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REGULATION OF ENTEROCHELIN SYNTHETASE IN *ESCHERICHIA COLI* K-12

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Enterochelin synthetase activity is controlled by both repression and feed-back inhibition mechanisms Inclusion of iron in growth media results in synthesis of all four (D, E, F and G) components of enterochelin synthetase being repressed The specific inhibition of L-serine activation (partial reaction catalyzed by the F component) by the end products, ferric-enterochelin and 2,3-dihydroxybenzoylserine, is shown to inhibit overall enterochelin synthetase activity

Enterochelin synthetase in *Escherichia coli* catalyzes the synthesis of enterochelin from 2,3-dihydroxybenzoic acid and L-serine and consists of at least four components, designated D, E, F and G [1] All four components are necessary for the synthesis of enterochelin [2] and the D component physically associates with the F and/or G component(s) in vitro [3] Intermediates produced during partial reactions appear to be protein bound [4,5] Four classes of *E coli* mutants defective in enterochelin synthetase (*entD, entE, entF* and *entG*), are available [4,6] Cell extracts prepared from such strains may be used in complementation assays for the detection of individual components of the enterochelin synthetase system [1]

Enterochelin is an iron-transport compound (siderophore) produced by *E coli* K-12 [7], *Klebsiella pneumoniae (Aerobacter aerogenes)* [8] and *Salmonella typhimurium* [9], and has also been called enterobactin [9] Under conditions of iron deficiency, *E coli* cells synthesize enterochelin and excrete it into culture fluids Here a stable ferric-enterochelin complex is formed which is believed to be transported into the bacterial cytoplasm Iron is released for cellular metabolism following enzymatic hydrolysis of the ligand to 2,3-dihydroxybenzoylserine by enterochelin esterase [10,11] The enterochelin precursor, 2,3-dihydroxybenzoate, can also form an iron-complex which may diffuse through the bacterial outer-membrane [12]

The expression of the *ent* genes varies in response to the extracellular level of iron Synthesis of enterochelin synthetase is repressed to 5% of the maximum level when 10 μM $FeCl_3$ are included in the minimal growth medium [10] In contrast, synthesis of the three enzymes concerned with the conversion of chorismate to 2,3-dihydroxybenzoate is repressed to 5% of maximal activity when 200 μM iron are included in the same medium [13] Although the enterochelin synthetase system is not inhibited by iron [14], the activation of L-serine (partial reaction catalyzed by the F component) is inhibited by AMP and by inorganic pyrophosphate (PP_i) [15] We present here, evidence that synthesis of each of the individual components of enterochelin synthetase is repressible and that negative-feedback inhibition of the biosynthetic pathway occurs

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TABLE I

ACTIVITIES OF ENTEROCHELIN SYNTHETASE AND ITS INDIVIDUAL COMPONENTS IN CELL EXTRACTS PREPARED FROM STRAIN AN194

Cell extracts were prepared from AN194 cells grown in appropriately supplemented minimal media to which either 100 μM 2,2'-dipyridyl (derepressed) or 50 μM $FeCl_3$ (repressed) had been added

Source of cell extract	Specific activities of enterochelin synthetase and its individual components (nmol L-serine converted in 15 min/mg protein)				
	enterochelin synthetase activity	D activity	E activity	F activity	G activity
AN194 (derepressed)	33 21	160 41	209 32	64 03	54 21
AN194 (repressed)	2 26	4 46	3 76	2 77	3 07

In order to determine whether synthesis of each of the individual components of enterochelin synthetase is repressed when adequate amounts of iron are included in growth media, purified cell extracts [1] were prepared from AN194 cells [16] grown under different conditions The *E coli* strain AN194 is able to convert 2,3-dihydroxybenzoic acid to enterochelin normally and was used as a source of cell extract containing the complete complement of proteins necessary for synthesis of enterochelin Cells were grown in appropriately supplemented minimal medium to which had been added either 100 μM 2,2'-dipyridyl (to reduce the availability of iron) or 50 μM $FeCl_3$ The results obtained (Table I) when the two types of cell extract were assayed for both overall enterochelin synthetase activity [1], and the presence of individual components of enterochelin synthetase [1], are consistent with the view that synthesis of all four components is repressed in cells grown in the presence of adequate levels of iron

Activation of L-serine (F component activity) has been shown previously to be inhibited by AMP and by PP_i [15] In Fig 1 the inhibition of overall enterochelin synthetase activity by AMP is compared with that of the ATP-PP_i exchange activities [1] catalyzed by the F and E components Purified cell extract was prepared from the *E coli* strain AN273 [16], which also contains a complete complement of enterochelin synthetase components, and partially purified F and E components were prepared as described previously [1] The ATP-PP_i exchange activity associated with F component, and the overall enterochelin synthetase activity, were found to be inhibited to the same extent by a given concentration of AMP The exchange activity associated with E component was only slightly inhibited by AMP These results indicate that inhibition of overall enterochelin synthetase activity by AMP may occur as a result of specific inhibition of the partial reaction catalyzed by F component

The effects of ferric-enterochelin and 2,3-dihydroxybenzoylserine on both overall enterochelin synthetase activity and the ATP-PP_i exchange activities associated with the F and E components of enterochelin synthetase, are shown in Fig 2 Each compound inhibits both overall enterochelin synthetase activity, and activation of L-serine catalyzed by F component In terms of the concentration of 2,3-dihydroxybenzoylseryl-groups present, inhibition by 2,3-dihydroxybenzoylserine is approx 3-times greater than that of ferric-enterochelin However, on a molar basis, the two compounds are equally effective The ATP-PP_i exchange activity catalyzed by the E component was not significantly inhibited by either compound It seems that activation of L-serine by the F component of enterochelin synthetase is a rate-limiting partial reaction and that inhibition of this activity by AMP, ferric-enterochelin or 2,3-dihydroxybenzoylserine results in parallel inhibition of enterochelin synthesis No significant inhibition of any of the three reactions was obtained when L-serine, 2,3-dihydroxybenzoic acid or iron were included in reaction mixtures

