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Fermentation of Glucose in Glycerol-Stereochemistry of the Proton transfer mediated by Phosphoglucoisomerase:a Deuterium NMR proof.

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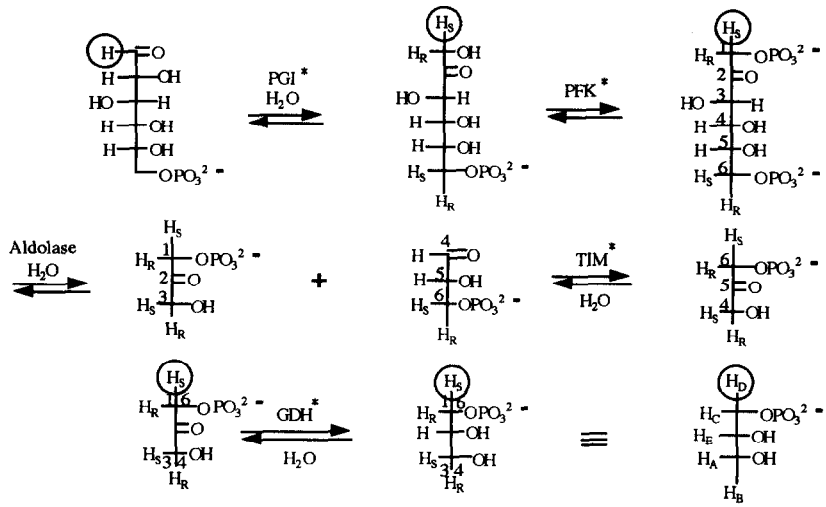
Abstract *: An unambiguous deuterium NMR evidence of the stereochemistry of the proton transfer mediated by phosphoglucoisomerase is given. The methodology used involves the fermentation of a slightly enriched [1-^{2}H]-glucose into glycerol, the latter being transformed further on into glycidyl acetate which is a more suitable structure for ^{2}H NMR studies.*

Introduction

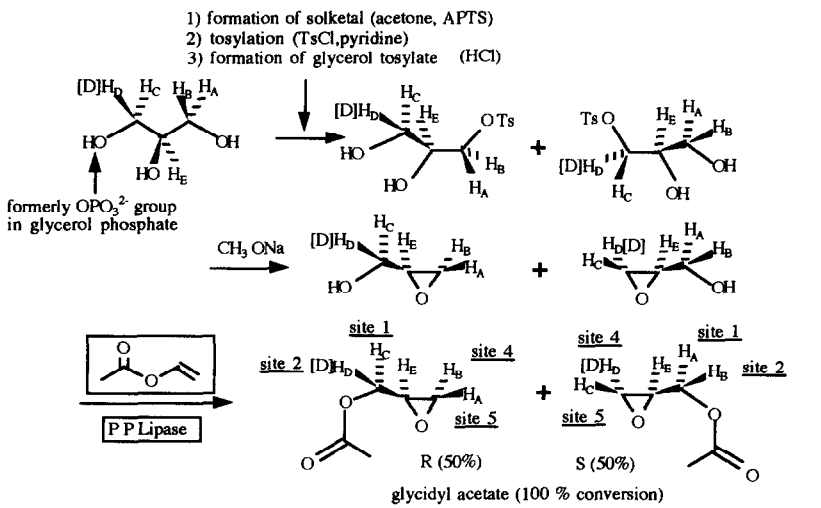
Phosphoaldoisomerases play an important role in glycolysis and in the fermentation of sugars. They catalyse the reversible isomerisation of an aldose phosphate into a ketose phosphate and the nature of the proton exchange (intra- and/or intermolecular) associated with this transformation still incites many works[1,2,3,4]. The stereochemistry of this proton transfer has also already been investigated[5,6,7]. For instance, it has been shown that the glucose-6-phosphate (G-6-P), fructose-6-phosphate (F-6-P) interconversion in tritiated water results in the incorporation of one tritium atom at the C-1 position of the latter and in the C-2 position of the former. Furthermore, the labelled position at the C-1 of the F-6-P synthesized in the presence of phosphoglucoisomerase (PGI) was not the same as that induced by phosphomannoisomerase[6]. The absolute configuration of the [1-^{3}H]-F-6-P formed was attributed on the basis of the following strategy[5,7]. The mixture of [2-^{3}H]-G-6-P and of [1-^{3}H]-F-6-P was treated with HIO_4 and the glycolic acid obtained was further stereospecifically oxidized in glycolic acid by means of glycolic acid oxidase. The final glyoxylic acid was shown to retain the tritium. As L-lactic acid, but not its enantiomer, was also oxidized by glycolic acid oxidase, it was suggested that the hydrogen removed in glycolic acid is sterically related to the α-hydrogen of L-lactic acid. This assumption was supported by the action of L-lactic dehydrogenase which labilizes the same proton of glycolic acid as does the oxidase[5].The aim of this work is to give a real proof in addition to these assumptions on the stereospecificity of the proton transfer mediated by the PGI.

