared if the reaction was allowed to proceed for longer time, indicating that the intermediate was consumed (Fig. 1).

The observed decrease in the rate of TPNH formation was due to the inhibitory effect of TPNH (21). The disappearance of the intermediate is to be attributed to the increase of concentration of TPNH, which enhances (see below) the decarboxylation of the β-keto acid.

Preparation and Isolation of Intermediate—The preparation of the intermediate of the oxidative decarboxylation of 2-deoxy-6-phosphogluconate was accomplished running the reaction in the presence of pyruvate and lactate dehydrogenase, in order to recycle the TPNH formed during the oxidation of the 2-deoxy-6-phosphogluconate. In order to follow the reaction, two samples of 50 μl each were withdrawn at each time interval and one sample was boiled for 5 min. The two samples were then acidified and kept 5 min under vacuum to eliminate the radioactive CO_2 formed by the decarboxylation of the intermediate. The radioactivity found in the boiled sample was attributed to unreacted 2-deoxy-6-phosphogluconate, the radioactivity of the unboiled sample was attributed to the sum of unreacted 2-deoxy-6-phosphogluconate and of the β-keto acid; this was possible since β-keto acids are decarboxylated on boiling. As shown in Fig. 2, during the course of the reaction there is an enzymatic decar-

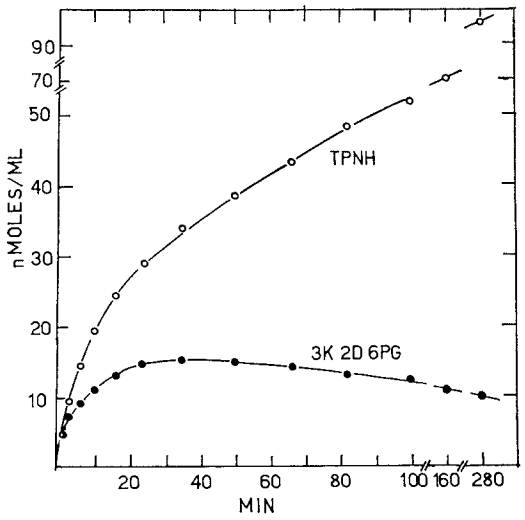

FIG. 1. Kinetics of the formation of an intermediate in the oxidative decarboxylation of 2-deoxy-6-phosphogluconate by 6-phosphogluconate dehydrogenase. Each milliliter of the reaction mixture contained 1.0 μmoles of 2-deoxy-6-phosphogluconate, 1.2 μmoles of TPN, 0.68 i.u. of 6-phosphogluconate dehydrogenase, 50 μmoles of Tris-HCl buffer, and 1 μmole of EDTA; the final pH was 8.0 and the temperature 20°. The concentration of TPNH formed was determined spectrophotometrically at 340 nm. The concentration of the intermediate 3-keto-2-deoxy-6-phosphogluconate (*3K 2D 6PG*) was determined by coupling with diazotized *p*-nitroaniline. *Abscissa*, reaction time; *ordinate*, nanomoles of TPNH, (○——○) and of 3-keto-2-deoxy-6-phosphogluconate (●——●).

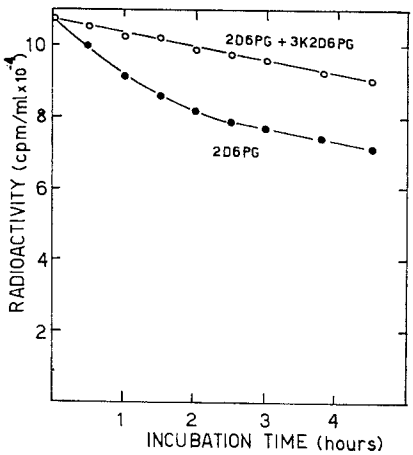

FIG. 2. Kinetics of the formation of the intermediate in the absence of TPNH, as determined by radioactivity measurements. Fifty milliliters of the reaction mixture, kept at 37°, contained 65 μmoles of radioactive 2-deoxy-6-phosphogluconate (*2D6PG*) (81,600 cpm per μmole), 100 μmoles of TPN, 200 μmoles of sodium pyruvate, 17 i.u. of 6-phosphogluconate dehydrogenase, 1 mg of lactate dehydrogenase, 2 mmoles of Tris-HCl buffer, and 5 mmoles of EDTA; the final pH was 8.0. At the time intervals indicated in the abscissa, the nonvolatile radioactivity of an unboiled sample (○——○) and of a boiled sample (●——●) was determined. *3K2D6PG*, 3-keto-2-deoxy-6-phosphogluconate.

boxylation of 2-deoxy-6-phosphogluconate, shown by the decrease of nonvolatile radioactivity (*open circles*) and the formation of a β-keto acid, shown by the further decrease, upon boiling, of nonvolatile radioactivity (*closed circles*). The formation of a β-keto acid was shown also by the increase of the concentration of a compound which gave a positive reaction with diazotized *p*-nitroaniline.

