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ACTIVITIES OF ASPARTATE (E.C. 2.6.1.1) AND ALANINE (E.C. 2.6.1.2) TRANSAMINASES, AND ALKALINE PHOSPHATASE (E.C. 3.1.3.1) IN HUMAN ERYTHROCYTES OF DIFFERENT GENOTYPES

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ABSTRACT

The activities of aspartate (E.C. 2.6.1.1) and alanine (E.C. 2.6.1.2) transaminases (AST and ALT, respectively), and alkaline phosphatase (ALP) (E.C. 3.1.3.1) were determined in erythrocytes obtained from 20 HbAA, 15 HbAS and 12 HbSS human subjects. The results showed that the three enzymes had different levels of activity in these genotypes. The mean ($\pm$ SD) activity levels of the enzymes, expressed in U/g Hb, obtained for HbAA, HbAS and HbSS samples were: 6.75 $\pm$ 0.99, 7.48 $\pm$ 1.19 and 13.37 $\pm$ 1.78 for AST; 4.26 $\pm$ 0.24, 4.33 $\pm$ 0.44 and 7.24 $\pm$ 1.49 for ALT; and, 69.24 $\pm$ 8.94, 64.26 $\pm$ 9.17 and 48.49 $\pm$ 4.35 for ALP. However, the activity levels of the different enzymes in HbAA were not significantly different ($p>0.05$) from that observed in HbAS erythrocytes. The activity levels of AST and ALT in HbSS erythrocytes were significantly higher ($p<0.05$) than that for HbAA erythrocytes. ALP showed significantly lower activity level ($p<0.05$) in HbSS than HbAS erythrocytes. There was no significant effect ($p>0.05$) of sex on the activity levels of the enzymes. These findings have far-reaching implications for the biochemical roles of these enzymes in the metabolism of the erythrocytes from the different genotypes studied.

Key words: transaminases, phosphatase, erythrocyte, genotypes, pyridoxal

INTRODUCTION

The measurement of enzyme activity levels in different samples, including body tissues and fluids, has remained of significance as a tool for understanding the molecular basis for several biochemical processes. One important biochemical activity relating to amino acid metabolism is transamination, catalyzed by transaminases. These enzymes require pyridoxal phosphate as coenzyme (Voet et al, 1999). It has been reported that erythrocytes accumulate pyridoxal phosphate, a phenomenon purported to be dependent on the haemoglobin content of the red cells (Mehansho and Henderson, 1980). This could further explain the observation of 5 and 10 times higher activities, respectively, of alanine and aspartate transaminases, in the red cell than serum (Henry et al, 1974; Moss et al, 1986).

In addition, erythrocytes of patients with sickle cell anaemia have been reported to show increased zinc content (Czajka-Narins, 1986). This divalent metal is an important prosthetic group for metallo-enzymes, including alkaline phosphatase. The present study undertakes a comparative determination of the levels of three enzymes – aspartate and alanine transaminases (AST & ALT), and alkaline phosphatase (ALP), in human erythrocytes from HbAA, HbAS and HbSS subjects with a view to ascertain if there are significant differences/similarities in the levels. The findings could aid our understanding of the relative importance of the biochemical activities involving these enzymes in the erythrocyte metabolism.

MATERIALS AND METHODS

Sodium and potassium ferricyanide were obtained from Sigma Co. Enzyme kits for aspartate and alanine transaminases (AST & ALT) and alkaline phosphatase (ALP) assays were from Boeringer. All other reagents used were of the highest grades commercially available.

Sample collection/analysis:

Blood was collected from 47 volunteers (20 HbAA, 15 HbAS and 12 HbSS; 26 males and 21 females), following at least an 8-hour fast, by veni-puncture, and emptied carefully into lithium heparinized sterile bottles. An aliquot of sample was used for electrophoretic genotyping for the unconfirmed haemoglobin genotype group us[illegible]

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TABLE 1: Activity levels of Aspartate transaminase from HbAA, HbAS and HbSS human erythrocytes. Data represent mean ± SD for duplicate determinations

	Enzyme activity (U / g Hb)		
	HbAA (n = 20)	HbAS (n = 15)	HbSS (n = 12)
Males (n=26)	6.90 ± 0.98(n = 12)	7.81 ± 1.01 (n = 7)	13.81 ± 1.80* (n = 6)
Females(n=21)	6.42 ± 1.00 (n = 8)	7.56 ± 0.98 (n = 8)	13.10 ± 1.51* (n = 6)
Average	6.75 ± 0.99	7.48 ± 1.99	13.37 ± 1.78*

*significantly different ($p<0.05$) from normal (HbAA)

TABLE 2: Activity levels of Alanine transaminase from HbAA, HbAS and HbSS human erythrocytes. Data represent mean ± SD for duplicate determinations

	Enzyme activity (U / g Hb)		
	HbAA (n = 20)	HbAS (n = 15)	HbSS (n = 12)
Males (n=26)	4.11 ± 1.01 (n = 12)	4.01 ± 0.95 (n = 7)	6.91 ± 1.30* (n = 6)
Females (n=21)	4.15 ± 0.98 (n = 8)	4.41 ± 0.99 (n = 8)	7.35 ± 2.00* (n = 6)
Average	4.26 ± 0.24	4.33 ± 0.44	7.24 ± 1.49*

*significantly different ($p<0.05$) from normal (HbAA)

the procedure described by Henry et al (1974). For erythrocyte preparation, the blood sample was centrifuged at 10, 000 g for 5 minutes, washed thrice with 10 volumes of normal saline (0.9% NaCl) and then diluted 1:20 with a stabilizing solution (0.27 mM EDTA, 0.27 mM 2-mercaptoethanol, pH 7.0) as described by Beutler (1984).

