STRUCTURE OF "ACTIVE GLYCOLIC ALDEHYDE"[+)]

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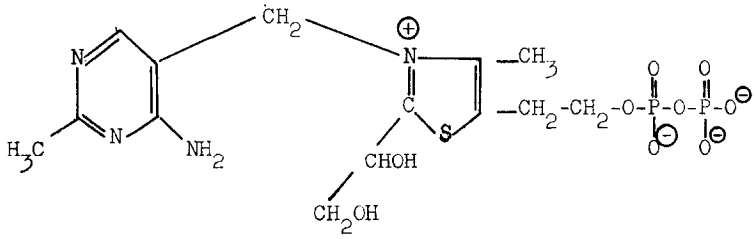
In our previous publications we have described the isolation of ^{14}C-labelled "active glycolic aldehyde" from an incubation mixture of transketolase with U-^{14}C-fructose-6-phosphate and TPP[+++)] (Holzer et al. 1962), as well as from an incubation mixture of 3-^{14}C-hydroxypyruvate and TPP with a preparation of pyruvate oxidase from pig heart muscle (da Fonseca-Wollheim et al. 1962). By analogy with the structure of "active acetaldehyde", it could be expected that "active glycolic aldehyde" is TPP whose position 2 of the thiazo ring is substituted by a 1,2-dihydroxyethyl group (abbreviated 2-(1,2-dihydroxyethyl)-TPP).



"active glycolic aldehyde" = 2-(1,2-dihydroxyethyl)-TPP = DETPP

We describe in this paper experiments which confirm this structure for the "active glycolic aldehyde" obtained from hydroxypyruvate.

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

^{14}C-labelled "active glycolic aldehyde" which contained about 50 % TPP was dephosphorylated with phosphatase and submitted to high voltage electrophoresis. UV fluorescence quenching spots III and IV appeared (part A of fig.1). The radioactivity was associated only with spot III. Spot IV was submitted to electrophoresis with authentic thiamine, as a control, and could be identified as such. Consequently, the ^{14}C-glycolic aldehyde-thiamine compound and thiamine can be separated electrophoretically.

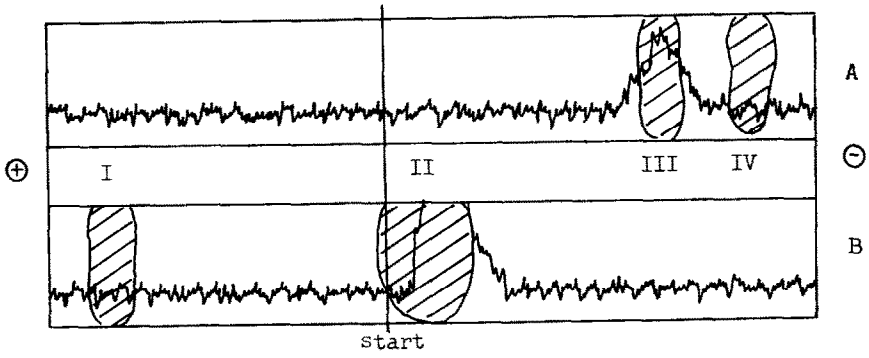
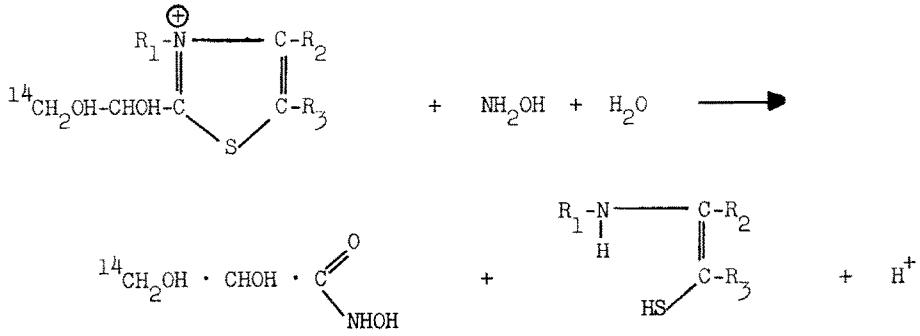


