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The Transketolase Exchange Reaction *in vitro*

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A reaction sequence for the non-oxidative pentose phosphate pathway was first proposed by Horecker, Gibbs, Klenow & Smyrniotis (1954) and Gibbs & Horecker (1954), from the results of experiments involving the reaction of [1-^{14}C]ribose 5-phosphate with enzyme preparations from acetone-dried powders of either rat liver or pea root tissue. From the ^{14}C distribution patterns found in glucose 6-phosphate formed in the reaction mixtures after 17h (rat liver) and 4h (pea root) it was proposed that fructose 6-phosphate is formed in the pentose pathway by a reaction catalysed by transaldolase (EC 2.2.1.2) (reaction 1):

$$\text{Sedoheptulose 7-phosphate} + \text{glyceraldehyde 3-phosphate} \rightleftharpoons \text{fructose 6-phosphate} + \text{erythrose 4-phosphate} \qquad (1)$$

and a reaction catalysed by transketolase (EC 2.2.1.1) (reaction 2):

$$\text{Xylulose 5-phosphate} + \text{erythrose 4-phosphate} \rightleftharpoons \text{fructose 6-phosphate} + \text{glyceraldehyde 3-phosphate} \qquad (2)$$

It was also suggested from the above results that the sum reaction (reaction 3) for the complete anaerobic segment of the pathway has the following stoicheiometry:

$$3[1\text{-}^{14}\text{C}]\text{Ribose 5-phosphate} \longleftrightarrow 2[1,3\text{-}^{14}\text{C}]\text{fructose 6-phosphate} + \text{glyceraldehyde 3-phosphate} \qquad (3)$$

and the reaction sequence of the pathway acted to form fructose 6-phosphate with only C-1 and C-3 labelled. Theoretically this scheme directed that the ratio of radioactivity in C-1 and C-3 of fructose 6-phosphate was 2:1, although experimentally Horecker *et al.* (1954) found the ratio to be 3:1.

When [1-^{14}C]ribose was metabolized by rabbit liver *in situ* during short-time experiments (1–5min) (Williams, Rienits, Schofield & Clark, 1971) it was found that the distribution of ^{14}C into the carbon atoms of the hexose 6-phosphates did not agree with the theoretical predictions of the pentose pathway reaction scheme. The following distribution of ^{14}C was found in fructose 6-phosphate after 5min metabolism of [1-^{14}C]ribose by rabbit liver *in situ*:

C-1, 80.5%; C-2, 2.9%; C-3, 1.5%; C-4, 3.9%; C-5, 0%; C-6, 11.2%. We have proposed (Williams *et al.* 1971) that this distribution of radioactivity was the result of the reactions (4), (5), (6) and (7), in which reaction (7), a rapid transketolase exchange reaction, is featured:

$$[1\text{-}^{14}\text{C}]\text{Ribose} + \text{ATP} \xrightarrow{\text{Ribokinase}} [1\text{-}^{14}\text{C}]\text{ribose 5-phosphate} + \text{ADP} \qquad (4)$$

$$[1\text{-}^{14}\text{C}]\text{Ribose 5-phosphate} \overset{\text{Isomerase}}{\rightleftharpoons} [1\text{-}^{14}\text{C}]\text{ribulose 5-phosphate} \qquad (5)$$

$$[1\text{-}^{14}\text{C}]\text{Ribulose 5-phosphate} \overset{\text{Epimerase}}{\rightleftharpoons} [1\text{-}^{14}\text{C}]\text{xylulose 5-phosphate} \qquad (6)$$

$$\text{Fructose 6-phosphate} + [1\text{-}^{14}\text{C}]\text{xylulose 5-phosphate} \underset{\text{exchange}}{\overset{\text{Transketolase}}{\rightleftharpoons}} [1\text{-}^{14}\text{C}]\text{fructose 6-phosphate} + \text{xylulose 5-phosphate} \qquad (7)$$

