CATABOLITE REPRESSION OF PROTOPORPHYRIN IX BIOSYNTHESIS IN *ESCHERICHIA COLI* K-12

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1. Introduction

It was reported previously [1] that streptomycin-dependent (SM-dependent) mutants of *Escherichia coli* strains B and K-12 cultured in the presence of a concentration of dihydrostreptomycin which is non-limiting for growth contain very little 503 nm pigment compared with wild-type parent cells. Until now, however, the principle underlying this difference was unknown. Recently, we showed [2] that the 503 nm pigment in yeast is identical with prototetrahydroporphyrin IX and that it is derived from protoporphyrinogen IX by auto-oxidation. It has also been established [3] that glucose repression of protoporphyrinogen oxidase results in the accumulation of its substrate – protoporphyrinogen IX – which can be readily detected spectrophotometrically in intact cells as prototetrahydroporphyrin IX. In addition, we have demonstrated [4,5] that the degree of catabolite repression of SM-dependent *E. coli* can be modified by varying the dihydrostreptomycin concentration of the growth medium. On the basis of these observations we postulated that SM-dependent mutants of *E. coli* cultured in the presence of a dihydrostreptomycin concentration which is non-limiting for growth are unable to accumulate protoporphyrinogen IX, as indicated by the low level of 503 nm pigment [1], because protoporphyrinogen oxidase is derepressed in these cells. As reported here, we have now obtained confirmation of this inference.

2. Materials, methods and results

SM-dependent mutants of *E. coli* cultured in a medium containing a dihydrostreptomycin concen-

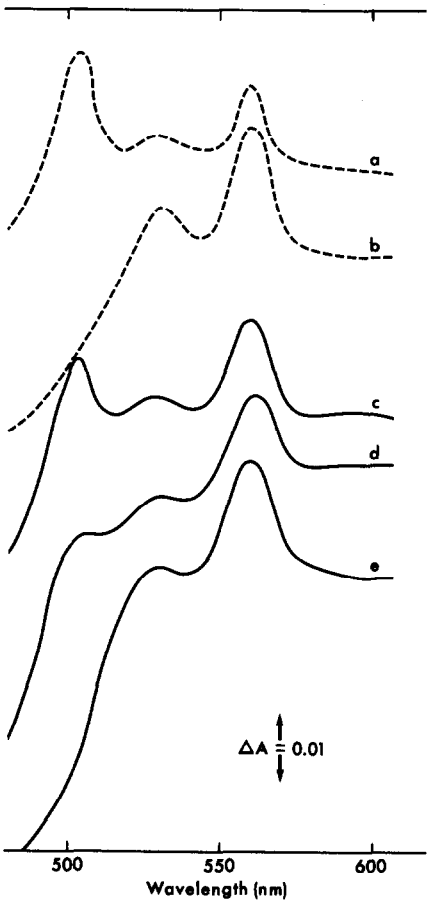

Fig.1. Difference spectra (reduced minus oxidised) or intact cells of wild-type (----) and SM-dependent mutants (——) of *E. coli* K-12. Cells were grown to mid log phase under forced aeration at 37°C in a minimal salts medium containing (a) 0.4% glucose; (b) 0.8% acetate; (c) 0.4% glucose and 10 μg DHSM/ml; (d) 0.4% glucose and 30 μg DHSM/ml; (e) 0.4% glucose and 1000 μg DHSM/ml. Difference spectra were determined as in [1].

Table 1
Relationship between catabolite repression and the level of prototetrahydroporphyrin IX in wild-type and SM-dependent mutants of *E. coli* K-12[a]

Strain	Carbon source	DHSM[b] (μg/ml)	Fumarase[c] (units[d]/ mg protein)	Relative level of prototetrahydroporphyrin[e]
K-12	Glucose	–	124 (1.00)[f]	1.00
SM-d K-12	Glucose	1000	449 (3.62)	0.00
SM-d K-12	Gluconate	1000	310 (2.50)	0.00
SM-d K-12	Glucose	10	130 (1.05)	0.94

[a] Cells were grown to mid log phase under forced aeration at 37°C in a minimal salts medium containing 0.4% glucose or 0.4% gluconate supplemented with dihydrostreptomycin as indicated.
[b] Dihydrostreptomycin.
[c] Assayed in cell-free extracts as described in [4].
[d] Unit is defined as $\triangle OD_{240\ nm} = 0.01$/min.
[e] Level relative to that determined in glucose-grown, wild-type *E. coli* K-12. Prototetrahydroporphyrin IX levels were measured by reduced minus oxidised difference spectrophotometry [1].
[f] Activity relative to that obtained in cell-free extracts prepared from glucose-grown, wild-type *E. coli* K-12.