Figure 1: High voltage electrophoresis of ^{14}C-DET before (part A) and after (part B) sulfite cleavage according to Williams et al. (1935). ^{14}C-DETPP obtained according to da Fonseca-Wollheim et al. (1962), containing about 50 % TPP was hydrolized to ^{14}C-DET and thiamine with acid phosphatase from wheat germ (Holzer et al. 1960). Part of this preparation (7000 cpm) was submitted to electrophoresis on Schleicher-and-Schüll-paper 2043b Mgl (40 v/cm; 80 mA; 0.1 M Sörensen-phosphate-buffer pH 7.0; 2 hours) (cf. pherogram A). On control pherograms we found authentic thiamine at position IV, ^{14}C-DET at position III, 2-(1,2-dihydroxyethyl)-4-methyl-5-(2-hydroxyethyl)-thiazol at position II and (2-methyl-4-amino-5-pyrimidyl)-methyl sulfonate at position I. The two last mentioned substances were obtained by cleavage of thiamine with sulfite. The largest part of ^{14}C-DET (110 000 cpm) was cleaved with $NaHSO_3$ according to Williams et al. (1935) (for details cf. Holzer and Beaucamp (1961)), neutralized and submitted to electrophoresis (cf. pherogram B). Two UV fluorescence quenching spots appeared. Spot I corresponds to the pyrimidine part of thiamine; spot II has the mobility of the thiazol part of thiamine and contains the entire radioactivity. The radioactivity distribution was determined with the radiopaperchromatograph FH 452 (Frieseke and Hoepfner, Erlangen). UV fluorescence quenching zones are indicated with oblique lines.

The main fraction of "active glycolic aldehyde" dephosphorylated with phosphatase was cleaved with sulfite according to Williams et al. (1935) and then submitted to electrophoresis. Part B of fig. 1 shows the two UV fluorescence quenching spots (I and II) which resulted. Spot I was identified by comparing its mobility with that of authentic (2-methyl-4-amino-5-pyrimidyl)-methyl sulfonate which was obtained by cleaving thiamine with sulfite (Willi-

ams et al. 1935). The radioactivity has a mobility similar to that of the thiazol part of thiamine (spot II). The thiazol part substituted with the ^{14}C-dihydroxyethyl group and the unsubstituted one have the same mobility in electrophoresis, as expected. This is demonstrated by the almost identical location of the maxima of radioactivity and UV fluorescence quenching (spot II in part B of fig. 1). Spot II containing both the thiazol part substituted with the ^{14}C-1,2-dihydroxyethyl group and the unsubstituted part, was eluted and submitted to paperchromatography in the solvent system butanol/glacial acetic acid/water (5 : 2 : 3) on Schleicher-and-Schüll-paper 2043b Mgl for 15 hours. Two UV fluorescence quenching spots with Rf-values 0.67 and 0.81 appeared. The position of the spot with the Rf-value of 0.81 corresponded to that of the thiazol part obtained by cleaving thiamine with sulfite. The entire radioactivity was associated with the spot whose Rf-value is 0.67. Consequently, as expected, the thiazol part substituted with a 1,2-dihydroxyethyl group is more hydrophilic than the not substituted one.

A cleavage with hydroxylamine was carried out for further proof of the structure presented in the introduction. Since the thiazol part of "active glycolic aldehyde" has the structure of a thioimide of glyceric acid, it could be expected that the formation of radioactive glyceryl hydroxamic acid would proceed according to the following equation:



Part A of fig. 2 shows a chromatogram of authentic glyceryl hydroxamic acid which was obtained by reaction of glyceric acid-methyl ester (from diazomethane and glyceric acid) with hydroxylamine. The hydroxamic acid was identified by its characteristic red color after spraying with $FeCl_3$. Part B from fig. 2 shows the chromatogram of ^{14}C-labelled "active glycolic aldehyde" cleaved with hydroxylamine. Radioactivity appears at the position of glyceryl hydroxamic acid (peak I). Its yield was approx. 5 %. A large part of the radioactivity remains close to the starting line. This radioactivity probably represents decomposition products of glyceryl hydroxamic acid and unreacted DETPP. The glyceryl hydroxamic acid preparation as well as the "active glycolic aldehyde" cleaved with NH_2OH contain a yellow fluorescing compound whose Rf-