Hiatt (1957) has also postulated the operation of a transketolase exchange reaction to account for similar ^{14}C distribution patterns in the glucose units of glycogen after the metabolism of [1-^{14}C]-xylose *in vivo*. We now report results of studies on transketolase-catalysed exchange reactions *in vitro* using rat liver transketolase. The results of two experiments are presented, which show (*a*) that transketolase catalyses an exchange between like aldehyde acceptor molecules (reaction 10, sum of partial reactions 8 and 9):

Fructose 6-phosphate + transketolase $\rightleftharpoons$ glycolaldehyde–enzyme + erythrose 4-phosphate (8)

[^{14}C]Erythrose 4-phosphate + glycolaldehyde–enzyme $\rightleftharpoons$ [^{14}C]fructose 6-phosphate + transketolase (9)

Fructose 6-phosphate + [^{14}C]erythrose 4-phosphate $\rightleftharpoons$ erythrose 4-phosphate + [^{14}C]fructose 6-phosphate (10)

and (*b*) that transketolase catalyses an exchange between the unlike aldehyde acceptor molecules erythrose 4-phosphate and glyceraldehyde 3-phosphate, as shown in the partial reactions (11) to (14) and the sum reaction, (7):

[1-^{14}C]Xylulose 5-phosphate + transketolase $\rightleftharpoons$ [2-^{14}C]glycolaldehyde–enzyme + glyceraldehyde 3-phosphate (11)

Fructose 6-phosphate + transketolase $\rightleftharpoons$ glycolaldehyde–enzyme + erythrose 4-phosphate (12)

[2-^{14}C]Glycolaldehyde–enzyme + erythrose 4-phosphate $\rightleftharpoons$ transketolase + [1-^{14}C]fructose 6-phosphate (13)

Glycolaldehyde–enzyme + glyceraldehyde 3-phosphate $\rightleftharpoons$ xylulose 5-phosphate + transketolase (14)

Exchange between like aldehyde acceptors. [4-^{14}C]-Erythrose 4-phosphate, prepared from [6-^{14}C]-glucose 6-phosphate by the method of Simpson, Perlin & Sieben (1966), was incubated with fructose 6-phosphate and rat liver transketolase (Horecker & Smyrniotis, 1955). The specific radioactivities of erythrose 4-phosphate and fructose 6-phosphate were determined at various time-intervals and the results are shown in Fig. 1(*a*). The specific radioactivity of erythrose 4-phosphate decreased and that of fructose 6-phosphate increased to reach equilibrium values at 30min. No change in the concentration of either metabolite was found to occur during the time-course of the experiment. Further, in an incubation identical with that described above, except that no transketolase had been added, no change in the specific radioactivity or concentration of erythrose 4-phosphate and fructose 6-phosphate was found.

After 30min of incubation 1.5ml of the reaction mixture described in the legend of Fig. 1(*a*) was deproteinized with 2.0ml of 0.6M-perchloric acid and neutralized to pH 5.5 with potassium hydroxide. Tris buffer, pH 7.5 (100μmol), was added, together with 0.5 unit of glucose phosphate isomerase (EC 5.3.1.9). After 30min at 37°C the reaction was stopped by heating at 100°C for 10min and the [^{14}C]glucose 6-phosphate dephosphorylated, purified and degraded as described by Williams *et al.* (1971). The following distribution of ^{14}C was found in glucose 6-phosphate: C-1, 0.8%; C-2, 0%; C-3, 0%; C-4, 1.2%; C-5, 2.0%; C-6, 96.0%. This distribution supports the postulated exchange reaction mediated by transketolase and shown in reaction (10).

