BBA 43164

The conversion of protoheme to heme *a* in Staphylococcus

Several workers[1–3] have proposed a scheme of biogenesis of heme *a* in which protohemin is a precursor. An alternative hypothesis is that heme *a* biosynthesis branches off the biosynthetic pathway at an earlier stage, possibly at the coproporphyrin level.

Studies with different *Staphylococcus aureus* mutants[2,4,5] which require added protohemin for the formation of cytochromes *a* and *b* suggest, but do not definitely prove, that heme *a* is formed from the added protohemin. The possibility remains that the added protohemin could act other than as a direct precursor.

In order to decide whether protohemin actually is a precursor of heme *a*, the protohemin requiring mutant *S. aureus* JT/52 has been grown aerobically in the presence of ^{14}C-labeled protoheme, the hemes and the free porphyrin formed have been extracted, purified and the specific activities determined.

Labeled protoheme was prepared from [^{14}C]glycine using reticulocytes from rabbits treated with phenylhydrazine[6]. The labeled protoheme formed was completely freed from accompanying labeled free porphyrin by extraction of ethereal solution with 15 % HCl (w/v). The protoheme was then crystallized[7].

S. aureus JT/52 was grown for 24 h at 37° with vigorous aeration in 16 l of a minimal medium[8] supplemented with 0.5 % (w/v) proteose–peptone (Difco) and containing 1 μg/ml of the ^{14}C-labeled heme, which had been previously dissolved in a mixture of pyridine–0.3 M NH_4OH (1:5, v/v). Difference spectra (reduced in the presence of lactate *minus* oxidized in the presence of air) of the harvested organisms showed the presence of cytochromes *a*, *o* and *b* as has been reported in other strains[9].

The hemes were extracted from 60 g wet weight of cells with acetone–HCl[10], taken into ether, washed with 1 % HCl and the ether then removed *in vacuo*. The oily residue was then taken into pyridine–water (1:1, v/v) and chromatographed on a Sephadex G-25 column equilibrated in the same solvent. By this procedure most of the carotenoids were separated from the hemes, the heme *a* was partially separated from the protoheme and the presence of a free porphyrin was revealed. The remaining carotenoids that contaminated the heme fraction were removed by extraction with ether, and the fraction containing the hemes was transferred into ether after the pyridine was removed *in vacuo*. The residual free porphyrin in the heme fraction was removed from the ethereal solution by extraction with 15 % HCl (w/v).

Iron was removed from the heme in acetic acid by the $FeSO_4$ procedure[11], the porphyrins taken into ether and most of the protoporphyrin extracted with 8 % HCl (w/v). The porphyrin *a*, which remained in the ether was separated from the residual protoporphyrin by chromatography on a cellulose column[12].

The porphyrin *a* was identified by its spectrum and by paper chromatography with a propanol–heptane (1.8:10, v/v) solvent, using porphyrin *a* prepared from dog heart as a reference compound. The free porphyrin extracted from the bacteria was identified as coproporphyrin by its spectrum and HCl number. The samples were spotted onto filter paper, treated with 30 % (w/v) H_2O_2, dried and counted in a Packard Tri-Carb scintillation spectrometer. The protoheme used for growth was also converted into the porphyrin and counted in the same way.

TABLE I

SPECIFIC ACTIVITIES OF PORPHYRINS

Concentrations calculated using molar extinction coefficients given in ref. 7.

Porphyrin		*Specific activity (counts/min per μmole)*
Protoporphyrin	added as heme	7700
Porphyrin *a*		7000
Protoporphyrin	extracted	5800
Coproporphyrin		370

Table I gives the specific activities of the porphyrins. The errors involved were estimated to be ± 10 %. The results show that the specific activities of the protoheme added to the medium and the heme *a* extracted from the cells are essentially equal within the errors of measurement. It is evident that the heme *a* was formed from the added protoheme and there was no significant synthesis *de novo*.

The finding of unlabeled coproporphyrin in the mutant cells confirms JENSEN's[13] earlier observations. This indicates that in this strain of *S. aureus*, synthesis of coproporphyrin *de novo* occurs in the presence of heme. In another hemin-requiring strain of *S. aureus*, this porphyrin synthesis was not detected[4].

This work was supported by Grant No. GB 4795 from the National Science Foundation and Grant No. GM 10285 of the Institute of General Medical Sciences, U.S. Public Health Service. We wish to thank Dr. M. MORRISON who suggested the use of Sephadex for the separation of hemes and Dr. D. ROLOFF for the gift of strain JT/52.

Department of Biochemistry, P. SINCLAIR
University of Kentucky Medical Center, D. C. WHITE
Lexington, Kry (U.S.A.) and
Institute of Medical Research, J. BARRETT
Royal North Shore Hospital,
Crows Nest (Australia)

1 R. LEMBERG AND A. BENSON, *Nature*, 183 (1959) 678.
2 M. KIESE, H. KURZ AND E. THOFERN, *Biochem. Z.*, 330 (1958) 541.
3 M. GRASSL, G. AUGSBURG, U. COY AND F. LYNEN, *Biochem. Z.*, 337 (1963) 35.
4 J. BARRETT, *Biochim. Biophys. Acta*, 54 (1961) 580.
5 J. JENSEN AND E. THOFERN, *Z. Naturforsch.*, 9b (1954) 596.
6 H. BORSOOK, C. DEASY, A. HAAGEN-SMIT, G. KEIGHLEY AND P. LOWY, *J. Biol. Chem.*, 196 (1952) 669.
7 J. FALK, *Porphyrins and Metalloporphyrins* (BBA Library, Vol. 2), Elsevier, Amsterdam, 1964, p. 186.
8 J. LASCELLES AND D. WOODS, *Brit. J. Exptl. Pathol.*, 33 (1952) 288.
9 H. TABER AND M. MORRISON, *Arch. Biochem. Biophys.*, 105 (1964) 367.
10 J. FALK, *Porphyrin and Metalloporphyrins* (BBA Library, Vol. 2), Elsevier, Amsterdam, 1964, p. 183.
11 D. B. MORELL AND M. STEWART, *Australian J. Exptl. Biol. Med. Sci.*, 34 (1956) 211.
12 D. B. MORELL, J. BARRETT AND P. CLEZY, *Biochem. J.*, 78 (1961) 793.
13 J. JENSEN, *Biochem. Biophys. Res. Commun.*, 8 (1962) 271.

Received March 6th, 1967