SITE OF GLUCOSE REPRESSION OF HEME BIOSYNTHESIS

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Incubation of aerobic cultures of *Saccharomyces cerevisiae* with high concentrations of glucose leads to the repression of heme biosynthesis [1] and to the accumulation of prototetrahydroporphyrin IX, a pigment with an absorption maximum at 503 nm, commonly designated P503 [2]. Previously we showed [3] that P503 is derived from protoporthyrinogen IX by auto-oxidation (see fig. 1, reaction 3). Thus, the accumulation of P503 in glucose-repressed cells indicates an antecedent accumulation of protoporphyrinogen IX, a postulated intermediate in heme biosynthesis [4, 5]. Here we report that the accumulation of protoporphyrinogen IX in glucose-repressed cells is due to a deficiency of the mitochondrial enzyme which converts it to protoporphyrin IX, the immediate

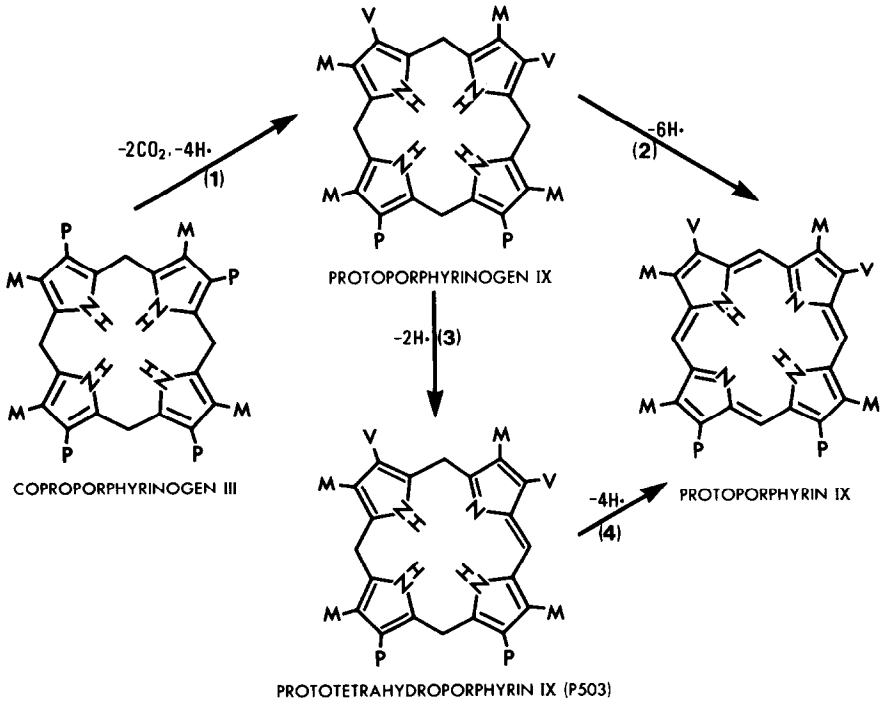

Fig. 1. The conversion of coproporphyrinogen III to protoporphyrin IX via protoporphyrinogen IX and P503. Reaction (1), coproporphyrinogenase; reactions (2) and (4), protoporphyrinogenase; reaction (3), autocatalytic.

precursor of heme.

The penultimate step of heme biosynthesis – the conversion of coproporphyrinogen III to protoporphyrin IX – involves the oxidative decarboxylation of two propionic acid side chains to vinyl groups and the oxidative removal of six hydrogen atoms from the porphyrinogen nucleus (see fig. 1). It has generally been supposed that a single enzyme, coproporphyrinogenase, catalyses the entire reaction [6–13]. However, as reported here, we have found two distinct enzymes which participate in this reaction. The first, coproporphyrinogenase, catalyses the conversion of coproporphyrinogen III to protoporphyrinogen IX (fig. 1, reaction 1), while the second, which has been called protoporphyrinogenase [4, 5], catalyses the oxidation of protoporphyrinogen IX and P503 to protoporphyrin IX (fig. 1, reactions 2 and 4).