Table 2
Specific activities of coproporphyrinogenase and protoporphyrinogen oxidase of wild-type and SM-dependent mutants of *E. coli* K-12[a]

Strain	Carbon Source	DHSM[b] (μg/ml)	Specific activity (nmoles product/mg protein/h) Coproporphyrinogenase[c]	Protoporphyrinogen oxidase[d]
K-12	Glucose	–	2.33	1.19
K-12	Acetate	–	2.65	4.36
SM-d K-12	Glucose	1000	2.43	4.30
SM-d K-12	Glucose	30	2.48	2.62
SM-d K-12	Glucose	10	2.17	1.80

[a] Cells were grown to mid log phase under forced aeration at 37°C in a minimal salts medium containing 0.4% glucose or 0.8% acetate supplemented with dihydrostreptomycin as indicated. Cells were disrupted by sonic treatment and centrifuged at 17 000 *g* for 30 min. The supernatant was recentrifuged at 150 000 *g* for 1 h to give a soluble cytoplasmic fraction and a membrane fraction. Coproporphyrinogenase activity was located almost exclusively in the soluble cytoplasmic fraction while all of the protoporphyrinogen oxidase activity was associated with the membrane fraction.
[b] Dihydrostreptomycin.
[c] Assayed as described in [7].
[d] Assayed as described in [8].

tration which is non-limiting for growth (1000 μg/ml) have relaxed catabolite repression, and catabolite repression is restored when the mutants are cultured in the presence of a level of antibiotic which is limiting for growth (10 μg/ml) (cf. level of catabolite-sensitive enzyme, fumarase, in cells grown in the presence of 10 and 1000 μg dihydrostreptomycin/ml, table 1). In the present studies, therefore, the extent of catabolite repression of SM-dependent mutants was controlled by varying the dihydrostreptomycin concentration of the growth medium, while in wild-type cells it was modified by varying the carbon source [6].

The level of prototetrahydroporphyrin IX (used here as a measure of the protoporphyrinogen IX content of the cells) in an SM-dependent *E. coli* varied inversely with the dihydrostreptomycin concentration of the growth medium (table 1 and fig.1). The highest level of prototetrahydroporphyrin IX was observed in glucose-grown, wild-type cells and in SM-dependent mutants grown in a medium containing 10 μg dihydrostreptomycin/ml. No prototetrahydroporphyrin IX was detected in cells of either wild-type or SM-dependent *E. coli* in which catabolite repression was relaxed.

The state of catabolite repression of the cells had little effect on the level of coproporphyrinogenase (table 2). In contrast, the level of protoporphyrinogen oxidase – like that of the catabolite-sensitive enzymes, fumarase and acetohydroxy acid synthase – increased as the degree of catabolite repression diminished (cf. tables 1 and 2 and [5]). The level of protoporphyrinogen oxidase was highest in acetate-grown, wild-type cells and SM-dependent mutants cultured in the presence of 1000 μg dihydrostreptomycin/ml.

In summary, cells in which catabolite repression is relaxed have a high level of protoporphyrinogen oxidase and contain very little protoporphyrinogen IX, whereas in catabolite-repressed cells these levels are reversed. These results strongly support the suggestion that protoporphyrinogen IX accumulates only in catabolite-repressed cells of *E. coli* because protoporphyrinogen oxidase, which converts it to protoporphyrin IX, is repressed in these cells. Control of catabolite-sensitive enzymes by dihydrostreptomycin in SM-dependent mutants of *E. coli* has now been demonstrated for several enzymes [4,5,9]. However, the relationship between this in vivo effect and the action of dihydrostreptomycin on general protein synthesis which has been observed in vitro [10] remains unresolved.

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References

[1] Kamitakahara, J. R. and Polglase, W. J. (1970) Biochem. J. 120, 771–775.
[2] Poulson, R. and Polglase, W. J. (1973) Biochim. Biophys. Acta 329, 256–263.
[3] Poulson, R. and Polglase, W. J. (1974) FEBS Lett. 40, 258–260.
[4] Coukell, M. B. and Polglase, W. J. (1969) Biochem. J. 111, 279–285.
[5] Whitlow, K. J. and Polglase, W. J. (1975) J. Bacteriol. 121, 9–12.
[6] Whitlow, K. J. and Polglase, W. J. (1974) FEBS Lett. 43, 64–66.
[7] Poulson, R. and Polglase, W. J. (1974) J. Biol. Chem. 249, 6367–6371.
[8] Poulson, R. and Polglase, W. J. (1975) J. Biol. Chem. 250, 1269–1274.
[9] Coukell, M. B. and Polglase, W. J. (1969) J. Gen. Microbiol. 57, 419–427.
[10] Pestka, S. (1967) Bull. N.Y. Acad. Med. 43, 126–148.