PROTOPORPHYRIN FORMATION FROM COPROPORPHYRINOGEN III BY CHROMATIUM CELL EXTRACTS*

Masataka Mori and Seiyo Sano

Department of Public Health, Faculty of Medicine,
Kyoto University, Kyoto, Japan

Received July 5, 1968

The final step of porphyrin biosynthesis, namely the conversion of coproporphyrinogen III to protoporphyrin IX involves the oxidative decarboxylation of two propionic acid side chains to vinyl groups. This transformation by liver mitochondrial enzymes requir molecular oxygen, which could not be replaced by alternative electron acceptors (Sano and Granick, 1961). Recently Sano (1966) showed the conversion of synthetic 2,4-bis(β-hydroxypropionic acid) deuteroporphyrinogen IX to protoporphyrin IX by enzymes from or by bovine liver mitochondria, suggesting that this compound resulting from an oxygenase-type reaction on coproporphyrinogen III is dehydrated and decarboxylated to protoporphyrin IX. The importance of these findings is their implication that oxygen was present in the earth's atmosphere at a time in evolution when protoporphyrin first appeared. Porphyrins, hemoproteins (cytochromes) and bacteriochlorophyll are however present in strictly anaerobic bacteria, e.g. Chromatium and Desulfovibrio desulfuricans. Clearly, anaerobic bacteria must employ an alternative synthetic pathway for protoporphyrin formation not involving molecular oxygen and this supposition has led us to the studies on anaerobic protoporphyrin formation in Chromatium strain D.

In the present paper, we wish to report that protoporphyrin IX was formed from neither coproporphyrinogen III nor δ-aminolevulinic acid under anaerobic

---

* This investigation was supported in part by research grants from the National Institutes of Health GM 11793-02 and 03 and by Fujiwara Foundation of Kyoto University.

conditions, but unexpectedly formed under aerobic conditions.

MATERIALS AND METHODS

Chromatium strain D was grown photosynthetically for 30 - 36 hours on the carbonate medium modified from Morita et al. (1965). The cells were collected by centrifugation, washed with 1 % NaCl previously bubbled through with nitrogen, suspended in 0.05 M Tris-HCl buffer, pH 7.4 (1 : 2, by weight) and disrupted in an Ohtake French pressure cell under nitrogen. The resulting suspension was centrifuged 20 min at 15,000 g under nitrogen, the residue discarded and the supernatant designated "French press extract". Acetone powder of Chromatium was also used as enzyme preparation.

δ-Aminolevulinic acid (ALA) was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo. Coproporphyrin III and protoporphyrin IX were prepared by the method previously described (Sano, 1966). trans-2,4-Diacrylic acid deuteroporphyrin IX was prepared as described by Sparatore and Mauzerall (1960). Porphyrinogens were prepared from the corresponding porphyrins according to the method of Sano and Granick (1961). Protoporphyrin IX enzymically formed was characterized on the basis of chromatography, spectral properties and rate of conversion into mesoporphyrin IX by reduction.

Coproporphyrinogen oxidase (coprogenase) activity was measured by the method of Sano and Granick (1961). Anaerobic incubation was carried out under oxygen-free nitrogen at 38°. Short time incubation less than 10 min at 60° under aerobic conditions not only prevented the destruction of porphyrin but also accelerated the reaction rate 3 to 4 times faster than that at 38°.

RESULTS

Anaerobic incubation with coproporphyrinogen III. --- No protoporphyrin was formed from coproporphyrinogen III by either French press extract or whole cell supension (Table 1) in the presence of a variety of electron acceptors such as NAD, NADP, FAD, FMN (5 μmoles, each), methylene blue, phenazine methosulfate, 2,6-dichlorophenol indophenol, 2,3,5-triphenyltetrazolium chloride,

TABLE 1

Conversion of coproporphyrinogen III to protoporphyrin IX by Chromatium. A, cells; B, French press extract.

A: 200 mg wet cells incubated in 30 ml in fresh culture medium with coproporphyrinogen III. B: 2 ml (60 mg protein) incubated at 37° in 0.1 M phosphate buffer, pH 6.5, in a total volume of 4 ml with coproporphyrinogen III.

	Incubation conditions	Incubation period (hours)	Coprogen added (mμmoles)	Porphyrin (mμmoles)	
				Copro. recovered	Proto. formed
A	Anaerobic/light	12	210	175.0	0
	Anaerobic/dark	12	210	182.0	0
B	N_2/dark	0.5	80	80.0	0
	N_2/dark	3	80	78.0	0
	N_2/light	0.5	80	75.7	0
	N_2/light	3	80	78.6	0
	Air/dark	0.33	80	41.5	18.8
	Air/dark	0.67	80	23.0	21.2

MnO_2, potassium ferricyanide, chloranil (10 μmoles, each), cytochrome c (7.3 μmoles), sodium tetrathionate and sodium fumarate (100 μmoles). No effect was shown by the addition to the incubation mixture of boiled extracts, coenzyme A (1.4 μmoles) plus ATP (3.0 μmoles), cysteine (4 μmoles, 40 μmoles), glutathione (4 μmoles, 40 μmoles), spinach ferredoxin (approx. 3 mg) and Chromatium ferredoxin (approx. 20 mg). Protoporphyrin IX was not obtained from diacrylic acid deuteroporphyrinogen.

