FORMATION OF SEDOHEPTULOSE-7-PHOSPHATE FROM ENZYMATICALLY OBTAINED "ACTIVE GLYCOLIC ALDEHYDE" AND RIBOSE-5-PHOSPHATE WITH TRANSKETOLASE[+)]

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By incubation of hydroxypyruvate with TPP[+++)] and a preparation of pyruvate oxidase from pig heart muscle, "active glycolic aldehyde" is formed (da Fonseca-Wollheim et al. 1962). "Active glycolic aldehyde" isolated from such incubation mixtures is TPP whose position 2 of the thiazol ring is substituted by a 1,2-dihydroxyethyl group (Bock et al. 1962). It could be expected on the basis of previous experiments of Racker et al. (1953) and Horecker and Smyrniotis (1953) that the incubation of "active glycolic aldehyde" and ribose-5-phosphate with transketolase would yield sedoheptulose-7-phosphate. This is demonstrated in this paper.

^{14}C-DETPP obtained from 3-^{14}C-hydroxypyruvate (da Fonseca-Wollheim et al.1962) was incubated with ribose-5-phosphate and purified apotransketolase (Srere et al. 1958) from yeast for 5 hours at 34°C. The deproteinized incubation mixture was applied to a column of Dowex 2-formate. Fig. 1 shows the chromatogram obtained by gradient elution with ammonium formate/formic acid buffer. 3 radioactivity peaks appear. The radioactivity of the water washed portion(peak I), when submitted to high voltage electrophoresis in neutral buffers, shows no mobility. It probably represents dephosphorylated sugars. Peak II represents TPP and DETPP which are not separated by this

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+++) Abbreviations: DETPP = 2-(1,2-dihydroxyethyl)-thiamine pyrophosphate; TPP = thiamine pyrophosphate

ion exchange chromatography method. Peak III appears at exactly the same position where authentic sedoheptulose-7-phosphate appears.

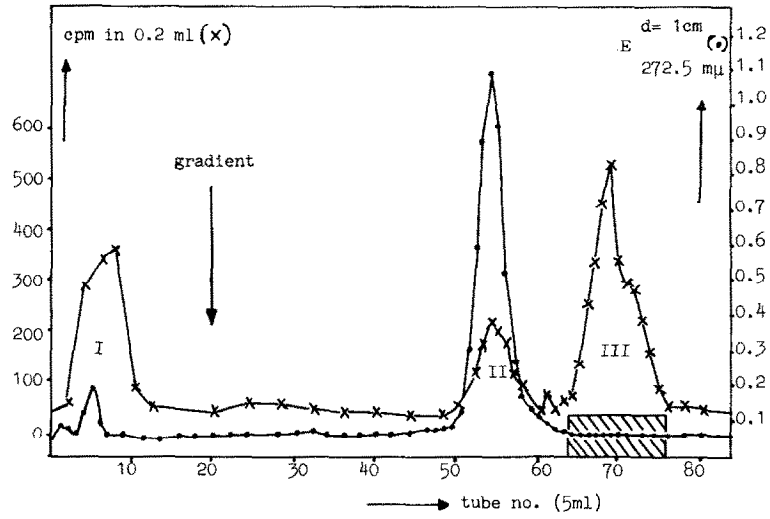

Figure 1: Ion exchange chromatogram with Dowex 2-formate of an incubation mixture of ^{14}C-DETPP and ribose-5-phosphate with transketolase. Radioactivity peak II: ^{14}C-DETPP; radioactivity peak III: ^{14}C-sedoheptulose-7-phosphate; oblique lined area: positive in the orcinol reaction.

Since ribose-5-phosphate, identified with the carbazol test (Dische and Borenfreund 1951), appears very near to sedoheptulose-7-phosphate in the ion exchange chromatography, use was made of the orcinol reaction for heptoses according to Dische (1953) for further identification. Fig. 2 shows the absorption spectra of the pooled fractions corresponding to peak III from the ion exchange chromatogram (fig. 1) and of authentic sedoheptulose-7-phosphate. The absorption spectra are identical. Ribose-5-phosphate shows no absorption at 610 mμ under the same conditions. Using the quantitative values of the orcinol reaction, the specific radioactivity of the sedoheptulose-7-phosphate fraction of the ion exchange chromatogram was calculated to be 1/4 of that of the used ^{14}C-DETPP. This can be explained by the fact that traces of pentose-phosphate isomerase and epimerase in the large amount of the used transketolase are responsible for the formation of some sedoheptulose-7-phosphate during the 5 hours period of incubation. Sedoheptulose-7-phosphate produced in this way is not radioactive and therefore dilutes the one produced from ^{14}C-DETPP.

Peak III (fig. 1), when submitted to paper chromatography for 12 hours on Schleicher and Schüll paper 2040b ausg. in the solvents system ethylene

glycol monomethyl ether / pyridine / acetic acid / water (80:40:10:10) (Runeckles and Krotkov 1957) moves with the same R_{Pi} (Rf relative to orthophosphate) as authentic sedoheptulose-7-phosphate (R_{Pi} approx. 0.68). Ribose-5-phosphate, under these conditions, has an R_{Pi} of approx. 0.77; TPP and DETPP show an R_{Pi} of approx. 0.01. The sugar-phosphates were identified with the aniline phthalate spray (Partridge 1949) and by the dipping technique of Runeckles and Krotkov (1957).

