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Partial purification of erythrocyte glucosephosphate isomerase

Glucosephosphate isomerase (glucose-6-phosphate ketol-isomerase, EC 5.3.1.9) catalyzes the conversion of glucose 6-phosphate to fructose 6-phosphate and is present in most biologically active materials. After its original description by LOHMANN[1] in extracts of yeast, heart, kidney, liver and skeletal muscles, glucosephosphate isomerase has been purified and crystallized from microbial[2], mammalian[3] and plant tissues[4]. Although the enzyme has been easily crystallized from many sources, glucosephosphate isomerase from erythrocytes has been purified with extreme difficulty and low yields[5,6]. Recently there has been additional interest in erythrocyte glucosephosphate isomerase. BAUGHAN *et al.*[7] has described a congenital nonspherocytic hemolytic disease in which erythrocyte glucosephosphate isomerase activity is markedly decreased. Multiple isoenzyme components of human glucosephosphate isomerase have also been demonstrated[8]. The purpose of this paper is to describe a purification procedure which overcomes previous difficulties and lends itself to isolation of glucosephosphate isomerase from small quantities of blood.

Glucosephosphate isomerase was measured spectrophotometrically by following the appearance of NADPH at 340 mμ. The assay system contained glycylglycine (8.3 μmoles/ml) (pH 8.1), NADP (0.165 μmole/ml) glucose-6-phosphate dehydrogenase (0.267 enzyme unit/ml), and fructose 6-phosphate (1.65 μmoles/ml).

DEAE-Sephadex A-50 was prepared by a modification of the procedure used by ARMSTRONG *et al.*[9]. It was allowed to swell in demineralized water for 18 h, and the supernatant decanted. The DEAE-Sephadex was then mixed with 0.5 M NaOH for approx. 30 min, and filtered through a Buchner funnel. The NaOH treatment was repeated twice. The DEAE-Sephadex was then mixed with demineralized water for 30 min and again filtered through a Buchner funnel. This procedure was repeated 3 times. 0.5 M Tris (pH 8.7) was then mixed with the DEAE-Sephadex and 2 M HCl added until the pH remained constant at 8.7. The DEAE-Sephadex was filtered with suction and washed twice with 0.1 M Tris (pH 8.7). This mixture was allowed to stand and the supernatant was decanted. The DEAE-Sephadex was washed twice with 0.01 M Tris (pH 8.7) and suspended in 0.01 M Tris (pH 8.7).

The blood was centrifuged at 1400 × *g* for 30 min, and the plasma and buffy

TABLE I

SUMMARY OF PURIFICATION PROCEDURES FOR HUMAN ERYTHROCYTE GLUCOSEPHOSPHATE ISOMERASE

	Vol. (ml)	*Total activity* (μmoles/min)*	*Recovery (%)*	*Specific activity** (μmoles/min per mg)*	*Purification*
Hemolysate	90	61.1	100.0	0.0221	
DEAE-Sephadex eluate	140	58.2	95.3	0.618	28.0
Calcium phosphate eluate	18	31.8	52.0	1.97	81.1
Ammonium sulfate precipitate redissolved	6	21.0	34.4	3.58	162.0

coat were removed. The erythrocytes were then washed twice with 2–3 vol. of isotonic saline. The packed red cells (usually 10 ml) were lysed by the addition of 9 vol. of demineralized water. An equal volume of DEAE-Sephadex was added to the hemolysate and allowed to mix for 30 min at 4°. The resulting mixture was permitted to run through a Buchner funnel with suction applied until the DEAE-Sephadex was reasonably dry. Under these conditions glucosephosphate isomerase is not adsorbed to the DEAE-Sephadex and is present in the eluate. The eluate was passed through a calcium phosphate column (32 mm × 25 mm), and the enzyme eluted with approx. 25 ml of 0.2 M potassium phosphate (pH 7.7). Ammonium sulphate (0.43 g/ml) was added to this fraction, allowed to mix for 15 min and centrifuged at 28 000 × *g* for 20 min. The precipitate was redissolved in a smaller volume of demineralized water.

The progress of enzyme purification during this procedure is summarized in Table I. In addition to human erythrocytes, the procedure has been used to isolate glucosephosphate isomerase from the erythrocytes of pigs, cattle, horses, and goats with equally satisfactory results. The enzyme is relatively stable at −15° and can be dialyzed against 0.01 M Tris–0.0024 M HCl (pH 8.7) with 1% EDTA for 16 h without significant loss of activity. The enzyme preparation isolated in Table I contained small amounts of lactate dehydrogenase (3.0% of the glucosephosphate isomerase activity), aldolase (1.3%), fructose-6-phosphate kinase (0.6%), glyceraldehyde-3-phosphate dehydrogenase (1.28%), and phosphoglycerate kinase (0.21%) but did not contain measurable amounts of glucose-6-phosphate dehydrogenase, hexokinase, 6-phosphogluconate dehydrogenase, pyruvate kinase, enolase, glutathione reductase, and glutamic oxaloacetate transaminase.

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