Results and discussion

The methodology worked out for this purpose uses the glycerol obtained in the fermentation of glucose. Thus, starting from a solution of glucose slightly enriched in deuterium at the C-1 position in the presence of Baker's yeast and bisulfite, a large amount of slightly enriched [1-^{2}H]-glycerol is obtained[8,9]. In the case of the stereochemistry defined above (1-S,2-R)[1-^{2}H]-glycerol should be formed (see Scheme 1).



Scheme 1 *: Structural relationship between the proton transfer mediated by PGI and the glycerol synthesized in the fermentation of glucose (* PFK= phosphofructokinase, TIM = triosephosphate isomerase, GDH = glycerolphosphate dehydrogenase).*



Scheme 2 *: Synthesis of 2,3-epoxy-1-acetate from glycerol.[site 1 represents the resonance of the deuterium (or the proton) at lower field on the ^2H NMR (or proton NMR) spectra].*

The glycerol cannot be analysed directly by means of ^2H NMR spectroscopy because the five protons are quasi isochronous. We have shown recently[10] that the glycidyl acetate obtained from glycerol[11,12,13] allows us to overcome this problem. In effect, the ^2H NMR spectrum of this molecule presents 6 very well resolved and identified resonances (see Figure 1a). The unambiguous attribution of the proton spectra and of the deuterium was made on the basis of chemical shifts and coupling constants considerations for the epoxy protons in connection with stereoselective label experiments for the exocyclic hydrogens[10]. For instance, the

values of $^2J_{H4H5}$ = -4.8 Hz , $^3J_{H4H3}$ = 4.2 Hz and $^3J_{H5H3}$ = 2.6 Hz (see Scheme 2) can be compared to those of the well known propylene oxide[14] for which $^2J_{gem}$ = - 5.0 Hz, $^3J_{trans}$ = 4.1 Hz and $^3J_{cis}$ = 2.7 Hz.