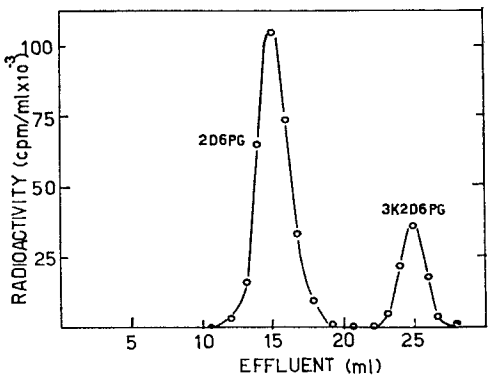

FIG. 3. Chromatographic separation of 2-deoxy-6-phosphogluconate (*2D6PG*) and 3-keto-2-deoxy-6-phosphogluconate (*3K2D6PG*). The mixture containing the two radioactive compounds, after charcoal treatment, was charged on a Dowex 1-Cl column (0.8 × 3 cm). All radioactivity was bound to the resin. The radioactive material was eluted from the resin by means of a linear gradient elution, whereby 75 ml of 1 M NaCl were allowed to flow in a well stirred reservoir containing 75 ml of quartz-distilled water. Fractions of 1 ml each were collected at a flow rate of 0.2 ml per min. The fractions were analyzed for radioactivity and formazan formation.

At the end of the experiments, *i.e.* when no further increase of the concentration of the intermediate was observed, the incubation mixture was treated with 600 mg of charcoal to absorb the enzymes and the coenzyme and centrifuged. The supernatant was then subjected to a column chromatography. Only two radioactive peaks were obtained (Fig. 3). The material contained in the first radioactive peak was identified as unreacted 2-deoxy-6-phosphogluconate, since there was no loss of radioactivity upon boiling and the reaction with diazotized *p*-nitroaniline was negative. The material of the second radioactive peak was identified as 3-keto-2-deoxy-6-phosphogluconate.

Identification of Intermediate as 3-Keto-2-deoxy-6-phosphogluconate—The material present in the second radioactive peak obtained after column chromatography was identified as 3-keto-2-deoxy-6-phosphogluconate by the following criteria: (*a*) it gave a formazan derivative upon coupling with diazotized *p*-nitroaniline; (*b*) it was radioactive and thus contained carbon 1 of 2-deoxy-6-phosphogluconate; (*c*) upon boiling there was complete loss of radioactivity and no more formazan formation; (*d*) by its chromatographic behavior, it appeared to be a phosphorylated compound containing a carboxyl group.

Additional evidence of a β-keto acid structure was furnished by the observation that the compound lost radioactivity (Fig. 4) and thus was decarboxylated by treatment with 4-aminoantipyrine; this reagent is known to decarboxylate β-keto acids (22). It is known that the oxidation by DPN-dependent dehydrogenases of 2-deoxygluconate (23) and gulonate (24) results in the formation of 2-deoxy-3-ketogluconate and 3-ketogulonate, respectively, both β-keto acids being decarboxylated by treatment with 4-aminoantipyrine.

A final proof of the proposed structure and that the compound isolated was a true intermediate of the oxidative decarboxylation of 2-deoxy-6-phosphogluconate was furnished by the fact that this 3-keto-2-deoxy-6-phosphogluconate was enzymatically reduced to 2-deoxy-6-phosphogluconate and decarboxylated to 1-deoxyribulose 5-phosphate.