Coproporphyrinogenase activity was detected when a soluble fraction prepared from yeast cells was incubated under anaerobic conditions in the presence of coproporphyrinogen III, $FeSO_4$, L-methionine, ATP and $NADP^+$ (cf. ref. [13]); the product, protoporphyrinogen IX, was identified by the spectrophotometric method described by Porra and Falk [5]. Extracts prepared from either glucose-repressed or derepressed cells contained similar amounts of coproporphyrinogenase.

Protoporphyrinogenase activity was associated

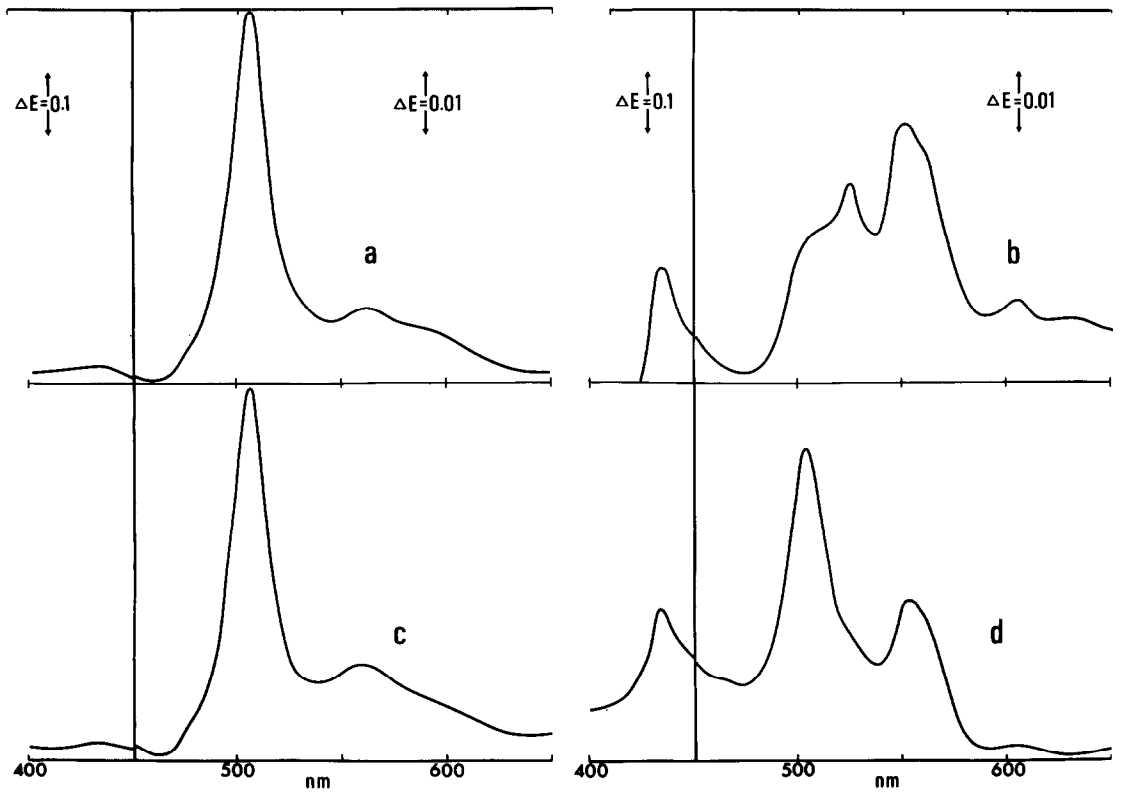

Fig. 2. Difference spectra showing the effect of cAMP on P503 in intact yeast cells. Cells were cultured anaerobically at 29°C in a 4% glucose–yeast extract–Tween 80–ergosterol medium to a cell density of 1 mg dry weight/ml of medium [2]. After harvesting, the washed cells were resuspended (25 mg dry weight/ml) in a 0.1% glucose–yeast extract adaptation medium in the presence and absence of 5 mM cAMP and incubated aerobically at 29°C. Reduced minus oxidised difference spectra were recorded at room temperature using a Cary 15 spectrophotometer. Cell suspensions in the sample cuvettes were reduced with endogenous substrate and the reference suspensions were oxidised with H_2O_2. Curve a, anaerobic cells immediately after resuspension in adaptation medium containing cAMP; curve b, cells incubated for 30 min in the presence of cAMP; curve c, anaerobic cells immediately after resuspension in adaptation medium (without cAMP); curve d, cells incubated for 30 min in adaptation medium (without cAMP).

