RIBOSEDIPHOSPHATE IN THE HUMAN ERYTHROCYTE

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Leloir (1951) first suggested the possible role of ribose-1,5-diphosphate (R-1,5-P) as coenzyme for phosphoribomutase, the enzyme catalyzing the conversion of ribose-1-phosphate (R-1-P), to ribose-5-phosphate (R-5-P). Klenow (1953) isolated R-1,5-P by column chromatography from an incubation mixture containing R-1-P, glucose-1,6-diphosphate (G-1,6-P), and phosphoglucomutase. More recently Tener and Khorana (1958) reported the chemical synthesis of α-D-ribofuranose-1,5-diphosphate. The chemically synthesized product and the natural compound showed identical behavior on chromatography and on treatment with cyclohexylcarbodiimide (Tener and Khorana, 1958). Although phosphoribomutase has not been isolated from erythrocytes, Guarino and Sable (1955), found that dialyzed human red cell hemolyzates can convert R-1-P to R-5-P in the presence of G-1,6-P.

The human erythrocyte has been shown to contain relatively high concentrations of two diphosphate compounds ordinarily thought of as coenzymes, namely 2,3-diphosphoglycerate (DPG), and G-1,6-P (Vanderheiden et al., 1958; Bartlett, 1959). Accumulation of two other diphosphate compounds in the erythrocyte, sedoheptulose-1,7-diphosphate (SDP), and octulose-1,8-diphosphate (ODP), was reported recently by Bucolo and Bartlett (1960), and by Bartlett and Bucolo (1960), when human blood was incubated with inosine and orthophosphate (Pi). A third "coenzyme", namely R-1,5-P has been isolated in this laboratory from erythrocytes incubated with inosine and Pi.

Heparinized fresh human blood (10 ml.) was incubated in air for one hour at 37°C with 30 mg. of inosine, 40 μc of Pi^{32} and 1 ml. of 0.35 M phosphate buffer pH 7.45 in saline solution. The blood was centrifuged, the plasma and white cells were removed and the red cells were washed once with approximately 10 volumes of saline solution. The washed cells were suspended in 3 ml. of saline, the proteins were precipitated with 2 volumes of cold 0.6 N perchloric acid and removed by centrifugation. The clear perchloric acid filtrate was neutralized with 10% KOH; the precipitate was removed by centrifugation and the neutral perchloric acid filtrate was lyophilized to dryness. The powdered material was transfered to a small centrifuge tube to which 0.5 ml. of cold water was added. The material was mixed thoroughly and the insoluble precipitate was removed by centrifugation. Aliquots of the clear supernatant were applied on Whatman 3MM filter paper and the phosphate esters were separated by high voltage electrophoresis (Ryle et al., 1955)[1]. A typical radioelectrograph of a 40 λ aliquot is shown in Fig. 1. The compounds identified are in order of their mobility: DPG, 3-phosphoglyceric acid (3-PGA), R-1,5-P, Pi, hexosediphosphate (HDP), SDP, ODP, dihydroxyacetonephosphate (DHAP), ATP, R-5-P, Glucose-6-phosphate (G-6-P), sedoheptulose-7-phosphate (S-7-P), ADP, IMP and AMP[1]. For identification purposes 240 λ batches of the clear supernatant were used in preparative electrophoresis. The sheets were exposed to No-Screen X-ray film, the radioactive spots were outlined, cut, and eluted with water in the cold. The eluates were pooled, lyophilized to dryness and the residue was dissolved in a small amount of water. The following results were obtained upon analysis of the compound eluted from the R-1,5-P spot:

1) the absorption spectrum of the orcinol compound was identical to that of R-5-P (Fig. 2).

2) the ratio ribose:total-phosphate:acid-labile-phosphate was 1:2.2:0.8 respectively (Table 1).

1- Details of the method of separation and identification of compounds will be reported elsewhere.

