A potential biochemical explanation for the genesis of porphyria cutanea tarda

Studies on the inherent biochemical defect in highly purified human erythrocyte uroporphyrinogen decarboxylase and its amplification by iron

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Received 12 June 1985

Familial porphyria cutanea tarda (PCT) is a photocutaneous disease in which subnormal activity of uroporphyrinogen decarboxylase is observed both in the liver and red cells. Hepatic iron plays a key role in the genesis of overt biochemical and clinical PCT. In this report, we have studied the properties of 10000-fold purified erythrocyte uroporphyrinogen decarboxylase preparations from two familial PCT patients and a non-porphyric control subject. The apparent Michaelis constants (K_m), determined by using uroporphyrinogen III substrate, were approx. 3.2-times higher for the enzyme from the diseased subjects ($K_m = \sim 1.0\ \mu M$) as compared to the normal ($K_m = 0.3\ \mu M$). Though both abnormal and normal enzymes were inhibited progressively with increasing concentrations of iron, the enzymes from diseased subjects exhibited greater susceptibility e.g. 0.1 mM Fe^{2+} inhibited the former about 50% and the latter about 20%. These observations suggest that (i) the inherent biochemical defect in PCT is the reduced enzyme-substrate affinity and (ii) the intrinsic abnormal conformation renders the PCT enzyme particularly susceptible to inhibition by iron.

Porphyria cutanea tarda *Uroporphyrinogen decarboxylase* *Human disease* *Erythrocyte* *Porphyrin* *Intracellular iron*

1. INTRODUCTION

Interest in uroporphyrinogen decarboxylase (EC 4.1.1.37), an enzyme which is involved in mammalian heme biosynthesis, has been heightened by recent data that suggest the genesis of human porphyria cutanea tarda (PCT) might be associated with an intrinsic abnormality of the enzyme [1] in combination with its interaction with microenvironmental factors in the hepatocyte such as iron [2]. The disease is expressed as a photocutaneous skin lesion due to the accumulation of porphyrins in the skin.

Recent observations of an approximate 50% reduction in uroporphyrinogen decarboxylase activity both in the liver and erythrocytes of familial PCT patients [3–5] and immunochemical studies [6], all suggest that the red cell and liver enzymes are encoded by the same gene. However, reduced enzyme activity per se should not lead to biochemically active disease because: (i) intrahepatic substrate concentrations normally are well below the Michaelis constant (K_m) of uroporphyrinogen decarboxylase, whose activity is 50–100-fold in excess of rate-limiting heme biosynthetic enzymes [7], and (ii) the clinical and biochemical expression of the enzyme defect in PCT is rarely spontaneous and almost invariably is associated with hepatic iron overload [8] usually with alcoholic liver disease. Reduction of storage iron by phlebotomy predictably effects clinical and biochemical remission [9,10] which is reversed by oral iron ingestion [11].

In this report, we have for the first time con-

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00145793/85/$3.30

ducted comparative kinetic studies of highly purified (about 10000-fold) erythrocyte enzyme preparations procured from 2 familial PCT patients and a non-porphyric control subject. The purpose was to address the twin questions (a) the nature of inherent uroporphyrinogen decarboxylase enzyme defect in PCT and (b) the susceptibility of defective enzyme to iron.

2. EXPERIMENTAL

Erythrocytes were collected from 2 unrelated patients with familial PCT and one non-PCT control subject: patient GB, has congenital porphyria thought to reflect bone marrow expression of familial PCT [12]. Patient AB, has classical overt familial PCT with liver disease diagnosed by criteria as described elsewhere [1]. The non-porphyric control subject FJMc has hemochromatosis with increased liver iron, normal hemolysate uroporphyrinogen decarboxylase activity and no biochemical evidence of PCT. Sources of chemicals have been described [1,2]. The enzymes were purified approx. 10000-fold with a specific activity of about 110 units/mg protein using pentacarboxylic porphyrinogen I as substrate. Purification procedures, the details of which will be published elsewhere (Mukerji et al., in preparation) included DEAE-cellulose chromatography, ammonium sulfate fractionation (40–70% saturation), Sephadex G-100 chromatography and affinity chromatography on uroporphyrin-Sepharose. The enzyme was assayed and the Michaelis constant (K_m) determined, using uroporphyrinogen III substrate, by HPLC analyses as described by Mukerji et al. [1,2].

