SEDOHEPTULOSE DIPHOSPHATE FORMATION BY THE HUMAN RED BLOOD CELL[1]

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Sedoheptulose 1, 7-diphosphate has been prepared enzymatically by the action of aldolase on triose phosphate and erythrose 4-phosphate; directly (Klybas, Schramm and Racker, 1959), or by using either transketolase and fructose 6-phosphate (Smyrniotis and Horecker, 1956), or transaldolase and sedoheptulose 7-phosphate (Horecker, 1957) as sources of erythrose 4-phosphate. The sedoheptulose diphosphate was found to be cleaved about half as fast as fructose diphosphate by muscle aldolase (Horecker, 1957). In a scheme to account for CO_2 fixation during photosynthesis, sedoheptulose diphosphate has been used as a precursor for the regeneration of ribulose diphosphate, considered to be the primary CO_2 acceptor (Bassham and Calvin, 1957). Sedoheptulose diphosphate has proved to be an efficient precursor for erythrose 4-phosphate and phosphoenolpyruvate in the biosynthesis of shikimic acid (Srinivasan, Katagiri and Sprinson, 1959). As a result of the discovery of a specific widely distributed C-1 phosphatase for sedoheptulose diphosphate, Racker and Schroeder (1958) and Couri and Racker (1959) have suggested that this enzyme may function instead of the generally accepted phosphatase hydrolysis of fructose diphosphate in the reversal of glycolysis during carbohydrate synthesis. In view of the potential biological importance

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of sedoheptulose diphosphate it is of interest that this compound was found to accumulate in significant amounts during the metabolism of ribose (from inosine) by the intact human red blood cell.

Following a 4 hour incubation of heparinized human blood at 38° with 15 μmoles per ml of inosine and 40 μmoles per ml of inorganic phosphate, the red cells were extracted with trichloroacetic acid and the extract chromatographed on a column of Dowex 1-chloride according to methods described previously (Bartlett, 1959). The 0.1 N NH_4Cl elution section which contained the sugar diphosphates was rechromatographed on a column of

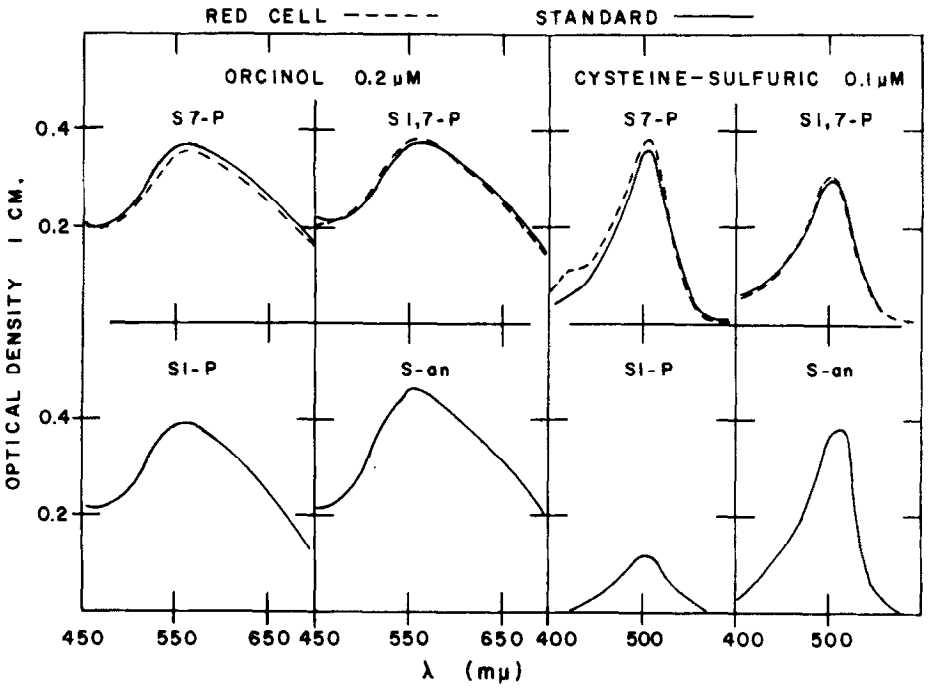

Fig. 1. Spectra of sedoheptulose (S) phosphates (P) and sedoheptulosan (S-an). Cysteine-sulfuric acid reaction: 0.1 μmoles of sample in 1.5 ml, 4.5 ml 95% H_2SO_4, 3' Δ 100°, 0.1 ml 3% cysteine-HCl, 30' Δ 60°. Orcinol reaction: 0.2 μmoles of sample in 3.0 ml, 3.0 ml orcinol reagent, 40' Δ 100°.

