A THIAMINE PYROPHOSPHATE-GLYCOLALDEHYDE COMPOUND ("ACTIVE GLYCOLALDEHYDE") AS INTERMEDIATE IN THE TRANSKETOLASE REACTION[+)]

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Thiamine pyrophosphate (TPP) is essential as coenzyme for the transfer of the two-carbon fragment catalyzed by transketolase (Horecker and Smyrniotis, 1953; Racker, de la Haba et al., 1953). It was to be expected that a thiamine pyrophosphate-glycolaldehyde compound, i.e. "active glycolaldehyde", would be an intermediate in the transketolase reaction by analogy with "active acetaldehyde" as an intermediate in the decarboxylation (Holzer and Beaucamp, 1959; 1961) and oxidation (Holzer, Goedde et al., 1960) of pyruvate. Datta and Racker (1959; 1961) isolated a "glycolaldehyde-enzyme-intermediate" which shows the transfer-reactions predicted for "active glycolaldehyde" and therefore probably is a transketolase bound "active glycolaldehyde". Applying methods which had made possible the isolation of "active acetaldehyde" from enzyme incubations (Holzer and Beaucamp, 1959; 1961; Holzer, Goedde et al., 1960) we tried to isolate "active glycolaldehyde" from incubation mixtures of transketolase with uniformly ^{14}C-labelled fructose-6-phosphate and TPP. Since no acceptor aldehyde is added, the complete reaction cannot proceed. It could be expected, however, that the TPP added to the incubation mixture become loaded with glycolaldehyde. In the present paper we describe a radioactive derivative of TPP, separated from such incubation mixtures using ionexchange chromatography. When incubated with transketolase and non-radioactive glycolaldehyde this ^{14}C-labelled derivative yields radioactive erythrulose and thus exhibits the properties of "active glycolaldehyde". These results have shortly been mentioned in a previously published review (Holzer, 1961).

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RESULTS

To obtain "active glycolaldehyde", transketolase was incubated with uniformly ^{14}C-labelled fructose-6-phosphate and TPP. After addition of hot methanol and centrifugation the supernatant was fractionated on a Dowex 2 column. From fig. 1 it can be seen that two radioactive peaks (I and II) appear within the range of the TPP peak (the latter identified by UV-spectrum,

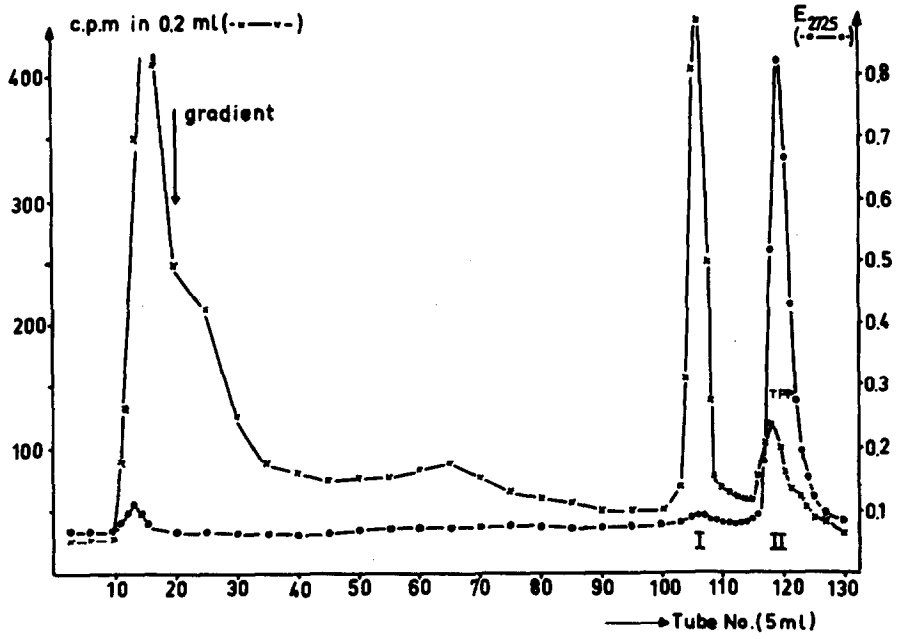

Figure 1: Ion exchange chromatography of a reaction mixture of uniformly ^{14}C-labelled fructose-6-phosphate with transketolase and TPP.
x —— x = radioactivity in 0.2 ml of a 5.0 ml-fraction,
o —— o = UV-absorption at 272.5 mμ (d = 1.0 cm).

