STUDIES ON UROPORPHYRINOGEN DECARBOXYLASE FROM CHICKEN ERYTHROCYTES

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1. Introduction

Uroporphyrinogen (URO'gen*) is decarboxylated stepwise to coproporphyrinogen (COPRO'gen) by the uroporphyrinogen decarboxylase (URO'gen carboxy-lyase E.C.4.1.1.d), the intermediate products being porphyrinogens with 7-, 6-, and 5-carboxylic groups [1–3]. This enzyme has been partially purified from rabbit reticulocytes [4, 5] and from *Rhodopseudomonas spheroides* [6].

To study the yet unsolved problems related to the enzymatic transformations of URO'gen to COPRO'gen and to determine whether one or more enzymes are involved in this process, a purified URO'gen decarboxylase from chicken erythrocytes was obtained.

The differential action of several chemical and physical agents on the different steps involved in the enzymic decarboxylation of URO'gen has been studied. The experimental results are reported in this communication.

2. Materials and methods

Perphyrins used were isolated by methods previously described [3]. URO'gen was prepared according to Mauzerall and Granick [4].

* Abbreviations:
URO'gen: (8-COOH porphyrinogen)
PHYRIA'gen: (7-COOH porphyrinogen)
COPRO'gen: (4-COOH porphyrinogen)
URO: (8-COOH porphyrin)
PHYRIA: (7-COOH porphyrin)
COPRO: (4-COOH porphyrin)

The incubation mixture contained 0.067 M potassium phosphate buffer pH 7.0, enzyme preparation, 10^{-3}M GSH, 10^{-4}M EDTA, and 3 μM porphyrinogen in a final volume of 4 ml. Standard assays were performed in Thunberg tubes at 37° for 1 hr in the dark. The reactions were stopped by the addition of conc. HCl to give a final concentration of 5% and free porphyrins were esterified by the usual method. The porphyrins with different number of carboxyls, were seperated as methyl esters by Falk and Benson's method of chromatography [7] and the amount of each one were determined spectrophotometrically and expressed as per cent of the total amount recovered.

Proteins were determined by the method of Lowry [8].

Polyacrylamide disc gel electrophoresis was carried out according to Raymond and Weintraub [9].

URO'gen decarboxylation is expressed as the sum of μmoles of the 7-, 6-, 5-, and 4-COOH porphyrinogens formed. PHYRIA'gen decarboxylation is expressed as the sum of μmoles of the 6-, 5-, and 4-COOH porphyrinogens formed.

3. Results and discussion

Haemolysate supernatant was obtained from normal chicken erythrocytes. The haemoglobin was removed by a batchwise DEAE cellulose (0.83 meq/g) treatment according to Hennessey et al. [10]. All the soluble enzymes of haem biosynthesis (δ-ALA dehydratase, porphobilinogenase and decarboxylase) were eluted with four volumes of 0.134 M potassium phosphate buffer (pH 7.0) for

Table 1
Intermediate accumulation in uroporphyrinogen decarboxylation

Incubation time (min)	URO remaining (μmoles)	Porphyrins formed (μmoles)			
		7-COOH	6-COOH	5-COOH	4-COOH
15	6.9	2.9	0.5	0.5	1.2
30	5.2	3.5	0.6	0.5	2.2
45	3.5	3.7	0.6	0.6	3.6

Purified enzyme (0.3 mg) was incubated with 3×10^{-6} M URO'gen III for the times indicated. Porphyrins formed were measured as described in the text. URO: 8-COOH porphyrin.

each volume of haemolysate supernatant. No advantage was gained by the addition of bovine serum albumin to stabilize the enzyme preparation. The eluates were fractionated by the addition of solid $(NH_4)_2SO_4$. The protein collected between 40–75% saturation was dialyzed against 3 mM phosphate buffer pH 7.0 and subjected to chromatography through DEAE cellulose equilibrated with the same buffer. Elution was conducted with the buffer in a stepwise gradient to 0.25 M KCl and gave rise to a profile which showed three protein peaks. The first peak was found to contain all of the decarboxylase activity. The enzyme thus obtained had a specific activity of 78 μmoles of URO'gen decarboxylated per hour and per mg of protein, being 220 times that of haemolysate supernatant. Details of this purification procedure will be given elsewhere [11]. This purified enzyme gave rise to a single symetrical peak when it was passed through a Sephadex G-100 column eluted with 0.134 M phosphate buffer pH 7.0 and it migrated as a single band in disc electrophoresis on polyacrylamide gel. The enzyme was stable for at least three weeks when it was kept frozen as ammonium sulphate precipitate.

