Vol. 90, No. 3, 1979 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 12, 1979 Pages 757-763

SYNTHESIS OF 5-DEOXY-D-XYLULOSE-1-PHOSPHATE BY HUMAN ERYTHROCYTES

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Received August 13,1979

SUMMARY

Human erythrocytes synthesize 5-deoxy-D-xylulose-1-phosphate from added acetaldehyde and endogenous dihydroxyacetone phosphate. Acetaldehyde is not only a substrate for the reaction but also undergoes oxidation, causing reversal of glycolysis and thus accumulation of the product.

In the course of a study of pathways of oxidation of acetaldehyde in perfused rat liver, chromatographic analysis of extracts of livers that had been infused with (1-^{3}H) acetaldehyde revealed that only small amounts of labeled reduced products were formed and that a fraction that was highly acidic, due to its content of phosphate esters, contained most of the non-volatile radioactivity in the extracts. Finding merely traces of radioactive reduction products prompted speculation that the more highly labeled fraction consisted of compounds that had become radioactive not by reduction by the labeled hydrogen of the acetaldehyde but rather by incorporation of the labeled aldehyde as a whole. In view of its phosphate ester composition, it seemed likely that the fraction contained 5-deoxy-D-xylulose-1-phosphate*, formed by the aldolase-catalyzed condensation of acetaldehyde and DHAP. This view gained support when it was found that authentic (4-^{3}H) DXP, synthesized from (1-^{3}H) acetaldehyde and unlabeled DHAP, moved in the same chromatographic fraction as the unknown phosphate ester fraction.

Formation of DXP from acetaldehyde and FDP was first described in 1936 by Meyerhof, Lohmann, and Schuster (1) using muscle extracts to catalyze the reaction:

*ABBREVIATIONS

5-deoxy-D-xylulose-1-phosphate: DXP; dihydroxyacetone phosphate: DHAP; fructose-1,6-diphosphate: FDP.

0006-291X/79/190757-07$01.00/0

acetaldehyde + FDP = DXP + DHAP

Synthesis of DXP was attributed to the actions of aldolase and triose phosphate isomerase in the extracts. The equilibrium constant of the reaction was found to be 0.7 at 40°.

The enzymes catalyzing formation of DXP are found in virtually every type of cell. For the purpose of studying conditions of synthesis of DXP at the cellular level, the mature erythrocyte offered two important inducements. One was that it is a cell in which glycolysis, which provides DHAP for the synthesis, is uncomplicated by the regulatory influences of mitochondrial respiration. Another was that DXP, formed in red blood cells, might react with hemoglobin, as does its structural analogue, fructose-1-phosphate (2). Formation in vivo of a stable adduct between hemoglobin and DXP would serve to integrate the concentration of acetaldehyde in the blood and thereby provide a means of evaluating alcohol intake.

The first step in this investigation was to ascertain whether DXP is formed in human red blood cells supplied with acetaldehyde. Results of the experiment described below revealed that, in the presence of acetaldehyde, not only was DXP formed in glycolyzing human red blood cells but also that it reached a concentration far exceeding that of FDP and DHAP in normal human erythrocytes.

MATERIALS AND METHODS

Synthesis of (1,2-^{14}C) acetaldehyde: This was accomplished by oxidation of (U-^{14}C) alanine with ninhydrin. The reaction was carried out in a 250 ml glass-stoppered flask having a center well and glass stirrups on the stopper and neck of the flask. Magnetic bars were used for stirring the contents of the center well and outer chamber.

2 mmoles of L-alanine (Sigma) and 1.90×10^8 cpm of L-(U-^{14}C) alanine (New England Nuclear) were dissolved in 8 ml of 0.25M KH_2PO_4 in the outer chamber of the flask and 5 ml of 1M $NaHSO_3$ were added to the center well. The reaction was started by adding 0.88 g of ninhydrin to the alanine solution. The flask was stoppered, using rubber bands stretched over the stirrups to prevent unseating, and placed on a magnetic stirrer-heater kept at 40° for the next 48 hours. The flask was then cooled in ice and the acetaldehyde-bisulfite solution in the center well transferred with washings to a 10 ml volumetric flask. The solution was found to contain 0.72×10^8 cpm. Taking into account the loss of one-third of the label as $^{14}CO_2$, the radioactive yield was 57%.