thiochrom test and paper chromatography). We have some evidence that peak I is "active fructose-6-phosphate", i.e. a TPP-fructose-6-phosphate compound. We shall report on this in a subsequent paper. Peak II, as demonstrated by the following experiments, represents "active glycolaldehyde". The peaks I and II separated by ion exchange chromatography can also be separated by paper chromatography (part A of fig. 2). Peak II eluted from the paper chromatogram A moves during high voltage electrophoresis (part B of fig. 2)

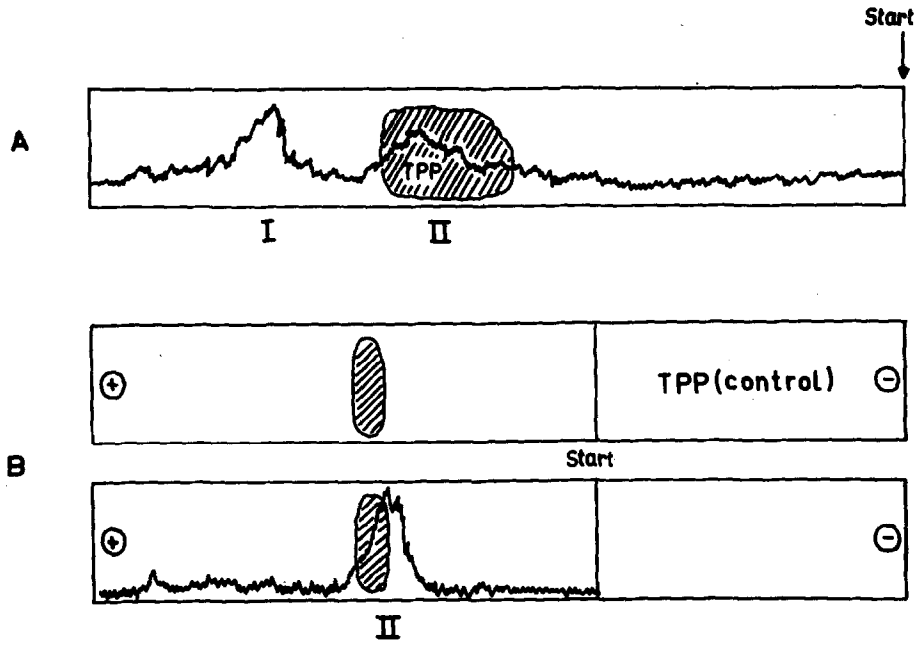

Figure 2: Paper chromatography and high voltage electrophoresis of "active glycolaldehyde".
A: Paper chromatography of the combined column fractions I and II from fig. 1 in the solvent system butanol/ethanol/water = 4:2:3 for 36 hours. Peak I and II correspond to those indicated in fig. 1.
B: High voltage electrophoresis of the eluate from peak II of part A.

uniformly and has a mobility similar to that of TPP. Therefore it could be a TPP-derivative with no additional dissociated groups. The radioactivity of this substance accounts for about 1 per cent of the added U-^{14}C-fructose-6-phosphate. One portion of the water eluted peak II from part A in fig. 2 (50,000 c.p.m.) was incubated with transketolase and non-radioactive glycolaldehyde in order to see whether it reacts as "active glycolaldehyde". After separating such an incubation mixture by paper chromatography we found one new radioactive peak, as shown in fig. 3, which had the same Rf-value as authentic erythrulose. After elution and rechromatography this peak again appeared at the position corresponding to authentic erythrulose. From these experiments we conclude that peak II, isolated from our incubation of transketolase with fructose-6-phosphate, is indeed "active glycolaldehyde". It can be assumed by analogy with "active acetaldehyde" that it is α,ß-dihydroxyethyl-thiamine pyrophosphate, with the dihydroxyethyl group attached to position 2 of the thiazole ring.