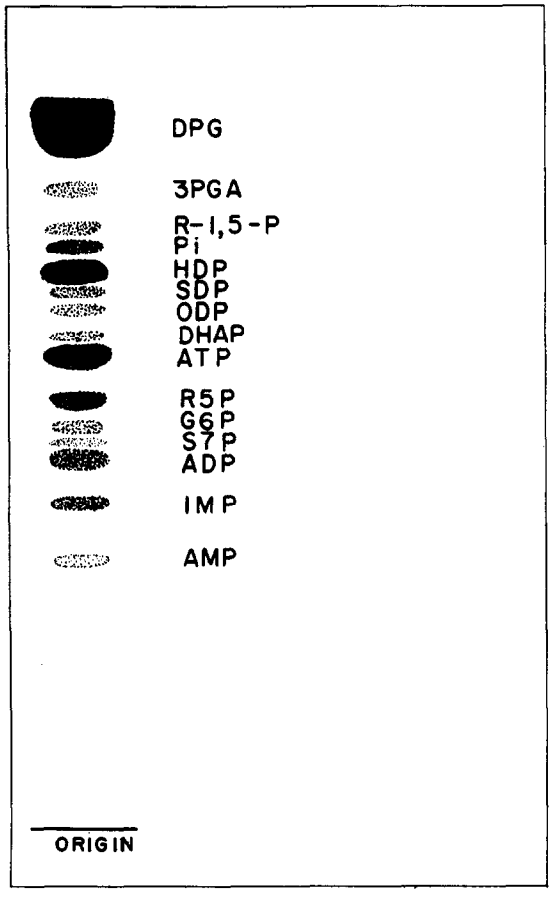

Figure 1

Electrophoretic separation of erythrocyte phosphate esters.

3) hydrolysis of the compound with 1 N HCl at 100°C for 7 minutes, gave rise to Pi and R-5-P (Fig. 3).

4) paper chromatography of the compound before and after hydrolysis, on Whatman # 1 paper, using n-propanol-ammonia-water solvent (60:30:10) (Klenow, 1953), gave spots with R_f corresponding to R-1,5-P,[1] Pi and R-5-P (Klenow, 1953, and Tener and Khorana, 1958) when detected by the molybdate reagent (Burrows et al., 1952). Moreover, when a similar chromatograph was sprayed with phloroglucinol reagent (Borenfreund and Dische, 1957), the isolated

1- I am indebted to Dr. H. Khorana for the sample of α-D-ribofuranose-1,5-diphosphate cyclohexylammonium chloride salt used in these experiments as the source of standard R-1,5-P.

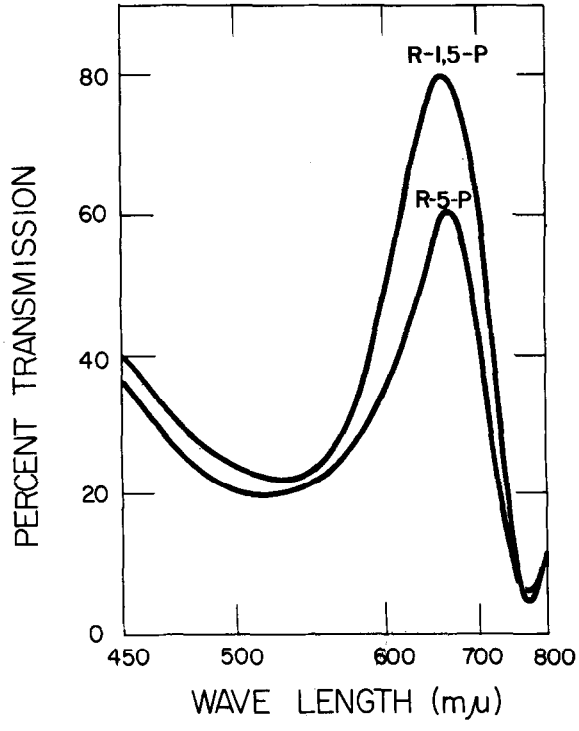

Figure 2

Absorption spectra of the orcinol compound of R-5-P and R-1,5-P. (Horecker 1951)

Table 1

Analysis of isolated R-1,5-P

	μM/ml.
Total phosphate*	3.6
Acid labile phosphate*	1.3
Ribose**	1.6
$\frac{\text{Total phosphate}}{\text{Ribose}}$ ratio	2.2
$\frac{\text{Acid labile phosphate}}{\text{Ribose}}$ ratio	0.8

* Bartlett (1959a)
** Horecker (1951)

compound gave the purple color of ribose at spots corresponding to R-1,5-P (before hydrolysis), and to R-5-P (after hydrolysis) (Table 2).

