

Determination of Erythrocyte Hydroxymethylbilane Synthase Activity and Its Application for Study of Acute Intermittent Porphyria

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Summary We describe a convenient procedure for measuring hydroxymethylbilane synthase activity in erythrocyte, which can be used to diagnose acute intermittent porphyria in either latent or acute stage. In assaying erythrocyte hydroxymethylbilane synthase with porphobilinogen as a substrate, we found that the hydroxymethylbilane synthase had an optimum pH at 8.2. The Km value of the enzyme estimated in 6 healthy persons was 14.0 ± 0.8 $\mu\text{mol/l}$ (mean \pm SE). The within-run and between-run coefficients of variation for our method were 3.8% and 4.4%, respectively. In 133 apparently healthy adults, the reference range of hydroxymethylbilane synthase with porphobilinogen as a substrate was estimated to range from 30.0 to 73.7 nmol of porphyrin formed per hour per milliliter of red blood cells. The age or sex difference was insignificant. Application of this method for diagnosis of acute intermittent porphyria and for identifying carriers of the disease was demonstrated in an affected Chinese family.

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Key words: acute intermittent porphyria, hydroxymethylbilane synthase, porphobilinogen

Acute intermittent porphyria (AIP) is an autosomal dominant inherited metabolic disease, in which delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) are formed excessively in the liver. It results from a partial deficiency of hydroxymethylbilane synthase (HMBS, EC 4.3.1.8; formerly called PBG deaminase or uroporphyrinogen I synthase) [1]. Acute episodes of AIP may be provoked by alcohol, stress, infection, starvation, hormonal changes, and administration of certain drugs [1,2]. About 5% of patients with attacks that are severe enough to require hospitalization will die [3]. As repeated attacks in the same patient are common, the mortality rate is reported to be 15 to 20% [3]. It is important to confirm the diagnosis and to identify potential or latent cases in the relatives of patients with this disease by measuring HMBS in erythrocyte for proper management and prevention of future acute episodes.

HMBS is an enzyme which catalyzes the deamination of PBG to form hydroxymethylbilane (Fig. 1). The hy-

droxymethylbilane is converted to uroporphyrinogen I nonenzymatically or to uroporphyrinogen III in the presence of uroporphyrinogen III synthase [1]. There are many methods for the determination of HMBS [4-8]. The enzyme activity may be measured by using either PBG or ALA as a substrate. Although ALA has been considered to be less expensive and more stable during storage than PBG, the use of ALA as a substrate, a coupled enzyme procedure, may present some disadvantages including lead inhibition of ALA dehydrase [6].

We describe a method which is suitable for a routine determination of erythrocyte HMBS activity using PBG as a substrate. The application of the test for studying a Chinese AIP family is demonstrated.

MATERIALS AND METHODS

Materials

Porphobilinogen, delta-aminolevulinic acid and copro-

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