Radioactive acetaldehyde was prepared as required from 1 ml aliquots of the acetaldehyde-bisulfite solution: 2 ml of 2M barium acetate was pipetted into one arm of a Rittenberg tube (3) and frozen by immersing the arm in dry ice-acetone. One ml of radioactive acetaldehyde-bisulfite solution was then overlaid on the barium acetate and also frozen. The tube was then evacuated and closed off. The frozen solutions were thawed and mixed by gentle shaking. The empty arm was immersed in the freezing mixture causing acetaldehyde and water to distill over. Distillation was continued until the reaction mixture was reduced to a powder. About 50% of the radioactive acetaldehyde bisulfite was recovered as free labeled acetaldehyde in the distillate. The acetaldehyde concentration, assayed with yeast alcohol dehydrogenase (4), was 17.0 mM. Its specific activity was 0.65×10^5 cpm/μmole.

Incubation of human erythrocytes with (1,2-^{14}C) acetaldehyde: 8.0 ml of (1,2-^{14}C) acetaldehyde (136 μmoles), 5.5 ml of water, and 1.5 ml of a solution containing 1.4M NaCl, 0.055M glucose, and 0.1M sodium phosphate buffer, pH 7.47, were pipetted into a 35 ml glass-stoppered tube. To this solution were added 15 ml of packed erythrocytes obtained from freshly drawn heparinized blood of a healthy donor. The suspension was kept at 37° for 3 hours and mixed from time to time by gentle swirling. It was then cooled in an ice bath and extracted while cold with perchloric acid. The extract, which was freed of protein and perchlorate by conventional procedures and lyophilized, contained 23% of the radioactivity added initially as (1-^{3}H) acetaldehyde. It was then chromatographed on a column of Dowex-1-formate using a method detailed in an earlier report (5).

Enzymatic assay of DXP: The sample, containing 0.05 to 0.2 μmole of DXP, 0.5 ml of NADH (1 mg/ml), and 2.0 ml of 0.01M K phosphate buffer, pH 7.4, were added to a cuvette to which 0.02 ml of aldolase (10 mg/ml, Sigma) was added to cleave DXP to acetaldehyde and DHAP. Addition then of 0.01 ml of yeast alcohol dehydrogenase (10 mg/ml, Sigma) caused oxidation of NADH by acetaldehyde, producing a fall in absorbance at 340 nm. When no further change in absorbance occurred, 0.01 ml of glycerol-3-phosphate dehydrogenase (1 mg/ml, Sigma) was added to bring about oxidation of NADH by DHAP, causing the absorbance at 340 nm to fall once more.

RESULTS

Results of the chromatographic analysis, illustrated in Figure 1, show separation of three radioactive fractions, the largest of which consisted of acetic acid, accounting for 54% of the radioactivity in the extract. Oxidation of acetaldehyde by human erythrocytes has recently been reported (6) and is confirmed by the present findings. From the amount of radioactivity in the fraction and the specific activity of the labeled acetaldehyde, the rate of oxidation of the aldehyde was estimated to be 0.4 μmole/hour/ml of cells.

Fraction 112-123, which still has not been identified, contained 12% of the radioactivity in the extract and fraction 132-144, which was presumed to contain DXP,