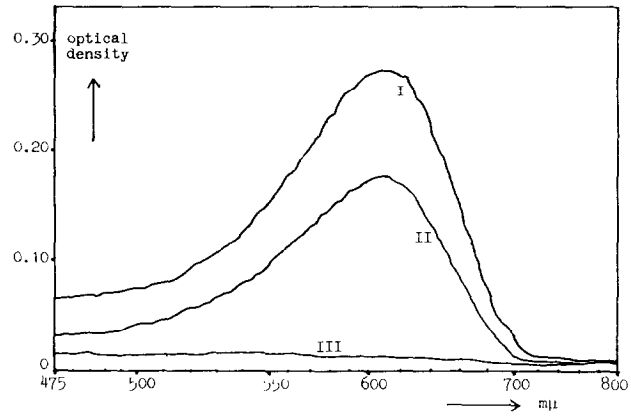

Figure 2: Absorption spectra in the orcinol reaction.
Curve I: With combined tubes no. 64-76 from the ion exchange chromatography shown in fig. 1; curve II: with 0.27 μmole/ml of authentic sedoheptulose-7-phosphate; curve III: with 1.0 μmole/ml of ribose-5-phosphate.

The radioactivity corresponding to peak III (fig. 1) moves in high voltage electrophoresis according to Wieland and Pfleiderer (1955) (40 v/cm; 0.2 M Sörensen-phosphate-buffer pH 7.0; 0°C; 90 min) 10-15 cm to the anode. Authentic sedoheptulose-7-phosphate has the same mobility, whereas ribose-5-phosphate moves 16.5 cm to the anode (identification with aniline phthalate spray).

EXPERIMENTAL

Formation of ^{14}C-sedoheptulose-7-phosphate from ^{14}C-DETPP and ribose-5-phosphate with transketolase

0.77 μmole of ^{14}C-DETPP ($2.3 \cdot 10^5$ cpm from tubes no. 412-460 of the ion exchange chromatogram of fig. 2 of da Fonseca-Wollheim et al. (1962)), 2 μmoles $MgCl_2$ and 10 μmoles ribose-5-phosphate Na-salt were dissolved in 0.5 ml 0.2 M tris buffer pH 7.4. After addition of 91 units of transketolase (Srere et al.1958) dissolved in 0.5 ml 0.025 M glycyl-glycine buffer pH 7.4, the mixture was incubated for 5 hours at 34°C and then deproteinized by adding

5 ml of 60°C methanol. After centrifugation, the supernatant was concentrated in a vacuum evaporator to almost dryness and then dissolved in 1 ml of water.

Separation of ^{14}C-sedoheptulose-7-phosphate by ion exchange chromatography (cf. fig. 1)

After adding 3 µmoles of TPP as marker to the above incubation, it was applied to a column of Dowex 2-formate, X 8, 200-400 mesh (30 cm · 2.3 cm^2). After washing with 100 ml of water, it was fractionated by gradient elution with a solution containing 0.5 M $HCOONH_4$ and 0.2 M HCOOH flowing into a mixing chamber containing 300 ml of water. 5 ml fractions were collected and the UV absorption at 272.5 mµ (d = 1 cm) of each fraction was measured. The radioactivity of 0.2 ml was determined after drying, using the methane flow counter FH 407 (Frieseke and Hoepfner, Erlangen). Peak III appears where authentic sedoheptulose-7-phosphate appears in several control experiments carried out under the same conditions. Authentic sedoheptulose-7-phosphate was prepared from hydroxypyruvate and ribose-5-phosphate with transketolase according to Srere et al. (1958). The yield of ^{14}C-sedoheptulose-7-phosphate (total radioactivity of peak III 6.3 · 10^4 cpm) is 27 % with respect to the initially used ^{14}C-DETPP (2.3 · 10^5 cpm).

Orcinol reaction, paperchromatography and high voltage electrophoresis of peak III

Tubes no. 64-76 were pooled, concentrated in vacuo to 2 ml and subjected to the orcinol reaction according to Dische (1953), using 1 ml of concentrated HCl instead of 0.4 ml (cf. fig. 2). 1/4 of a deproteinized incubation mixture from a parallel experiment to the one shown in fig.1, containing initially 2.8 · 10^5 cpm ^{14}C-DETPP incubated with 9.1 units of transketolase, was submitted to paperchromatography as described in the text. After applying the other 3/4 of the incubation mixture to a Dowex 2-formate column and fractionating by gradient elution, the tubes corresponding to the radioactive peak III in fig. 1 were pooled, vacuum-concentrated and the sugar phosphates precipitated as Ba-salts (Smyrniotis and Horecker 1956). After converting the Ba-salt to the Na-salt with Na_2SO_4, the sugar phosphates were submitted to electrophoresis as described in the text.

SUMMARY

^{14}C-labelled 2-(1,2-dihydroxyethyl)-TPP obtained from 3-^{14}C-hydroxypyruvate and TPP with a preparation of pyruvate oxidase from pig heart muscle, when incubated with ribose-5-phosphate and transketolase, yields ^{14}C-sedo-

heptulose-7-phosphate. Consequently 2-(1,2-dihydroxyethyl)-TPP has the characteristic property of "active glycolic aldehyde" to transfer glycolic aldehyde to an acceptor aldehyde when catalyzed by transketolase.

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