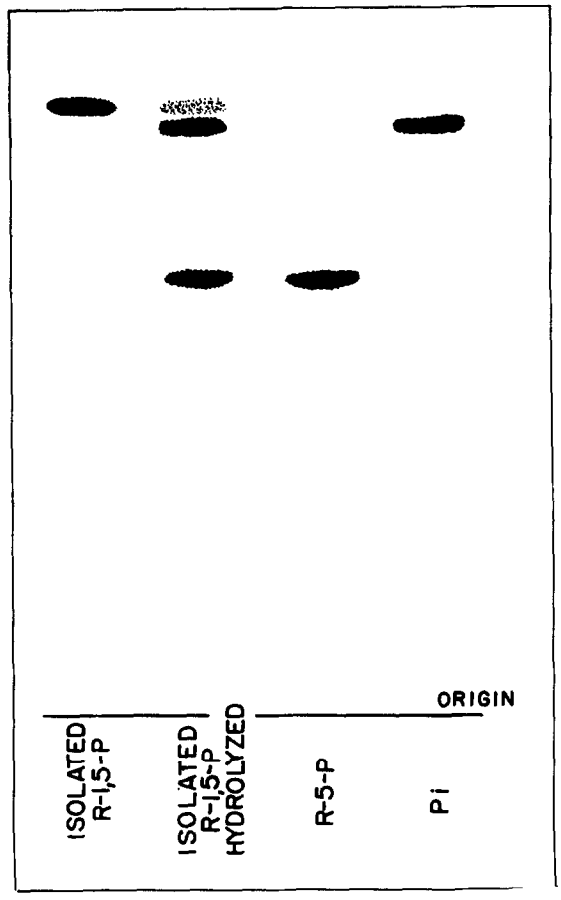

Figure 3

Electrophoretic separation of R-1,5-P and its hydrolysis products.

Table 2

Paper chromatography of isolated R-1,5-P

	Unwashed paper*		Washed paper*	
	R_f	Ribose spray**	R_f	Ribose spray**
R-1,5-P	0.05	Purple	0.13	Purple
R-5-P	0.16-0.20	Purple	0.29	Purple
Pi	0.0	-	0.21	-
Isolated R-1,5-P	0.05-0.08	Purple	0.06-0.10	Purple
Isolated R-1,5-P, hydrolyzed***	0.16-0.19	Purple	0.24	Purple
	0.0	-	0.19	-

* Hanes and Isherwood (1949)
** Borenfreund and Dische (1957)
*** 1 N HCl, 100°C, 7 minutes.

5) comparison of the electrophoretic mobility of the isolated compound with that of other phosphate esters of ribose, show that it is identical to

that of synthetic R-1,5-P (Fig. 4). Moreover, the relative electrophoretic mobility of the new phosphate ester of ribose with respect to that of other esters, lends support to the fact that it is a diphosphate compound. Similarly it appears, from the relative positions of CDP, SDP, HDP and R-1,5-P that the higher the mobility of the compound, the smaller the size of the carbohydrate moeity of the diphosphate ester, i.e. the closer the phosphate groups.