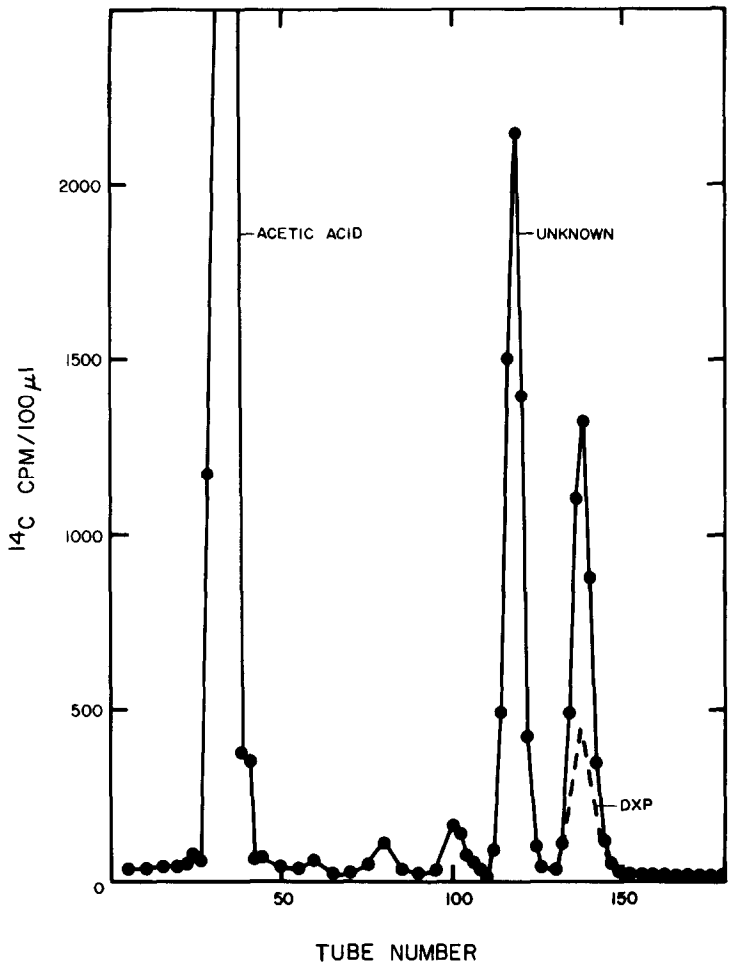

Figure 1: Doxex-1-formate chromatogram of extract of human red blood cells incubated with (1,2-^{14}C) acetaldehyde.

8%. It is interesting to note that, in similar experiments with (1-^{3}H) acetaldehyde, radioactive peaks were observed corresponding to both of these fractions, indicating that the aldehyde hydrogen of acetaldehyde had been retained in the products.

After pooling and lyophilizing fraction 132-144, the residue was dissolved in 3.0 ml of water. Aliquots of this solution were removed for enzymatic analysis for DXP.

The results shown in Figure 2A were given by analyzing 1 ml of the above solution by the method described earlier. The tracing in Figure 2B, given by a second 1 ml aliquot of the sample showed that, when aldolase was omitted, no acetaldehyde