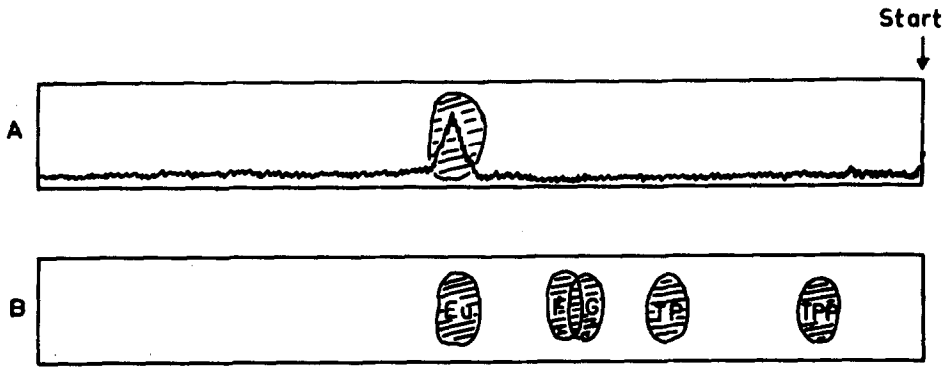

Figure 3: Paper chromatographic identification of radioactive erythrulose formed by incubation of ^{14}C-labelled "active glycolaldehyde" with transketolase and unlabelled acceptor-glycolaldehyde.
A: Radioactive erythrulose from "active glycolaldehyde".
B: Authentic reference substances (Eu = erythrulose; F = fructose; G = glucose; TP = thiamine monophosphate).

Krampitz, Suzuki and coworkers (1961) have reported on a substance prepared non-enzymatically from formaldehyde and TPP which yields sedoheptulose-7-phosphate when incubated with ribose-5-phosphate and gram quantities of transketolase. Whether this substance is identical with the "active glycolaldehyde" described in the present paper, can be decided only after the experimental details about the work of Krampitz, Suzuki et al. are available.

EXPERIMENTAL

Preparation and purification of "active glycolaldehyde": 9 µmoles U-^{14}C-fructose-6-phosphate with 7 µC ^{14}C (prepared by phosphorylation of U-^{14}C-fructose with purified yeast hexokinase and adenosine triphosphate) were incubated for 180 min at 30° C with 9.2 mg (specific activity 2 units/mg) of purified transketolase from bakers yeast (Srere, Cooper et al., 1958), 6 µmoles TPP and 5 µmoles $MgCl_2$ in 0.5 ml 0.15 M glycylglycine-buffer pH 7.4. In more recent experiments we have found that there is no apparent increase in radioactivity of peak I and II (fig. 1) between 10 and 180 min incubation time. The reaction was stopped by addition of 10 volumes hot methanol. After centrifugation the vacuum-concentrated supernatant was applied to a column of Dowex-2-Cl^-, x 8, 200-400 mesh, 40 cm x 6.3 cm^2, and fractionated in 5 ml portions, first by washing with 100 ml H_2O dest., then by gradient elution with 2 x 10^{-3} N HCl, the mixing chamber containing 250 ml H_2O. UV-absorption at 272.5 mµ (d = 1.0 cm) and radioactivity were registered as shown in fig. 1. Under these conditions fructose-6-phosphate

remains fixed to the resin; it can be eluted with 50-100 ml of 0.02 N HCl. The TPP-fractions containing radioactivity were combined, neutralized, concentrated under vacuum, and paper chromatographed in a solvent system butanol/ethanol/water = 4:2:3 for 36 hours (cf. part A of fig. 2). The radioactivity on the chromatogram was measured using a radiopaperchromatograph; the same procedure was used in part B of fig. 2 and in part A of fig.3. Peak II was eluted with water, and submitted to high voltage electrophoresis (Schleicher and Schüll-paper 2043b Mgl; 0.1 M phosphate buffer pH 7.0; 30 V/cm; 90 mA; 120 min). Authentic TPP migrated 5.8 cm towards the anode, the labelled "active glycolaldehyde" 5.4 cm (cf. part B of fig. 2).

Formation and identification of erythrulose: "Active glycolaldehyde" (50,000 c.p.m., eluted from peak II, part A, fig. 2) was incubated with 5 µmoles unlabelled glycolaldehyde, 0.5 µmoles $MgCl_2$ and 1.6 mg transketolase (7 units/mg) in 0.5 ml 0.1 M tris-(hydroxymethyl-) aminomethane buffer pH 7.4 at 30° C for 5 hours. After addition of hot methanol and centrifugation the vacuum-concentrated supernatant was mixed with 0.5 µmoles of unlabelled erythrulose as carrier. This mixture was applied to a Dowex-2 column x 8, 200-400 mesh, Cl^-, and Dowex-50, x 8, 200-400 mesh, H^+, each 10 cm x 2.4 cm^2, which were separated by a thin layer of glass-wool. The column was washed with about 80 ml of distilled water. The vacuum concentrated effluent containing the erythrulose was paper chromatographed in a solvent system butanol/acetic acid/water = 5:2:3 for 18 hours. There was one radioactive peak which after rechromatography exhibited a Rf-value of 0.45, identical with the Rf-value of authentic erythrulose localized with an $AgNO_3$-spray (cf. fig. 3).

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