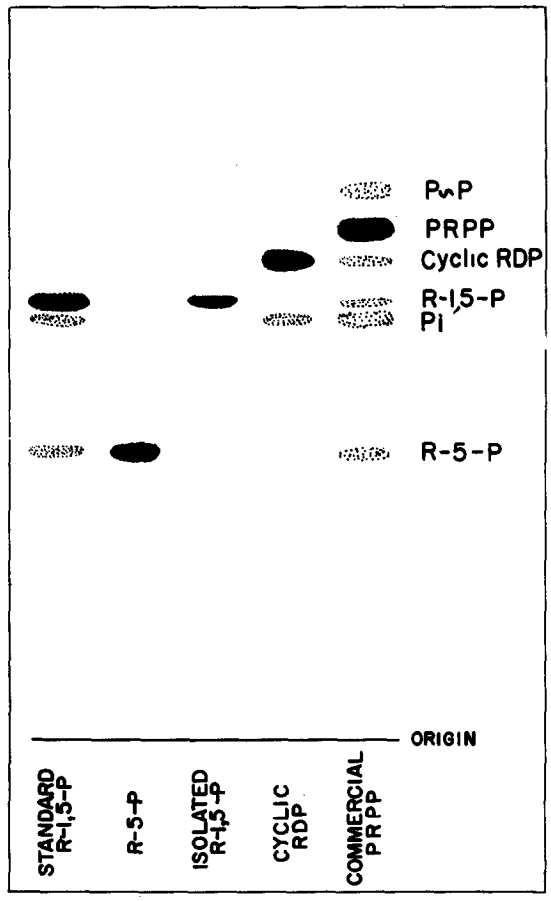

Figure 4

Electrophoretic separation of phosphate esters of ribose.

RDP = 5-phosphorylribose 1,2-cyclic phosphate prepared from commercial PRPP (Khorana, 1958).
PRPP = 5-phosphorylribose-1-pyrophosphate (Pabst).

The true role of the newly isolated diphosphate compound in red cell metabolism has not been established. However, because of the relatively lower concentrations of R-1,5-P (< 1μM/100 ml. RBC) in fresh erythrocytes, as compared to DPG (360-500μM/100 ml. RBC) and G-1,6-P (18-30μM/100 ml. RBC) one may assume that its role is indeed the catalysis of the intramolecular phosphate transfer between R-1-P and R-5-P. The concentration of R-1,5-P increases (1-2μM/100 ml. RBC) when R-1-P is formed in the erythrocyte as a result of nucleoside phosphorylase action on the added inosine. This increase in concentration suggests the existence of a regulatory mechanism in the red cell responsible for the efficiency of the enzymatic conversion of R-1-P to R-5-P when the erythrocyte is confronted with large concentrations of substrate.

This appears to be the first time that, through isolation of R-1,5-P, direct evidence is reported for the existence of this compound in living cells.

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REFERENCES

Bartlett, G. R., J. Biol. Chem., 234, 449 (1959).
Bartlett, G. R., J. Biol. Chem., 234, 466 (1959a).
Bartlett, G. R. and Bucolo, G., Biochem. Biophys. Research Commun., 3, 474 (1960).
Borenfreund, E. and Dische, Z., Arch. Biochem. Biophys., 67, 239 (1957).
Bucolo, G. and Bartlett, G. R., Biochem. Biophys. Research Commun., 3, 620 (1960).
Burrows, S., Grylls, F. S. M. and Harrison, J. S., Nature, 170, 800 (1952).
Guarino, A. J. and Sable, H. Z., J. Biol. Chem., 215, 515 (1955).
Hanes, C. S. and Isherwood, F. A., Nature, 164, 1107 (1949).
Horecker, B. L., Smyrniotis, P. Z. and Klenow, H., J. Biol. Chem., 205, 661 (1953).
Khorana, H. G., Fernandes, J. F. and Kornberg, A., J. Biol. Chem., 230, 941 (1958).
Klenow, H., Arch. Biochem. Biophys., 46, 186 (1953).
Leloir, L. F., Fortschr. Chem. Org. Naturstoffe, 8, 47 (1951).
Ryle, A. P., Sanger, F., Smith, L. F. and Kitai, R., Biochem. J., 60, 541 (1955).
Tener, G. M. and Khorana, H. G., J. Am. Chem. Soc., 80, 1999 (1958).
Vanderheiden, B. S., Huennekens, F. M. and Gabrio, B. W., Federation Proc. 17, 327 (1958).