Enzymatic Modifications of 3-Keto-2-deoxy-6-phosphogluconate—The 3-keto-2-deoxy-6-phosphogluconate, incubated with TPNH and 6-phosphogluconate dehydrogenase, underwent two simultaneous transformations: it was in part reduced to 2-deoxy-

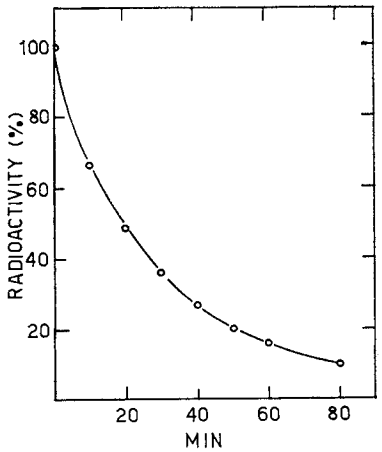

FIG. 4. Kinetics of the decarboxylation of 3-keto-2-deoxy-6-phosphogluconate by 4-aminoantipyrine. One milliliter of the reaction mixture contained 1 μmole of radioactive 3-keto-2-deoxy-6-phosphogluconate (81,600 cpm per μmole), 1 mmole of acetate buffer, pH 3.8, and 5 μmoles of 4-aminoantipyrine. At the time intervals indicated in the abscissa, a sample was kept under vacuum for 2 min and then counted.

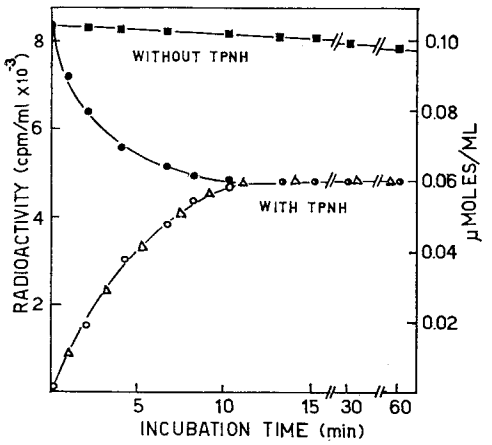

FIG. 5. Enzymatic reduction and decarboxylation of 3-keto-2-deoxy-6-phosphogluconate. Each milliliter of the reaction mixture contained 0.1 μmole of radioactive 3-keto-2-deoxy-6-phosphogluconate (81,600 cpm per μmole), 0.1 μmole of TPNH, 0.68 i.u. of 6-phosphogluconate dehydrogenase, 50 μmoles of Tris-HCl buffer, and 1 μmole of EDTA; the final pH was 8.0. The oxidation of TPNH was followed spectrophotometrically at 340 nm (△). The formation of 2-deoxy-6-phosphogluconate was measured by the increase of nonvolatile radioactivity after 4-aminoantipyrine treatment (○). The decarboxylation was measured by the decrease of nonvolatile radioactivity (●). If TPNH was omitted from the reaction mixture, neither decarboxylation nor reduction occurred (■). *Abscissa*, incubation time; *left ordinate*, nonvolatile radioactivity; *right ordinate*, micromoles of TPNH oxidized (△) and 2-deoxy-6-phosphogluconate formed (○).

6-phosphogluconate and in part decarboxylated to 1-deoxyribulose 5-phosphate. The reduction to 2-deoxy-6-phosphogluconate was shown by the parallel oxidation of TPNH (followed spectrophotometrically) and the increase of radioactivity stable to heat and 4-aminoantipyrine treatments; the decarboxylation was shown by the decrease of nonvolatile radioactivity (Fig. 5). At the end of the reaction the lack of a positive reaction with diazotized *p*-nitroaniline confirmed that all of the 3-keto-2-deoxy-6-phosphogluconate had been consumed. At this point the addition of TPN (to shift the equilibrium existing between the 2-deoxy-6-phosphogluconate and 3-keto-2-deoxy-phosphogluconate toward this last compound) and $MgCl_2$ (to accelerate the decarboxylation of the β-keto acid) caused a complete loss of radioactivity, indicating that the radioactive compound produced by the enzymatic reduction of the 3-keto-2-deoxy-6-phosphogluconate was oxidized and decarboxylated and thus was the 2-deoxy-6-phosphogluconate.

In our experimental conditions, the rates of decarboxylation and reduction were of the same order of magnitude (Table I, Experiments 3 and 4). When either TPNH or enzyme were omitted in the incubation mixture, no decarboxylation occurred. There was also no decarboxylation if TPNH was substituted with TPN, DPN, or DPNH. These results indicated that the reduced coenzyme was essential to the decarboxylation reaction.