value is 0.6. Chromatography of ^{14}C-labelled "active glycolic aldehyde" cleaved with NH_2OH yields radioactivity at the same position. Therefore this substance probably is a derivative of glyceryl hydroxamic acid; it does not give the characteristic red iron complex. When peak I was rechromatographed together with authentic glyceryl hydroxamic acid in the same solvent or in propanol/10 % ammonium carbonate/5 N NH_4OH (6 : 2 : 1) or in butanol/dimethyl-formamide/H_2O (4,5 : 0.5 : 5.0), radioactivity and the $FeCl_3$-reaction were detected in each case at the same position. Also in high voltage electrophoresis (conditions cf. fig. 1) radioactivity and $FeCl_3$-reaction were detected at the same position (about 2-3 cm from the starting line towards the anode). However, due to the low stability of the substance, only a part of the radioactivity and the authentic glyceryl hydroxamic acid was found at this position after rechromatography and electrophoresis.

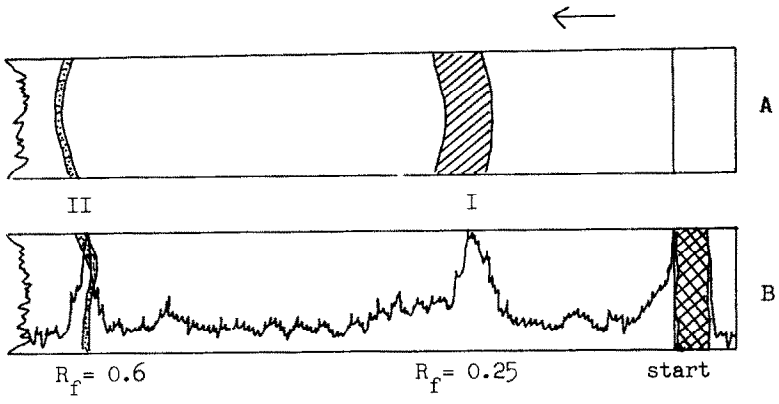


Figure 2: Radiopaperchromatogram of ^{14}C-glyceryl hydroxamic acid obtained by cleaving ^{14}C-DETPP with hydroxylamine.
5 ml 5 N KOH and 5 ml 5 M $NH_2OH \cdot HCl$ were added to 1 µmole ^{14}C-DETPP ($0.3 \cdot 10^6$ cpm) in 1.5 ml H_2O (pH = 7). The mixture was heated for 20 minutes in boiling water. After standing for 10 hours at room temperature, the mixture was vacuum evaporated to dryness, desalted by extraction with absolute alcohol and applied to a column of amberlite IR-120, 200-400 mesh, H^+, 30 cm $\cdot$ 3.14 cm^2, and eluted with water at 2°C. The radioactive fractions were collected and lyophilized. The residue was dissolved in water and submitted to paperchromatography for 13 hours on Schleicher-and-Schüll-paper 2043b Mgl in the solvent system butanol/ethanol/water (4 : 1 : 1). Part B shows the radioactivity distribution determined with the radiopaperchromatograph FH 452 and the UV fluorescence quenching zone (criss-cross lined area).
The glyceryl hydroxamic acid used in chromatogram A for comparison, was obtained by cleaving D,L-glyceric acid-methyl ester with hydroxylamine. This ester was prepared from D,L-glyceric acid and diazomethane. A strong positive Fe^{3+}-reaction was observed at position I (oblique lines). Both, the control solution and the incubation with ^{14}C-DETPP yield a fluorescing band (dotted area in position II).

SUMMARY

^{14}C-labelled "active glycolic aldehyde" obtained from 3-^{14}C-hydroxypyruvate and a preparation of pyruvate oxidase was cleaved with sulfite according to Williams et al. (1935). The pyrimidine and the thiazol parts thus produced, can well be separated in high voltage electrophoresis. The radioactivity was associated with the thiazol part. ^{14}C-glyceryl hydroxamic acid is formed by cleavage of ^{14}C-labelled "active glycolic aldehyde" with hydroxylamine. This shows that the ^{14}C-labelled 1,2-dihydroxyethyl group is attached at position 2 of the thiazol ring of TPP. Consequently, "active glycolic aldehyde" is, as expected by analogy with "active acetaldehyde", a 3-(2-methyl-4-amino-5-pyrimidyl)-methyl-2-(1,2-dihydroxyethyl)-4-methyl-5-(pyrophosphoryl-2-hydroxyethyl)-thiazolium salt (short designation: 2-(1,2-dihydroxyethyl)-TPP).

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