Aerobic experiments with coproporphyrinogen III. --- Protoporphyrin was formed when coproporphyrinogen III was incubated aerobically with French press extract or acetone powder. The effect of oxygen concentration (Fig. 1) on coprogenase activity with Chromatium was compared to that for the bovine liver mitochondrial enzyme (Porra and Falk, 1964). Maximal protoporphyrin formation occurred at about 40 % (v/v) oxygen concentration, indicating a similar Km for

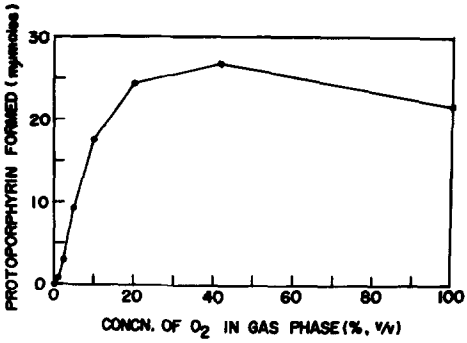

Fig. 1. Effect of oxygen concentration on Chromatium coprogenase activity. In 4 ml of 0.25 M phosphate buffer, pH 6.5, 300 mg acetone powder incubated 10 min at 60° with 98 mμmoles of coproporphyrinogen III in Thunberg tubes under mixtures of N_2 and O_2.

Chromatium and mitochondrial enzyme. Slight inhibition was observed at 100 % oxygen as in the case of mitochondrial enzyme (Porra and Falk, 1964). The pH optimum was 6.5 in 0.25 M potassium phosphate buffer with equal activity in Tris-HCl buffer. Protoporphyrin formation was linear with time up to 30 min at 38°, and to 10 min at 60°, and with enzyme amount to 300 mg of acetone powder. Heating the enzyme at 80° for 5 min or boiling destroyed the activity. It was stable for several months in acetone powder at -20°.

Aerobic and anaerobic experiments with δ-aminolevulinic acid. --- Porphyrin formation from δ-aminolevulinic acid by Chromatium cell suspension under various conditions is shown in Table 2. Porphyrin formation was only less than 140 mμmoles after 24 hours incubation. Coproporphyrin III was predominant when the incubation was carried out in the dark. Protoporphyrin IX was not formed. Addition of ferric iron (10 μM) plus sodium fumarate (0.02 M) in anaerobic system under strong light illumination failed to produce any protoporphyrin or to increase porphyrin synthesis. On the other hand, porphyrins were formed in large amount from ALA by French press extract of Chromatium under anaerobic conditions (Table 2). About half of the added ALA was converted to porphyrins when incubation was carried out anaerobically. Aerobic incubation gave a small amount of protoporphyrin, although the total

TABLE 2

Porphyrin formation from ALA by Chromatium.
A, cells; B, French press extract.

A: 300 mg wet cells incubated at 34° with ALA (Expt. No. 1, 2 : 3.0 μmoles; Expt. No. 3, 4 : 11.9 μmoles) in either 1 % NaCl (Expt. No. 1 to 3) or fresh culture medium (Expt. No. 4), in a total volume of 20 ml. The concentrations of ferric chloride and sodium fumarate were 10 μM and 0.02 M. B: 2 ml of French press extract incubated in 0.1 M Tris-HCl buffer, pH 7.4, with 5 μmoles of ALA in a total volume of 4 ml.

	Expt. No.	Incubation conditions with additions	Incubation period (hours)	Porphyrin formed (mμmoles)		
				Uro.	Copro.	Proto.
A	1	Air/dark	20	5.8	28.8	0
	2	N_2/dark	20	18.2	119.0	0
	3	Fe^{3+} N_2/light and fumarate	24	67.9	13.2	0
	4	Fe^{3+} N_2/light and fumarate	24	66.0	11.0	0
B	5	Air/dark	4	13.1	49.6	6.5
	6	N_2/dark	4	29.0	266.0	0
	7	N_2/light	16	15.4	262.0	0

generated by the photosynthetic system. Porra and Lascelles (1965), however, could not demonstrate any coprogenase activity in cell-free extracts of anaerobically grown Rhodopseudomonas spheroides. Recently Ehteshamuddin (1968) reported anaerobic protoporphyrin formation from coproporphyrinogen III by Pseudomonas sonic extracts in the presence of glutathione, although we could not demonstrate it in our system. We are still continuing the studies on the mechanism of protoporphyrin formation in anaerobic bacteria.

ACKNOWLEDGEMENTS

We are indebted to Dr. S. Morita, University of Tokyo and Dr. M. Cusanovich, University of California, San Diego, for supplying Chromatium strain D. We also thank Dr. T. Horio, Institute for Protein Research, Osaka University, for his generous gift of spinach ferredoxin.

REFERENCES

Sano, S. and Granick, S., J. Biol. Chem., 236, 1173 (1961)
Sano, S., J. Biol. Chem., 241, 5276 (1966)
Morita, S., Edwards, M. and Gibson, J,, Biochem. Biophys. Acta, 109, 45 (1965)
Sparatore, F. and Mauzerall, D., J. Org. Chem., 25, 1073 (1960)
Porra, R. J. and Falk, J. E., Biochem. J., 90, 69 (1964)
Lascelles, J., Ciba Found. Symp. : Porphyrin Biosynthesis and Metabolism. p. 265, Ed. by Wolstenholme, G. E. W. and Millar, E. C. P., London : J. and A. Churchill Ltd.
Porra, R. J. and Lascelles, J., Biochem. J., 94, 120 (1965)
Ehteshamuddin, A. F. M., Biochem. J., 107, 446 (1968)