Kinetic and Electrophoretic Studies of Human Erythrocytes Deficient in Pyrimidine 5'-Nucleotidase

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Summary. The mutant enzyme of a patient with hereditary pyrimidine 5'-nucleotidase deficiency was analyzed biochemically. Partially purified by DEAE-Sephadex and concentrated by ultrafiltration, the enzyme had a high $K_m$ for the substrate uridine monophosphate. Utilization of the substrate cytidine monophosphate was normal, but utilization of adenosine monophosphate was greatly increased. The enzyme was stable to heat; the pH optimum was acidic. Electrophoresis of the enzyme revealed a very faint, slower than normal band.

Introduction

Since the initial report of hereditary erythrocyte pyrimidine 5'-nucleotidase (P5N) deficiency by Valentine et al. (1974), several cases have been reported from various parts of the world (Ben-Bassat et al., 1976; Oda and Tanaka, 1976; Vives-Corrons et al., 1976; Miwa et al., 1977; Rosa et al., 1977a). Deficiency of erythrocyte P5N is accompanied by hemolytic anemia, basophilic stippling, and accumulation of pyrimidine nucleotides (Valentine et al., 1974). Certain characteristics of the normal human erythrocyte P5N have been reported (Paglia and Valentine, 1975; Torrance et al., 1977). However, kinetic and electrophoretic studies have been described in only one case of a mutant enzyme (Rosa et al., 1977a and b). This report presents the biochemical characterization of the enzyme from our patient with P5N deficiency.

Patient

A 41-year-old Caucasian woman with chronic hemolytic anemia was found to have P5N deficiency. Her RBC P5N activity was 19% of the normal mean. Hematologic values were as
follows: red cell count \(2.8 \times 10^{12}/l\), hemoglobin concentration 11.9 g/dl, packed cell volume 37%, and reticulocyte count 29%. Marked basophilic stippling was noted. Reduced glutathione content was 1160 \(\mu g/10^{11} RBC\) (normal range 590–770). A survey of the family members and certain metabolic studies of the propositus have been reported briefly (Oda and Tanaka, 1976). There was no consanguinity in the parents of the patient or in other members of the family. Therefore, the patient probably is a double heterozygote for RBC P5N.

**Materials and Methods**

Heparinized blood freshly obtained by venipuncture was washed three times with 0.85% saline, and the buffy coat was carefully removed after each centrifugation. Kinetic measurements on the enzyme using crude hemolysate as described by Rosa et al. (1977a) were unsuccessful because the patient's red cell P5N activity was too low. We therefore partially purified and concentrated the enzyme solution according to the first purification step of Torrance et al. (1977). Packed red cells were hemolyzed with 10 mM Bis-Tris-HCl buffer at pH 6.5, containing 0.7 mM mercaptoethanol and 2.7 mM EDTA. Red cell stroma was extracted by toluene and removed after centrifugation. The hemolysate was applied to a DEAE-Sephadex A-50 column. The column was washed with the same buffer until all hemoglobin was removed; then the non-heme protein was eluted with a buffer containing 150 mM NaCl. Fractions were assayed for P5N activity using uridine 5'-monophosphate (UMP) as substrate, and those fractions demonstrating enzyme activity were pooled and concentrated by ultrafiltration. This method of partial purification was essentially the same as that employed by Rosa et al. (1977a). P5N activity was measured by the method of Valentine et al. (1974), using 25 mM Tris-HCl buffer, pH 8.0, containing 10 mM MgCl₂ and UMP as substrate at a final concentration of 2.3 mM. Kinetics for the substrate UMP were studied by varying the concentration of the substrate. The apparent \(K_m\) for the substrate was determined by the Lineweaver-Burk plot. For substrate-specificity determinations, cytidine 5'-monophosphate (CMP) and adenosine 5'-monophosphate (AMP) were used in place of UMP. Electrophoresis was performed by the method of Anderson et al. (1975) using starch as the supporting gel. After electrophoresis, the P5N band was stained in two steps. Filter paper soaked in 10 ml reaction mixture containing 0.1 M Tris-HCl buffer, pH 7.8; 20 mM UMP; 5 mM glutathione; and 80 mM MgSO₄ was overlaid on the gel plate and incubated for 2 h. The inorganic phosphate (Pi) liberated by the reaction was stained by the method of Fiske and Subbarow (1925). Heat stability of the enzyme was tested at 45°C. The pH optimum was determined using 0.1 M Tris-maleate as buffer.

**Results and Discussion**

The mean \(K_m\) UMP of normal partially purified P5N enzyme was 0.93 mM. The \(K_m\) UMP of the partially purified enzyme of the patient was 3.8 mM (Fig. 1). Rosa et al. (1977a) also found a high \(K_m\) in their patient. They determined kinetics using a buffer of pH 6.0, the optimum pH of their patient's P5N enzyme. However, at that pH some activity may be from acid phosphatase, which is present in erythrocytes and acts as a nonspecific phosphatase. We therefore utilized a reaction pH of 8.0, where the effect of acid phosphatase can be neglected.

Partially purified patient erythrocyte P5N enzyme activity was 82.5% with CMP and 107% with AMP as substrates relative to UMP as substrate. The partially purified control enzyme had values of 71% and 20% with CMP and AMP, respectively. Both Paglia and Valentine (1975) and Rosa et al. (1977a) reported no utilization of AMP by patient enzyme or by normal enzyme using