with mitochondrial extracts prepared from yeast cells [2, 3]. Molecular oxygen was essential for its activity and could not be replaced by any of a range of alternate electron acceptors tested. Protoporphyrinogenase activity was present in mitochondrial extracts prepared from cycloheximide-treated cells but it was not detected in mitochondrial extracts isolated from cells grown in the presence of chloramphenicol for 2 hr. In contrast, coproporphyrinogenase activity was present in cells which had been exposed to chloramphenicol but no activity was found in cycloheximide-treated cells. These results indicate that protoporphyrinogenase is formed in mitochondria and coproporphyrinogenase in the cytoplasm.

Protoporphyrinogenase activity was found only in the mitochondrial extracts prepared from glucose-derepressed cells. It was not detected in those prepared from glucose-repressed yeast cells [2]. Since ferrochelatase (the only enzyme distal to protoporphyrinogenase in the heme biosynthetic pathway) has been detected in glucose-repressed yeast cells [14] the evidence presented here strongly indicates that the repression of heme biosynthesis by glucose is due primarily to the absence of protoporphyrinogenase activity. The accumulation of protoporphyrinogen IX in glucose-repressed cells, as indicated by the accumulation of P503 [2], lends additional support to this interpretation.

The results presented in fig. 2 show that the addition of adenosine 3', 5'-monophosphate (cAMP) to glucose-repressed cells caused a rapid disappearance of P503 accompanied by the appearance of an aerobic cytochrome spectrum. These data suggest that the glucose effect on heme biosynthesis, like its effect on many other processes [15–18], is reversed by cAMP. The existence of a glucose-sensitive enzyme at this site in heme biosynthesis may provide the basis for an understanding of such diverse phenomena as the remission in acute porphyria frequently observed following administration of glucose as well as the repression by glucose of mitochondrial biogenesis [1].

Acknowledgements

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References

[1] Ephrussi, B., Slonimski, P. P., Yotsuyanagi, Y. and Tavlitsky, J. (1956) C. R. Lab. Carlsberg Ser. Physiol. 26, 87–98.
[2] Poulson, R., Boon, J. and Polglase, W. J. (1974) Canad. J. Biochem. 52, in press.
[3] Poulson, R. and Polglase, W. J. (1973) Biochim. Biophys. Acta 329, 256–265.
[4] Sano, S. and Granick, S. (1961) J. Biol. Chem. 236, 1173–1180.
[5] Porra, R. J. and Falk, J. E. (1964) Biochem. J. 90, 69–75.
[6] Granick, S. and Mauzerall, D. (1958) Federation Proc. 17, 233.
[7] Porra, R. J. and Falk, J. E. (1961) Biochem. Biophys. Res. Commun. 5, 179–184.
[8] Batlle, A. M. del C., Benson, A. and Rimington, C. (1965) Biochem. J. 97, 731–740.
[9] Jacobs, N. J., Jacobs, J. M. and Brent, P. (1970) J. Bacteriol. 102, 398–403.
[10] Miyake, S. and Sugimura, T. (1968) J. Bacteriol. 96, 1197–2003.
[11] Hsu, W. J. and Miller, G. W. (1970) Biochem. J. 117, 215–220.
[12] Ehteshamuddin, A. F. M. (1968) Biochem. J. 107, 446–447.
[13] Tait, G. H. (1972) Biochem. J. 128, 1159–1169.
[14] Reithmuller, G. and Tuppy, H. (1964) Biochem. Z. 340, 413–420.
[15] Makman, R. S. and Sutherland, E. W. (1965) J. Biol. Chem. 240, 1309–1314.
[16] Ullman, A. and Monod, J. (1968) FEBS Letters 2, 57–60.
[17] Pastan, I. and Perlman, R. L. (1971) Nature New Biol. 229, 5–7.
[18] Fang, M. and Butow, R. A. (1970) Biochem. Biophys. Res. Commun. 41, 1579–1583.