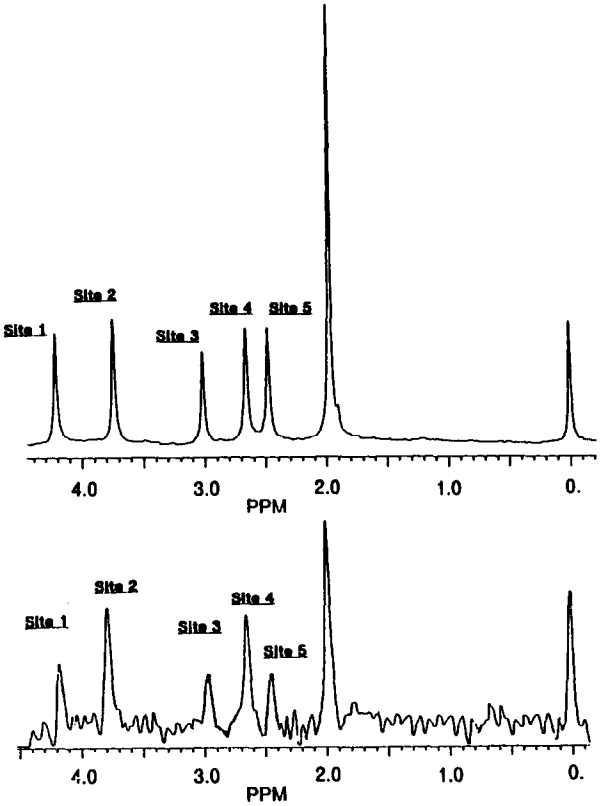


Figure 1 *: ^2H NMR spectrum of non deuterated glycidyl acetate (1a) and of the enriched deuterated esters (1b) obtained from the fermentation of the enriched [1-^2H]-glucose. (spectrometer : Bruker AM 400, solvent : CCl_4 , reference : hexamethyldisiloxane).*

Thus, taking in account that the cyclisation of glycerol tosylate is a pure SN_2 process[10], the postulated (1-S,2-R)[1-^{2}H]-glycerol should be transformed in (2-R,3-S)[3-^{2}H]-2,3-epoxy 1-propylacetate (50 %) and in its (1-S,2-R)[1-^{2}H] quasi-enantiomer (50%) (see Scheme 2).

The fermentation of a mixture of 3.10^{-4} M of [1-^{2}H]-glucose (97%) plus 0.6 M of non deuterated glucose affords a mixture of non deuterated and of [1-^{2}H]-glycerols which was converted further on into glycidyl acetates. The deuterium NMR spectra of the latter showed two "enriched" resonances corresponding to the sites 2 and 4 (see Figure 1b). The "enriched" site 2 involves a contribution of the H_D deuterium of the R, of the H_B deuterium of the S-natural glycidyl acetates and of the (1-S,2-R)[1-^{2}H]-isotopomer while the "enriched" site 4 takes its origin in the H_B deuterium of the R, in the H_D deuterium of the S-natural glycidyl acetates and in the (2-R,3-S)[3-^{2}H]-isotopomer. This clearly shows that the conclusions made by Rose et al.[5,7] on the stereochemistry of the proton transfer mediated by the PGI were right , otherwise a label on the sites 1 and 5 would have been observed.

Experimental

Deuterium NMR spectroscopy

The ^2H NMR spectra were obtained at 61.4 MHz with a Bruker AM 400 spectrometer equipped with a fluorine device. The following acquisition parameters were used : broad band decoupling, frequency window : 1,200 Hz, memory size :16 K, digital resolution 0.15 Hz, exponential multiplication associated with a line broadening of 1 Hz for the spectrum (1a) and of 2Hz for (1b). The number of scans was about 3,000. The solvent was CCl_4.

Fermentation of glucose into glycerol [8,9]

The anaerobic fermentation with baker's yeast is monitored in a medium composed of : $(NH_4)_2SO_4$ (1g/l), one crystal of KCl, $CaCl_2$ and $MgCl_2$, Na_2HPO_4 (0,5 g/l), Na_2SO_3 (20 g/l), anhydrous non deuterated glucose (108g/l, 0,6M) and deuterated [1-^2H]-glucose (54 mg/l, 3.10^{-4} M). The temperature was regulated at 32°C with periodic stirring. After two hours 15 g of Na_2SO_3 were added . Then, 10 days after the beginning, the fermentation was stopped. At this time no initial sugar was present. The yeasts were removed by centrifugation (2500 rpm) and the ethanol was distilled. The remaining liquid was then decoloured with active charcoal and the water eliminated under reduced pressure. The glycerol extracted from the residue with a mixture of ethanol/ether (1vol/1.5 vol), was dried over P_2O_5 under dynamic vacuum (5.10^{-2} Torr) for 8 hours .The resulting glycerol was converted without further purification into glycerol acetonide (Dean-Stark apparatus with excess acetone in benzene, catalyst: APTS; from this procedure, 23g of ethanol and 30 g of glycerol were obtained.

Conversion of glycerol into glycidyl acetate

The racemic mixture of glycidol was prepared according to the Sowden[11] and Baldwin[12] methods and was esterified to 100% conversion in the presence of porcine pancreatic lipase (EC 3.1.1.3, Sigma, type VI-S) with vinyl acetate as an acyl donor[13].

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