ERYTHRONIC ACID 4-PHOSPHATE : AN INTERMEDIATE OF INOSINE METABOLISM IN HUMAN RED CELL HEMOLYSATES

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Several reports have been published on the metabolism of inosine and the identification of its metabolites (Gabrio et al., 1956; Bucolo et al., 1960; Bartlett et al., 1960; Lionetti et al., 1961; Vanderheiden, 1961; Brownstone et al., 1961). In the course of a study of inosine metabolism with human hemolysates in this laboratory, a new phosphorus compound has been isolated by Dowex 1-formate column chromatography. Further studies revealed that this compound corresponds to erythronic acid 4-phosphate.

The incubation mixture contained DPN, 5 μmoles; ATP, 10 μmoles; potassium phosphate buffer at pH 7.4, 900 μmoles; inosine, 1000 μmoles and 50 ml. of hemolysate which was prepared by adding 3 volumes of water to washed human erythrocytes. After 90 minute incubation at 37°, the reaction was stopped by the addition of ice-cold perchloric acid. Acid-soluble phosphates were analyzed by ion-exchange chromatography on Dowex 1-formate, employing a formic acid elution system (Hurlbert et al., 1954). A peak containing a phosphate ester was eluted between ADP and 3-phosphoglyceric acid; this was a hitherto unknown metabolite of sugar metabolism. After formic acid and ammonium formate were removed by cation-exchange treatment and lyophilyzation, the compound was subjected to various analyses. Authentic erythronic acid 4-phosphate was isolated by column chromatography after bromine oxidation of erythrose 4-phosphate, which was prepared by the method of Klybas et al. (1959).

Color reactions of the compound were tested with several reagents. Among these, the color obtained with the chromotropic acid reagent was relatively specific (cf. Bartlett, 1959). The spectrum was identical with authentic erythronic acid (and its 4-phosphate) as shown in Fig. 1. Paper chromatography of the compound on Whatman No.1 paper was carried out using four different solvents —— Solvent I, iso-propanol-trichloroacetic acid - ammonia - water (75 : 5g : 0.3 : 25); Solvent II, t-butanol - picric acid - water (20 : 1g : 5); Solvent III, methanol - ammonia - water (6 : 1 : 3); Solvent IV, iso-propanol - iso-butanol - ammonia - water (40 : 20 : 1 : 39). The natural and the synthetic phosphate esters migrated to the same positions and were located at different areas from other hydroxycarboxylic acid phosphates. For example, the value of Rf in Solvent I were 0.47 for erythronic acid 4-phosphate; 0.51

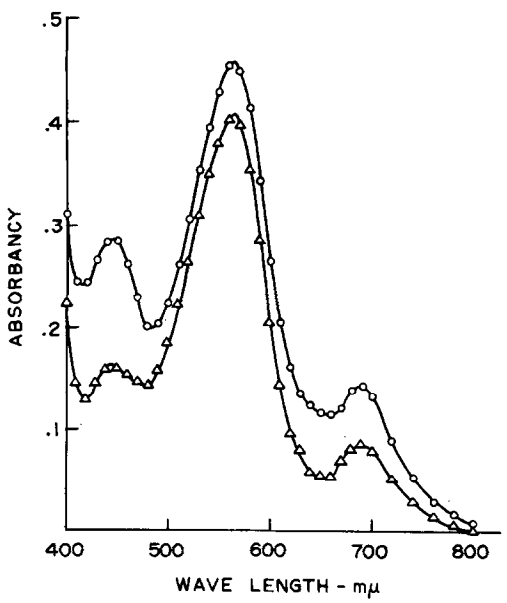

Fig. 1 Absorption spectra with chromotropic acid reagant. Synthesized erythronic acid 4-phosphate (Δ — Δ) and the compound from incubation mixture (O — O) showed the same color development specific to erythronic acid.

for 3-phosphoglyceric acid; 0.39 for 5-phosphoribonic acid; 0.33 for 6-phosphogluconic acid. When other developing solvents were used, Rf values were 0.48 in Solvent II; 0.45 in Solvent III; 0.19 in Solvent IV. The compound had one mole of acid stable phosphate for each mole of erythronic acid (Table I). It was hydrolyzed by prostatic acid and intestinal alkaline phosphatases to erythronic acid and inorganic phosphate. The compound was converted to the lithium salt (Hashimoto et al., 1962), and submitted to elementary analysis. The results were as follows:

	C	H	P
Found	21.02 %	3.54 %	11.98 %
Theoretical	20.54 %	2.58 %	13.24 %

Table I. Analyses of the new intermediate and erythronic acid 4-phosphate.

	Erythronic Acid	Phosphate
Erythronic acid 4-P	1.00	1.00
The New Intermediate	1.04	1.00

Erythronic acid was determined by the color reaction with chromotropic acid reagent. Phosphate was determined by the method of Fiske and SubbaRow (1925). Inorganic phosphate was not liberated by hydrolysis of 30 minute heating in N sulfuric acid at 100°C.

The compound was treated with periodate. Glyoxylic acid was isolated and identified as 2,4-dinitrophenylhydrazone derivative. It was formed in amounts equivalent to the amount of erythronic acid used, showing that the phosphate group is esterified to position 4.

Erythronic acid 4-phosphate accumulated to the extent of about 15 μmoles in the incubation mixture: it was not detected if inosine was replaced by glucose. Radioactive inorganic phosphate added to the incubation mixture was incorporated into the compound. The

specific activity was the same as that of 3-phosphoglyceric acid and other phosphates. These data show that this compound is an intermediate of inosine metabolism. Studies on the metabolism of erythronic acid 4-phosphate are now in progress.

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