Exchange between unlike aldehyde acceptors. To demonstrate the transketolase-catalysed exchange between the unlike aldehyde acceptors erythrose 4-phosphate and glyceraldehyde 3-phosphate (reactions 11 to 14), [1-^{14}C]xylulose 5-phosphate was added to a reaction mixture composed of xylulose 5-phosphate, erythrose 4-phosphate, fructose 6-phosphate and glyceraldehyde 3-phosphate maintained at equilibrium by transketolase. The specific radioactivities of fructose 6-phosphate and xylulose 5-phosphate were determined at various time-intervals and the results are shown in Fig. 1(*b*). The specific radioactivity of fructose 6-phosphate increased as the specific radioactivity of xylulose 5-phosphate decreased until equilibrium values were attained. The exchange was dependent on the presence of transketolase and no change in the concentration of any of the reactants (fructose 6-phosphate, glyceraldehyde 3-phosphate, xylulose 5-phosphate and erythrose 4-phosphate) was detected. Of the radioactivity contained in fructose 6-phosphate 98.2% was found in the CO_2 derived from C-1 when decarboxylated by the enzymes glucose phosphate isomerase, glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate

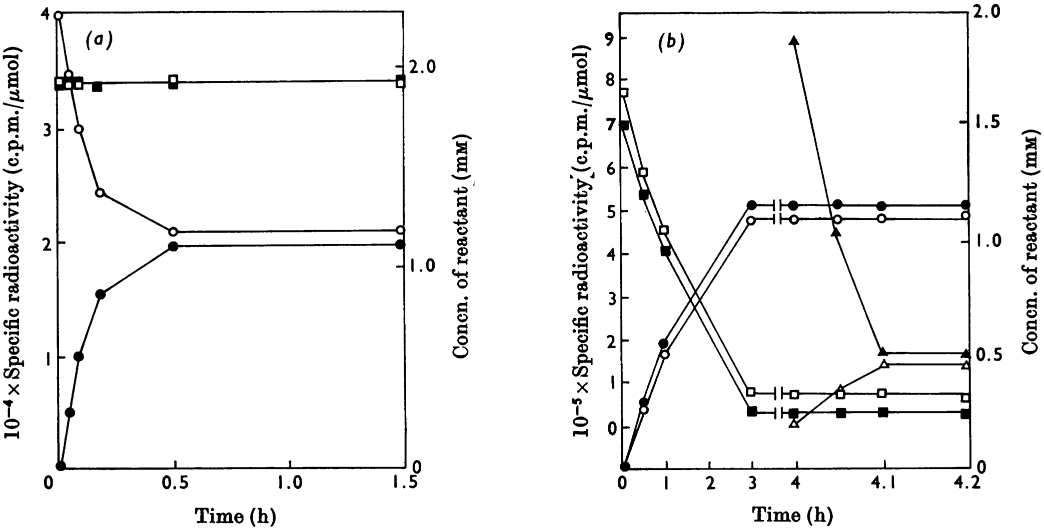

Fig. 1. (*a*) Transketolase-catalysed exchange between erythrose 4-phosphate and fructose 6-phosphate. The following reagents were incubated in a volume of 3.30ml at 25°C for 90min: [4-^{14}C]erythrose 4-phosphate (2.6×10^5 c.p.m., 6.5μmol); fructose 6-phosphate (6.5μmol); crystalline rat liver transketolase (5munits; approx. 4 units/mg; activity determined at pH7.4 and 25°C with xylulose 5-phosphate and erythrose 4-phosphate as substrates); glycylglycine–KOH buffer, pH7.4 (500μmol). Samples (0.3ml) were removed into 0.4ml of ice-cold 0.6M-$HClO_4$ at the times shown. After removal of the protein by centrifugation, the solutions were neutralized to pH6.5 with 2M-KOH and the precipitate of $KClO_4$ was removed by centrifugation at 5000*g* for 10min. The KOH-neutralized solutions were treated with plant acid phosphatase, deionized and chromatographed on 3MM Whatman paper by the procedure described by Williams *et al.* (1971). Each region of the chromatogram corresponding to erythrose and fructose was cut out and the material eluted. Radioactivity was determined with a scintillation counter on a sample corresponding to one-tenth of the total volume of eluted material, with a Triton X-100 scintillant (Patterson & Green, 1965). Fructose was determined by the method of Klotzsch & Bergmeyer (1965) and erythrose with the phenol–sulphuric acid reagent (Dubois, Gilles, Hamilton, Rebers & Smith, 1956). By using the results obtained for the radioactivity and sugar content of each eluted region, the specific radioactivities of erythrose (and hence erythrose 4-phosphate) (○) and fructose 6-phosphate (●) were calculated. The concentrations of erythrose 4-phosphate (Racker, 1965*b*) (□) and fructose 6-phosphate (Hohorst, 1965) (■) were each determined enzymically in a small portion of the neutralized solution before dephosphorylation. Each value is the mean of duplicate determinations from two experiments. (*b*) Transketolase-catalysed exchange between [1-^{14}C]xylulose 5-phosphate and fructose 6-phosphate. The following reagents were incubated in a volume of 4.60ml at 25°C: xylulose 5-phosphate (6.90μmol; grade