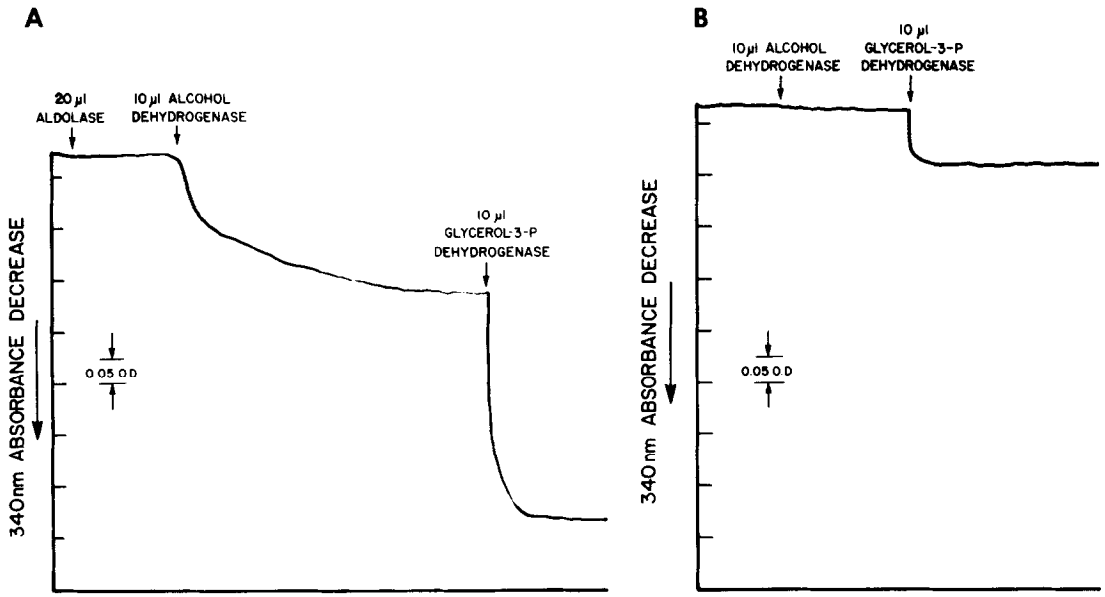

Figure 2: A- Changes in absorbance at 340 nm following additions of aldolase, alcohol dehydrogenase, and glycerol-3-Pi dehydrogenase to assay mixture. B- Effect of omitting aldolase.

was formed. It will be seen, however, that addition of glycerol-3-phosphate dehydrogenase caused NADH to become oxidized, indicating the presence of pre-formed DHAP. When this was substracted from the amount of DHAP formed after adding aldolase (Figure 2A), the quantities of acetaldehyde and DHAP released by cleavage of DXP by aldolase were found to be stoichiometrically equal.

The total amount of DXP in fraction 132-144 was found to be 0.60 µmole, accounting for 26% of the radioactivity in the fraction. To ascertain whether, by this assay, the amount of NADH oxidized after adding alcohol dehydrogenase was equivalent to the amount of ethanol produced, an aliquot of the assay mixture was transferred, after the reactions had gone to completion, to one arm of a Rittenberg tube (3):

> The arm containing the sample was placed in a dry ice-acetone bath, the tube evacuated and then closed off. The position of the two arms was reversed so that the sample, while still frozen, distilled into the arm immersed in the freezing bath. After distilling the sample to dryness, aliquots of the reaction mixture and the distillate were counted.

From the results of this assay it was found that 28% of the radioactivity in the cuvette was attributable to (1,2-^{14}C) ethanol. Agreement between the amount of alcohol formed and the amount of NADH oxidized by acetaldehyde was thus within 10% of theory.

DISCUSSION

Synthesis of DXP from added acetaldehyde and endogenous DHAP was observed to take place in human erythrocytes. When calculated from the starting concentration of acetaldehyde, the yield of DXP was small; but as acetaldehyde was added in great excess, it is apparent that the yield of DXP was limited by the concentration of DHAP. Taking into account the water content of the cells, 70% of cell volume (7), the concentration of DXP was 57 μM, more than 10 times the concentration of FDP and more than 5 times the concentration of DHAP in normal human red blood cells (7). To produce this degree of DXP accumulation, kinetic and/or thermodynamic influences, brought into play by acetaldehyde, must have been operative.

The aldehyde dehydrogenase system of human red blood cells is NAD-dependent (6) and accordingly can influence glycolysis directly by interacting with the glyceraldehyde-3-Pi dehydrogenase system. As the oxidation-reduction potential of the acetaldehyde/acetate system is 0.31 volts more negative than the glyceraldehyde-3-Pi, Pi/glycerate-1,3-Pi system (8), reduction of glycerate-1,3-Pi by acetaldehyde is virtually complete, the effect of which on glycolysis would be to cause DHAP and hence DXP to accumulate. In keeping with this explanation of the present results, exposure of glycolyzing human erythrocytes to 175 μM acetaldehyde for 4 days caused lactate production to fall from 0.92 μmole/hour/ml to 0.33 μmole/hour/ml while glucose disappearance rose slightly from 1.6 μmole/hour/ml to 1.9 μmole/hour/ml.*

The ubiquitous distribution of aldolase raises a number of questions concerning metabolic effects of acetaldehyde and possibly of DXP itself. One reaction of DXP is its

* Hoberman, H. D. Unpublished observations.

addition to hemoglobin. This is described in the paper which follows.

ACKNOWLEDGEMENT

This was supported by grant 5R01 AA02469-02 from the National Institute on Alcohol Abuse and Alcoholism.

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