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OCTULOSE PHOSPHATES FROM THE HUMAN RED BLOOD CELL[1]

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We have been isolating the carbohydrate intermediates which were formed by the red cell when normal heparinized human blood was incubated in air for 4 hours at 38° with 15 µmoles per ml of inosine and 40 µmoles per ml of Pi, using methods described previously (Bartlett, 1959). During the separation of the sugar monophosphate fraction on a column of Dowex 1-formate with gradient elution from 0 to 1 N formic acid, a peak of unknown phosphate eluted just before the sedoheptulose and hexose monophosphates. This material gave a spectrum (Fig. 1) similar to that described by Dische (1958) for octoses in the cysteine-sulfuric acid reaction as did a sample of authentic octose[2]. Octulose 8-phosphate was suspected, Racker and Schroeder (1957) having obtained a small yield of this compound by the action of transaldolase on fructose diphosphate and ribose 5-phosphate, intermediates present in the red cell under the conditions of our experiment.

When the hexose diphosphate fraction from the red cells was rechromatographed on a column of Dowex 1-formate with gradient elution from 0 to 1 N pH 3.0 ammonium formate, the large fructose diphosphate peak was preceded by two smaller phosphorus containing peaks. The

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[2] Generously supplied by Dr. N. H. Richtmyer.

middle one proved to be predominantly sedoheptulose diphosphate (to be reported separately). The first peak gave the octose color reaction and was in the position to be expected for an octulose diphosphate. Dische (1958) has presented evidence for the formation of octulose diphosphate when fructose diphosphate and ribose 5-phosphate were incubated with aldolase.

To obtain standards for comparison with the red cell unknowns the preparation of octulose mono- and diphosphates was attempted. 1.2 mmoles of dihydroxyacetone phosphate and 2.0 mmoles of ribose 5-phosphate were incubated with 80 mg of crystalline muscle aldolase in 300 ml of 0.02 N pH 7.4 Tris for 3 hours at 38°. After precipitation of the barium salts and removal of barium with Dowex 50, the mixture was chromatographed on a column of Dowex 1-formate. 0.23 mmoles of a phosphate giving the octose color eluted as a narrow peak ahead of 0.12 mmoles of fructose diphosphate. When an aliquot of the octose diphosphate was treated with aldolase, DPNH and glycerolphosphate dehydrogenase the expected decrease in 340 mμ absorption from the reduction of dihydroxyacetone phosphate and an equivalent increase in the orcinol reaction for ribose 5-phosphate were obtained, strongly supporting the conclusion that the compound was octulose 1,8-diphosphate. The red cell unknown diphosphate was cleaved with aldolase in the same manner. Moreover both compounds were hydrolyzed at the same rate in acid (Fig. 2).

To prepare the monophosphate 120 μmoles of the octulose diphosphate prepared with aldolase were hydrolyzed for 1 hour in 1 N HCl at 100° and the products chromatographed on a column of Dowex 1-formate using 0 to 1 N formic acid for elution. 54 μmoles of a phosphate giving the octose color appeared in the same elution position as the red cell unknown monophosphate. By analogy with the properties of fructose diphosphate the product of the acid hydrolysis of octulose 1,8-diphosphate would be octulose

8-phosphate. Acid hydrolysis curves on the purified compounds (Fig. 2) were consistent with the view that the diphosphate contained a relatively labile and a stable phosphate group and the monophosphate, a stable phosphate. Surprisingly, however, the monophosphate from the red cell was relatively acid-labile and its hydrolysis curve (Fig. 2) indicated that it might be the octulose 1-phosphate.