The fact that TPNH causes, in the presence of the enzyme, the decarboxylation of 3-keto-2-deoxy-6-phosphogluconate, explains why in the presence of pyruvate and lactate dehydrogenase, *i.e.* in conditions where there is no accumulation of TPNH, it is possible to accumulate the 3-keto-2-deoxy-6-phosphogluconate.

DISCUSSION

In previous papers we have reported some basic properties of 6-phosphogluconate dehydrogenase from *C. utilis* (for review see Reference 25). In order to obtain more information on the mechanism of action of the enzyme, on the nature of the intermediate product of the oxidative decarboxylation of 6-phosphogluconate, and to clarify why the intermediate products of the oxidative decarboxylations of isocitrate and malate were never trapped, we have used as substrate of the 6-phosphogluconate dehydrogenase the 2-deoxy-6-phosphogluconate and observed that this substrate analogue is first dehydrogenated to 3-keto-2-deoxy-6-phosphogluconate and then decarboxylated to 1-deoxyribulose 5-phosphate.

The dehydrogenase activity of the enzyme on 2-deoxy-6-phosphogluconate is only 1.5% of that on 6-phosphogluconate. Hence the possibility that this low activity could be due to an enzymatic contaminant in an otherwise physicochemically homogeneous preparation cannot be overlooked. The evidences for a single enzyme catalyzing the oxidation of both 6-phosphogluconate and 2-deoxy-6-phosphogluconate are the following: (*a*) both activities require TPN, and the enzyme has the same affinity for the coenzyme using both substrates; (*b*) the two substrates differ only in one substituent at carbon 2; (*c*) the two substrates undergo same transformations; (*d*) the ratio between the dehydrogenase activity on the two substrates is constant during all steps of the 340-fold purification procedure of the enzyme; (*e*) the same ratio is obtained using the enzyme prepared from three different sources; (*f*) to our knowledge no other enzyme has been reported to catalyze the oxidative decarboxylation of 2-deoxy-6-phosphogluconate. Finally, the lower activity on 2-deoxy-6-phosphogluconate can be partially due to the fact that, in this case, the decarboxylation is the rate-limiting step, whereas in the case of 6-phosphogluconate the rate-limiting step is the dehydrogenation (13).

Following the enzymatic oxidation of 2-deoxy-6-phosphogluconate, the transient formation of an intermediate having the characteristics of a β-keto acid has been detected; this compound accumulates in the first minutes of the reaction and then disappears being decarboxylated. If the reaction is instead carried out in the presence of pyruvate and lactate dehydrogenase, to efficiently recycle the TPNH, the substrate is oxidized to a β-keto acid which is not, in the absence of TPNH, decarboxylated, and can hence be accumulated, purified, and identified as 3-keto-2-deoxy-6-phosphogluconate. The 3-keto-2-deoxy-6-phosphogluconate prepared in this way is a true intermediate of the oxida-

tive decarboxylation of 2-deoxy-6-phosphogluconate; indeed, in the presence of TPNH and enzyme, it is reduced to 2-deoxy-6-phosphogluconate and decarboxylated to 1-deoxyribulose 5-phosphate.

Since the enzyme, in the absence of TPNH, is unable to catalyze the decarboxylation of the 3-keto-2-deoxy-6-phosphogluconate, it appears that the presence of the reduced coenzyme is essential to the decarboxylation step. TPNH is essential also in the tritium exchange reaction between the ribulose 5-phosphate and water, catalyzed by the 6-phosphogluconate dehydrogenase (15), and it has been proved that in this reaction the reduced coenzyme does not have a redox role (25). A redox role for the reduced coenzyme in the decarboxylation step would implicate the reduction of 3-keto-2-deoxy-6-phosphogluconate to 2-deoxy-6-phosphogluconate which should in turn be decarboxylated bypassing the 3-keto-2-deoxy-6-phosphogluconate; this process is to be excluded, since it has been observed that the 2-deoxy-6-phosphoglutonate, obtained by reduction of the β-keto compound, is, in our experimental conditions, not further transformed.