Dowex 1x8-formate (100-325 wet mesh) with gradient elution from 0 to 1N pH 3.0 ammonium formate. Eluting just ahead of the fructose diphosphate

was a phosphorus peak which gave a color typical of sedoheptulose in the cysteine-sulfuric acid reaction (Dische, 1953). This material appeared in the same elution position and gave the same spectra in the cysteine-sulfuric acid and orcinol (Mejbaum, 1939) tests (Fig. 1) as did a purified sample of reference sedoheptulose diphosphate which we prepared from fructose 6-phosphate and fructose diphosphate with spinach transketolase and crystalline muscle aldolase (Smyrniotis and Horecker, 1956). Both compounds were split quantitatively by aldolase to dihydroxyacetone phosphate as measured by the decrease in the optical density of DPNH at 340 mμ in the presence of glycerol phosphate dehydrogenase (Table 1) and by the disappearance of the cysteine-sulfuric color. Forty-six per cent of the phosphorus of the

Table 1
Aldolase Assay of Sedoheptulose Diphosphates

Sedoheptulose diphosphate	Micromoles Phosphorus	Micromoles Δ DPNH
Standard	1.01	0.46
Red Cell	1.06	0.47

The assay system contained 1.3 μmoles of DPNH, 50 μg of aldolase (Boehringer), 50 μg of glycerolphosphate dehydrogenase (Boehringer) and 250 μmoles of pH 7.5 Tris in a total volume of 3.0 ml. The reaction time was 30 min. at room temperature. The Δ μmoles of DPNH were obtained from the change in O.D. at 340 mμ using $E=6.0 \times 10^3$.

standard and 47 per cent of the red cell compound were hydrolysed to inorganic phosphate by heating for 20 minutes at 100° in 1N H_2SO_4. The enzyme-synthesized and the red cell sedoheptulose diphosphates were treated with potato acid phosphatase and the free sugars chromatographed on paper using n-butanol, ethanol, H_2O (40:11:19). Both migrated as a single spot (Rf 1.3 compared to sucrose) which gave the blue color reaction typical of heptulose with the orcinol-trichloroacetic acid reagent (Bevenue and Williams, 1951).

The sugar monophosphate section which was eluted from the Dowex 1-chloride column was rechromatographed on Dowex 1-formate with gradient elution from 0 to 1 N formic acid. Just preceding and overlapping with the hexose monophosphates was a small phosphorus area which showed the characteristic sedoheptulose color in the cysteine-sulfuric acid test. This material gave approximately the same spectra in the cysteine-sulfuric and orcinol reactions (Fig. 1) and was in the same elution position as a sample of sedoheptulose 7-phosphate which we prepared from ribose 5-phosphate with spinach transketolase (Horecker, Smyrniotis and Klenow, 1953). The standard sedoheptulose 7-phosphate and the red cell compound were both relatively stable to acid, giving 11.2 and 9.8 per cent inorganic phosphate respectively after 20 minutes at 100° in 1N H_2SO_4. A sample of sedoheptulose 1-phosphate which was prepared with muscle aldolase from fructose diphosphate and D-erythrose (Horecker, Smyrniotis and Klenow, 1953) was, as expected, much more acid labile than the 7-phosphate, 52 per cent of its phosphorus being hydrolysed to inorganic phosphate after 20 minutes in 1N H_2SO_4 at 100°. The sedoheptulose 1-phosphate gave a much lower color yield in the cysteine-sulfuric reaction than did the standard sedoheptulose 7-phosphate or the red cell monophosphate.

Until isolation and purification of larger amounts makes possible a more definitive identification the evidence at hand suggests that the red cell monophosphate is the C-7 derivative. There seems to be little doubt that the diphosphate is the C-1, 7 compound. Under the experimental conditions the concentrations of the sedoheptulose mono and diphosphates were 0.05 and 0.45 μmoles respectively per ml of red cells.

It seems probable that the sedoheptulose diphosphate was formed by the action of the red cell aldolase on erythrose 4-phosphate and dihydroxy-

acetone phosphate, intermediates which would be produced from the metabolism of ribose phosphate, although there remains a possibility that it could have resulted from the action of ATP and phosphofructokinase on sedoheptulose 7-phosphate (Ling, Byrne and Lardy, 1955). Details of the pattern of metabolic intermediates which were found in the red cell will be presented elsewhere but it should be pointed out here that under the experimental conditions the oxidation of triose phosphate appeared to be rate limiting, leading to a considerable accumulation of fructose diphosphate and favoring the synthetic direction for aldolase action. If the red cell sedoheptulose diphosphate was produced by aldolase it is of interest that this enzyme is apparently able to compete successfully with transketolase for the supply of erythrose 4-phosphate.

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