Figure 1

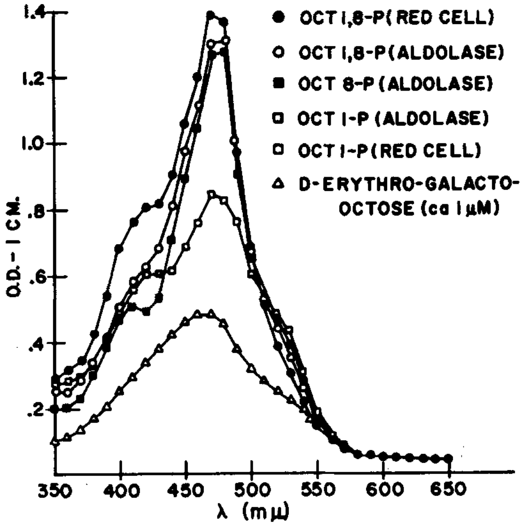

Figure 2

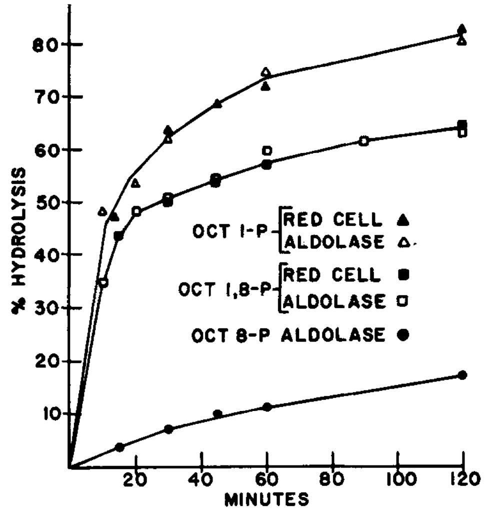

Fig. 1. Spectra of octulose phosphates (1.0 µM) in the cysteine-sulfuric acid reaction: 1.5 ml sample, 4.5 ml 95% H_2SO_4, 3' △ 100°, 0.1 ml 3% cysteine-HCl, 30' △ 60°.

Fig. 2. Rate of hydrolysis of octulose phosphates in 1.0 N HCl at 100°.

From the study of Jones and Sephton (1960) it was expected that some octulose 1-phosphate would be formed from the action of aldolase on ribose and dihydroxyacetone phosphate. 100 µmoles of dihydroxyacetone phosphate and 200 µmoles of ribose were incubated overnight at 38° with 10 mg of crystalline muscle aldolase. The barium salts were precipitated with ethanol, the barium removed with Dowex 50 and the products separated on a column of Dowex 1-formate by gradient elution with 0 to 1 N formic acid. 5 µmoles of a phosphate giving the octose color eluted in the correct position for an octulose monophosphate. Its phosphorus was relatively labile to acid hydrolysis although the rate slowed considerably short of complete hydrolysis, perhaps due to phosphate migration. The red cell octulose monophosphate gave the same cysteine-sulfuric acid spectrum and acid hydrolysis curve as did the octulose 1-phosphate prepared with aldolase.

The free sugars obtained by the action of potato acid phosphatase on the red cell octulose mono- and diphosphates and the diphosphate synthesized with aldolase, were chromatographed on paper using n-butanol, ethanol, H_2O (40:11:19). The three gave identical Rf values (1.2 compared to sucrose) and each gave the characteristic crimson spot changing to grey when sprayed with a trichloroacetic-orcinol reagent (Charlson and Richtmyer, 1960; Jones and Sephton, 1960).

The concentration of octulose mono- and diphosphate in the red cells under the above experimental conditions was 0.06 and 0.2 µmoles respectively per ml of red cells.

As far as we know this is the first report of the presence of octulose in animal tissue. Charlson and Richtmyer (1960) isolated an octulose (D-glycero-D-manno-octulose) from avocado and sedum. In addition to the references quoted above, Dische (1958); Racker and Schroeder (1957), on the enzyme synthesis of octulose, Jones and Sephton (1960) have described the preparation of several octuloses by the action of aldolase on fructose diphosphate and free pentoses. From the known properties of aldolase and the intermediates probably involved it would be expected that the configuration of the red cell octuloses would be D-glycero-D-altro-octulose.

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