III; Sigma Chemical Co., St Louis, Mo., U.S.A.); erythrose 4-phosphate (7.5μmol); thiamin pyrophosphate (1.0μmol); $MgCl_2$ (25μmol); glycylglycine–KOH buffer, pH7.4 (750μmol); transketolase (35munits). Samples (0.2ml) of the incubation mixtures were removed into 0.25ml of ice-cold 0.6M-$HClO_4$ at the times shown. The concentration of erythrose 4-phosphate (Racker, 1965*b*) (□), xylulose 5-phosphate (Racker, 1965*a*) (■), fructose 6-phosphate (Hohorst, 1965) (●) and glyceraldehyde 3-phosphate (Bücher & Hohorst, 1965) (○) were each determined on a small portion of the KOH-neutralized solution. After equilibrium was reached, as indicated by no further change in the concentrations of any of the reactants (i.e. at 4h), 0.02μmol (1.2×10^6 c.p.m.) of [1-^{14}C]xylulose 5-phosphate was added (the [1-^{14}C]xylulose 5-phosphate used in these experiments contained 30.2% of [1-^{14}C]ribulose 5-phosphate). Immediately after the addition of the radioactive material and at the times shown 0.2ml samples were removed from the incubation mixture and treated as described above. After determination of each of the reactants the remainder of the neutralized deproteinized sample was treated with plant acid phosphatase, deionized and chromatographed on 3MM Whatman paper by the procedure described by Williams *et al.* (1971). Each region of the chromatogram corresponding to fructose and xylulose was cut out and the material eluted. The radioactivity of each eluated sample was determined as described for Fig. 1(*a*). Fructose was determined by the method of Klotzsch & Bergmeyer (1965) and xylulose with cysteine–carbazole reagent (Dische & Borenfreund, 1951). By using the results obtained for the radioactivity and sugar content of each eluted region the specific radioactivities of fructose (and hence fructose 6-phosphate) (△) and xylulose 5-phosphate (▲) were calculated. Each value is the mean of duplicate determinations from two experiments.

dehydrogenase (EC 1.1.1.44) in the presence of $NADP^+$.

The results indicate that transketolase catalyses the exchange reaction predicted from the studies *in situ* (Williams *et al.* 1971), and this reaction is analogous to the exchange catalysed by transaldolase (Ljungdahl, Wood, Racker & Couri, 1961), the other group-transferring enzyme of the nonoxidative pentose phosphate pathway scheme. Calculations from the results of Figs. 1(*a*) and 1(*b*) indicate that the rate of the exchange reaction is rapid and is three- to eight-fold greater than the rate of the chemical reaction. Similar experiments with crystalline yeast enzyme (Sigma; type IV) gave results identical with those shown in Fig. 1 and support the conclusion that the isotope-exchange property of transketolase is not restricted to the rat liver enzyme. Finally, it is suggested that the greater than theoretically predicted amounts of ^{14}C found by Horecker *et al.* (1954) in C-1 of glucose 6-phosphate after 17h incubation may have resulted from the transketolase-catalysed exchange reaction. The failure to recognize the exchange reactions catalysed by transketolase and transaldolase in the studies on the reaction mechanism of the pentose phosphate pathway (Horecker *et al.* 1954; Gibbs & Horecker, 1954) may have led to an over-simplification of the pathway reaction sequences.

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