The erythrocyte samples obtained were then frozen and thawed for immediate use, or stored in frozen state until used (usually for not more than 10 days). A 0.02 ml aliquot of the prepared samples (haemolyzate) was used for the determination of haemoglobin concentration using Drabkin's solution (Drabkin and Austin, 1935).

Enzyme assays:

The assay of aspartate transaminase was based on the original method of Reitman and Frankel as reported by Varley et al (1980). For the procedure, 0.1 ml of haemolyzate preparation was added to 0.5 ml of buffered substrate (100 mM phosphate, pH 7.4; 2 mM 2-oxoglutarate and 200 mM L-aspartate). The mixture was incubated for 30 minutes at 37°C and the reaction terminated by the addition of 0.5 ml of 1 mM dinitrophenyl hydrazine in 1 M hydrochloric acid. The mixture was left for 20 minutes at room temperature before the addition of 5 ml of 0.4 M sodium hydroxide. The resultant solution was mixed, and the absorbance read, after 5 minutes, at 546 nm against a blank solution prepared by mixing all reagents and sample without any prior incubation.

Standardization was performed using pyruvate solutions of varied concentrations in the millimolar range. The optical densities obtained were used to make a standard curve from which AST activities were computed as described by Varley et al (1980).

The assay of alanine transaminase was similar to that for aspartate transaminase, with DL-alanine replacing L-aspartate in the above protocol. Both activities were expressed in international unit / gHb.

Alkaline phosphatase activity followed the procedure of the original method of King and Armstrong as reported by Varley et al (1980), using p-nitrophenyl phosphate (di-sodium salt) as substrate in 1M di-ethanolamine buffer (pH 9.8) containing 0.5mM $MgCl_2$. For the determination, 0.02ml of haemolyzate was added to 1ml of the buffered substrate solution and incubated at 37°C. The absorbance of the reaction mixture was read at 30 seconds interval at 405nm for 3 minutes. The alkaline phosphatase activity in international unit per gram of haemoglobin was calculated based on the formula:

[Enzyme activity] = (2760 x ΔA) / [Hb] where ΔA

TABLE 3: Activity levels of Alkaline phosphatase from HbAA, HbAS and HbSS human erythrocytes. Data represent mean ± SD for duplicate determinations

	Enzyme activity (U / g Hb)		
	HbAA (n = 20)	HbAS (n = 15)	HbSS (n = 12)
Males (n=26)	70.10 ± 3.90 (n = 12)	65.15 ± 8.20 (n = 7)	50.10 ± 3.51[a] (n = 6)
Females (n=21)	68.20 ± 4.50 (n = 8)	60.95 ± 5.30 (n = 8)	41.20 ± 5.20[a] (n = 6)
Average	69.24 ± 8.94	64.26 ± 9.17	48.49 ± 4.35[a]

[a] significantly different ($p<0.05$) from normal (HbAA) and HbAS genotypes

represents change in absorbance.

Statistical analysis:

The student's T-distribution table as adapted from Pearson and Harley (1966) was applied with test of significance taken at the cut-off of 95% confidence level (p = 0.05 to 0.001).

RESULTS AND DISCUSSION

The results for the activity determinations in haemolyzates from the three human genotypes studied are shown in Tables 1 and 2 for aspartate and alanine transaminases. In the case of AST, the mean ± SD of 6.75 ± 0.99, 7.48 ± 1.99 and 13.37 ± 1.78 (U / g Hb) were obtained for erythrocytes from HbAA, HbAS and HbSS subjects, respectively. The values obtained for ALT were: 4.26 ± 0.24, 4.33 ± 0.44 and 7.24 ± 1.49 for HbAA, HbAS and HbSS subjects, respectively. However, there was no significant difference (p>0.05) for the activities of both enzymes from HbAA and HbAS subjects. No sex-dependent effects were noted.

The activity level pattern observed was HbSS>HbAS>HbAA, a finding that correlates with observations made for lactate dehydrogenase in human serum (Neely et al, 1969; Serjant, 1985). The observed activity differences could be related to the genotype variations of the erythrocyte haemoglobin, a suggestion made by Mehansho and Henderson (1980) on the accumulation of the prosthetic group, pyridoxal phosphate, by human erythrocytes.

The results for alkaline phosphatase activities are shown in Table 3. The activity levels, as mean ± SD in U / g Hb were 69.24 ± 8.94, 64.26 ± 9.17 and 48.49 ± 4.35, for HbAA, HbAS and HbSS erythrocytes. When compared statistically, there were significant differences (p<0.05) between HbAA and HbSS, HbAS and HbSS, but no significant difference was obtained between HbAA and HbAS. Again, this variability could arise from the differences in the genotypes of the erythrocytes. Indeed, it had been reported that HbSS subjects have increased erythrocyte zinc content (Czjakar-Narin, 1986), and this cation is a prosthetic group for alkaline phosphatase.

The findings in the present report indicate variations in the levels of the different enzymes (AST, ALT and ALP) in the red cells from the three genotypes studied. These, taken with other reports, suggest that such variations could arise from the nature of the haemoglobin present.

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