3. RESULTS

The data reported in fig.1 and table 1 show that the apparent Michaelis constants (K_m) calculated from the Lineweaver-Burk double reciprocal plots, using uroporphyrinogen III as substrate, of the highly purified uroporphyrinogen decarboxylase from the erythrocytes of familial PCT patients (GB and AB) were about 3.2-times higher as compared to the K_m values determined for similarly purified red cell enzyme from the hemochromatosis patient (FJMc). The K_m values of the enzymes were 1.00 and 0.96 μM for the PCT patients GB and AB, respectively, as compared to 0.31 μM for the non-porphyric control subject (FJMc).

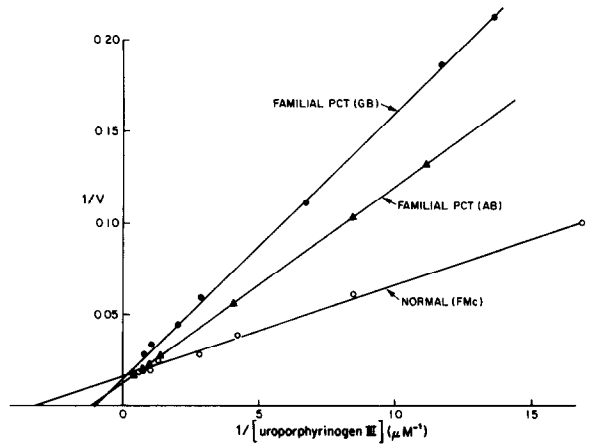

Fig.1. Lineweaver-Burk double reciprocal plots of rate versus average uroporphyrinogen III substrate concentration (0.05–6.0 μM) for 10000-fold purified red cell uroporphyrinogen decarboxylase from a normal control (FMc) (○) and 2 patients GB (●) and AB (▲) with familial PCT. The lines passing through the experimental points were drawn after calculation using linear regression analysis.

Table 1

Michaelis constants (K_m) of uroporphyrinogen decarboxylase highly purified (10000-fold) from the erythrocytes of non-porphyric control and patients with familial porphyria cutanea tarda

Human subjects[a]	n[b]	K_m[c] (μM)	r^2[d]
Non-porphyric control (FJMc)	2	0.31	0.99
Familial PCT (GB)	2	1.00	0.99
Familial PCT (AB)	2	0.96	0.99

[a] Patients, FJMc was a hemochromatosis patient who had normal red cell uroporphyrinogen decarboxylase activity. GB had postulated bone-marrow expression of familial PCT, and AB had classic familial sub-type of the disease

[b] n, number of assays per patient

[c] K_m values, determined as described in the text using uroporphyrinogen III as substrate, were measured together under identical assay conditions

[d] r^2, coefficient of determination for the line from which the kinetic constants were derived