From our results it appears that, in the reaction catalyzed by the 6-phosphogluconate dehydrogenase, the coenzyme has at least a dual role: (*a*) a redox role in the first step (dehydrogenation); and (*b*) a non-redox role in the decarboxylation and in the tritium exchange reactions. If the enzymatic decarboxylation of 3-keto-2-deoxy-6-phosphogluconate includes two steps, (*a*) the loss of CO_2 to form an enolate, and (*b*) the enzymatic ketonization to form the product which is liberated, it could be possible that the reduced coenzyme has a role only in the ketonization process and not in the decarboxylation. Studies are in active progress in this laboratory to establish the mechanism(s), not connected with an oxidoreduction, by which the reduced coenzyme stimulates both the decarboxylation and the tritium exchange reactions. It is to be recalled that TPNH is essential for the tritium exchange reaction catalyzed by isocitrate dehydrogenase (26) and that TPN stimulates the decarboxylation of oxalacetate catalyzed by the malic enzyme (3–7).

Our results indicate also that, in the presence of enzyme and TPNH, the chemical equilibria existing between the 3-keto-2-deoxy-6-phosphogluconate and its reduction and decarboxylation products are unfavorable to the accumulation of the intermediate. Also in the cases of 2-deoxygluconate dehydrogenase (23) and β-hydroxy acid dehydrogenase (24), the chemical equilibrium is shifted toward the reduced substrate. Coupled with the considerations on the rates of the partial reactions (exposed in the introduction of this paper), this finding could explain why the oxalosuccinate, the oxalacetate, and the 3-keto-6-phosphogluconate were never trapped as intermediates of the oxidative decarboxylation of isocitrate, malate, and 6-phosphogluconate.

The 3-keto-6-phosphogluconate, very likely formed by the enzymatic oxidative decarboxylation of the 6-phosphogluconate, has an hydroxyl group at carbon 2; the electron-attracting effect of this group is expected to develop at carbon 2 of 6-phosphogluconate, an electron deficiency which results in an easy release of the CO_2; in these conditions the dehydrogenation could be the rate-limiting step, as experimentally found (13). Using instead as substrate the 2-deoxy-6-phosphogluconate, the decarboxylation is slower and is the rate-limiting step, in fact, the formation of the β-keto acid has been observed. It appears therefore that the hydroxyl group present at carbon 2 of 6-phosphogluconate has a dual role in the mechanism of action of the enzyme: an orientation role for the substrate in the active site of the enzyme (in fact the 6-phosphogluconate has a K_m for the enzyme 10-fold lower than 2-deoxy-6-phosphogluconate) and a chemical role favoring the decarboxylation of the intermediate.

We were unable so far to obtain a direct chemical identification of the product of the enzymatic oxidative decarboxylation of 2-deoxy-6-phosphogluconate. This product should be the 1-deoxyribulose 5-phosphate on the basis of the analogy existing between the reaction we have studied and the oxidative decarboxylations of 6-phosphogluconate (27), 2-deoxygluconate (23), and gulonate (24) which yield ribulose 5-phosphate, 1-deoxyribulose, and xylulose, respectively. To the best of our knowledge the 1-deoxyribulose 5-phosphate was never reported in the literature.

When the oxidative decarboxylation of 2-deoxy-6-phosphogluconate was carried out in tritiated water, the pentose phosphate obtained contained 1 atom of tritium per residue of phosphate. Incubating this tritiated 1-deoxyribulose 5-phosphate with TPNH and enzyme in the conditions described (15) for the tritium exchange between ribulose 5-phosphate and water, no appreciable exchange of tritium was obtained. This result was not unexpected; comparing the possibilities of tritium exchange of ribulose 5-phosphate and 1-deoxyribulose 5-phosphate, it appears that for the second compound the rate of tritium exchange should be one-third of that of the first compound, since all three hydrogens are equivalent. Furthermore the hydroxyl group present at carbon 1 of ribulose 5-phosphate has both an orientation effect for the substrate in the active site of the enzyme and a labilization effect for the hydrogen exchangeable with the medium; in 1-deoxyribulose 5-phosphate, which lacks this hydroxyl, both effects are absent and thus the rate of tritium exchange should be much lower.

Finally a last point is of interest from the metabolic point of view. 2-Deoxyglucose is a well known metabolic inhibitor (28). It can be phosphorylated to 2-deoxyglucose 6-phosphate, oxidized to 2-deoxy-6-phosphogluconate and then metabolized in different ways (28). From our experimental results it appears that 2-deoxy-6-phosphogluconate can be also oxidatively decarboxylated to 1-deoxyribulose 5-phosphate, following another step of the oxidative pathway of the metabolism of glucose. Research is in progress to elucidate the further metabolism of 1-deoxyribulose 5-phosphate and the possible action of this compound as metabolic inhibitor.

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