Studies of the decarboxylating activity as a function of time (table 1) showed that the COPRO formed increased proportionally to incubation time. After 15 min of incubation, the increase in COPRO paralleled the decrease observed in the remaining URO and the amount of intermediates accumulated (7-, 6-, and 5-COOH porphyrinogens) remain constant. Moreover, this accumulation of intermediates was also observed when enzyme preparations from different purification steps were used. This would suggest that a kind of steady state has been reached and therefore, the rates of appearance and disappearance of 7-, 6-, and 5-COOH porphyrins are the same. The relatively greater amount of

Table 2
Effect of chemical agents on decarboxylation

Effectors	URO'gen decarboxylation	PHYRIA'gen decarboxylation
	Relative activity	
None	1	1
10^{-4}M EDTA	1.06	1.54
10^{-4}M EDTA + 10^{-3}M GSH	1.28	2.48
10^{-4}M EDTA + 10^{-3}M GSH + Boiled extract from rat liver	1.43	3.37
O_2 (aerobic incubation)	0.89	0.62
2×10^{-1}M NaCl	0.76	0.50

Reaction mixture contained, 3×10^{-6}M URO'gen III, 0.6 mg of purified enzyme and the additions as indicated. Incubation time: 1 hr. Boiled extract was obtained from rat liver homogenate by heating at 100°. 1 ml of this extract was added to the incubation mixture.

Table 3
Effect of heating on decarboxylase activity

Substrates	Heating time (min)	URO'gen decarboxylated (μmoles)	Recovery of the activity %	PHYRIA'gen decarboxylated (μmoles)	Recovery of the activity %
	0	8.7	100	5.2	100
URO'gen III	5	4.1	47	0.0	0
22.8 μmoles	15	3.2	37	0.0	0
PHYRIA'gen III	0			10.9	100
21 μmoles	10			2.2	20

The enzyme preparation was heated at 60° for the times indicated, immediately cooled in ice bath and assayed for enzyme activity. Incubations were carried out at 37° for 2 hr.

PHYRIA'gen accumulated (table 1) may be explained by an initial higher velocity of PHYRIA'gen formation. The removal of the first carboxyl groups from URO'gen would thus be faster than that of the subsequent ones. A similar difference in the behaviour of the first and second step of decarboxylation from URO'gen, was also observed when the influence of several chemical agents was studied (table 2). As it can be seen, EDTA, GSH and boiled liver extract enhanced the enzyme activity, while NaCl and aerobiosis decreased it. The effect of these agents was more pronounced in the removal of the second carboxyl i.e. PHYRIA'gen decarboxylation. The activity of this second stage could be calculated since the amount of PHYRIA'gen accumulated allow one to suppose enzyme saturation. On the other hand, preheating of the enzyme for the times indicated in table 3 resulted in a loss of activity that was different according the step catalyzed. Thus, when the enzyme was preheated at 60° for 5 min, a 50% of URO'gen decarboxylase activity still remained while PHYRIA'gen decarboxylase activity was completely lost. When this second decarboxylation step was studied with PHYRIA'gen as substrate, similar result was obtained, confirming that the second stage was more susceptible to heat than the first one. The difference observed in the percentage of inactivation obtained with both substrate could be due to a non-detectable amount of the intermediates formed (6-, 5- and 4-COOH porphyrinogens) because of the diminished formation of PHYRIA'gen by heat inhibition.

The fact that the purified enzyme yields COPRO' gen as the end product from URO'gen with an accumulation of intermediates (7-, 6- and 5-COOH porphyrinogen) indicate that it exhibits four enzyme activities each corresponding to a decarboxylation step. On the other hand, the finding that these enzyme activities were affected in different degree by the physical and chemical agents discussed above, would indicate that they could be associated to different proteins. However, the decarboxylase behaves as a single protein entity by the procedure used here. Further work is in progress to see if the decarboxylase can be dissociated into more than one protein.

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