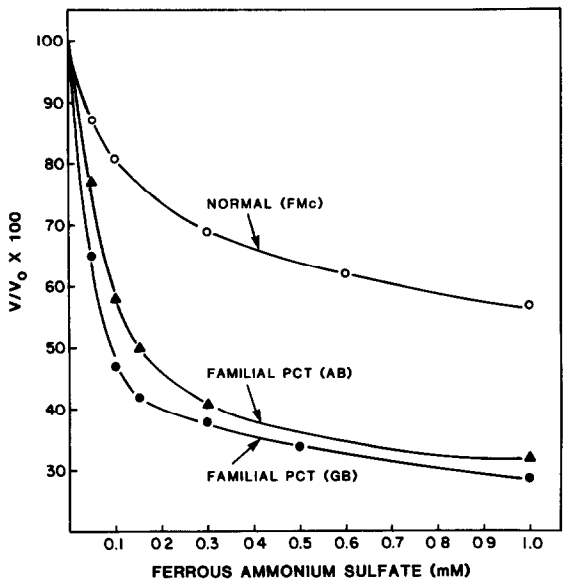

Fig.2. Inhibition of the activities of uroporphyrinogen decarboxylases, purified to 10000-fold from the red cells of a normal control, FMc (○) and 2 familial PCT patients GB (●) and AB (▲), by ferrous iron used as ferrous ammonium sulfate. V, activity in the presence of Fe^{2+}; V_o, control activity. Enzyme activities were measured by HPLC method using pentacarboxylic porphyrinogen I as substrate.

Results presented in fig.2 show progressive inhibition of the enzyme activities both from normal subject and PCT patients by increasing concentration of ferrous ammonium sulfate. However, the inhibitions by iron of the abnormal enzymes were significantly higher as compared to the normal enzyme (about 50 and 20% inhibitions, respectively, by 0.1 mM Fe^{2+}).

4. DISCUSSION

Recent observations of a proportional relationship between catalytic activity and immunoreactivity (CRIM-negative) for red cell uroporphyrinogen decarboxylase [13–15] both in familial and sporadic forms of PCT as well as in normal controls imply that the observed 50% reduced enzyme activity in familial PCT [3–5] represents diminished amounts of 'normal' enzyme. The data in this study show for the first time that the Michaelis constants (K_m) of highly purified (10000-fold) enzyme preparations from familial PCT was about 3.2-fold higher than similarly purified red cell enzyme from the non-porphyric control subject with respect to uroporphyrinogen III substrate (fig.1 and table 1). These data strongly support and extend our previous observations with about 200-fold purified red cell uroporphyrinogen decarboxylase preparations [1] that the inherent biochemical defect in human PCT is subnormal enzyme-substrate affinity. This would suggest that the antiuroporphyrinogen decarboxylase antibodies used for immunoquantitation studies [13–15] did not recognize the antigenic site(s) of the mutant enzyme in PCT, or that the kinetic abnormalities are due to point mutation in the structural gene.

The observations of in vitro inhibition of uroporphyrinogen decarboxylase activities by iron in the highly purified erythrocyte uroporphyrinogen decarboxylase enzyme preparations (fig.2) are new, and contrast with the conflicting data reported elsewhere of no [16], insignificant [17] or inhibition with excess (2–4 mM) Fe^{2+} [18,19], or only following pre-incubation of the enzyme and iron with cysteine [20]. The noteworthy observations are that mutation confers higher susceptibility to inhibition by iron of the abnormal enzymes as compared to the normal uroporphyrinogen decarboxylase. These results support our previous observations [1] that the enzyme from PCT patients might have an intrinsically abnormal conformation.

The data presented in this study, suggest that perturbation of hepatic decarboxylation of porphyrinogens by reduced substrate affinity could be enhanced by the 2 independent effects of ferrous iron i.e. direct competitive inhibition, and oxygen-dependent effects on the substrate and/or enzyme as reported by us elsewhere [2]. The dual prerequisites i.e. a metabolic lesion in heme biosynthesis and its expression by hepatic iron tie in nicely with the well known observations (i) that only a small proportion of patients with hemochromatosis or alcoholic liver disease develop PCT, (ii) that in family studies less than 20% of relatives with demonstrated evidence of red cell uroporphyrinogen decarboxylase deficiency have porphyria and (iii) why, in PCT patients where reduction of hepatic iron has led to biochemical remission, the diminished hepatic uroporphyrinogen decarboxylase activity is not restored to normal [21,22].

ACKNOWLEDGEMENTS

This work was supported by National Institutes of Health Research Grant PHS AM30094-01. We are grateful to Dr Kevin Smith (Department of Chemistry, University of California, Davis, CA) for kindly allowing us to analyze porphyrins by high performance liquid chromatography in his laboratory.

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