Intracellular ferric-enterochelin and 2,3-dihydroxybenzoylserine may be regarded as 'end products' in

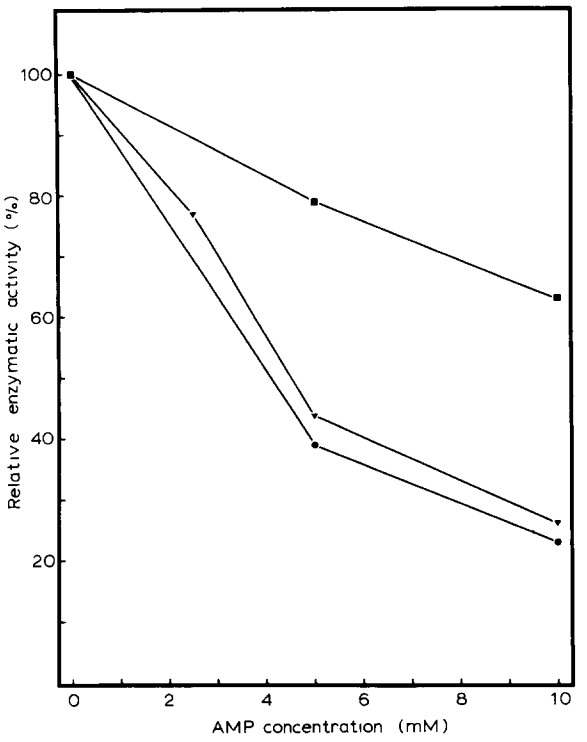

Fig 1 Inhibition by AMP of enterochelin synthetase activity and of the ATP-PP_i exchange activities catalyzed by the F and E components of enterochelin synthetase Purified cell extract (540 μg protein) prepared from strain AN273 was used as source of enterochelin synthetase activity (▼———▼) Semi-purified F component (63 μg protein) and E component (10 μg protein) were assayed for ATP-PP_i exchange activity in the presence of 2 mM L-serine (●———●) or 2 mM 2,3-dihydroxybenzoic acid (■———■)

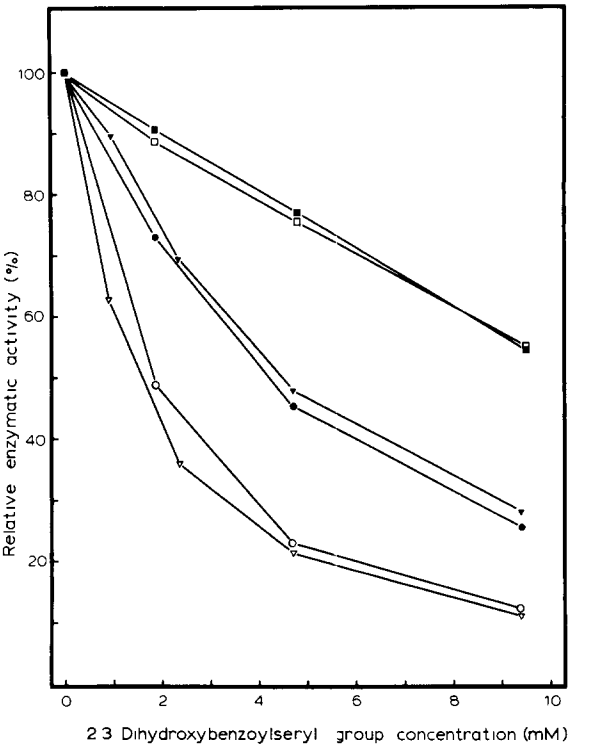

Fig 2 Inhibition by ferric-enterochelin and by 2,3-dihdyroxybenzoylserine, of enterochelin synthetase activity and of the ATP-PP_i exchange activities catalyzed by the F and E components of enterochelin synthetase Purified cell extract (540 μg protein) prepared from strain AN273 was used as a source of enterochelin synthetase activity during examination of inhibition by ferric-enterochelin (▼———▼) and 2,3-dihydroxybenzoylserine (▽———▽) The activity of semi-purified F component (63 μg protein) was assayed (by measuring ATP-PP_i exchange in the presence of 2 mM L-serine) to examine the inhibition by ferric-enterochelin (●———●) and 2,3-dihydroxybenzoylserine (○———○) The activity of semi-purified E component (10 μg protein) was assayed (by measuring ATP-PP_i exchange in the presence of 2 mM 2,3-dihydroxybenzoic acid) to examine the inhibition by ferric-enterochelin (■———■) and 2,3-dihydroxybenzoylserine (□———□)

the enterochelin iron-transport system, and inhibition of enterochelin synthetase by these compounds should provide effective feed-back control of the system The effectiveness of such control becomes evident when one examines the operation of the system in mutant Fes^- (strain carrying a mutant *fes* allele, and therefore unable to hydrolyse intracellular ferric-enterochelin [16]) or Fep^- (strain carrying a mutant *fep* allele, and therefore unable to take up extracellular ferricenterochelin [7]) strains Cell extracts prepared from otherwise isogenic $Fes^- Fep^+$, $Fes^+ Fep^-$ and $Fes^+ Fep^+$ strains, grown under conditions which allow derepressed synthesis of enzymes concerned with conversion of 2,3-dihydroxybenzoic acid to enterochelin, all show high specific activities of enterochelin synthetase Stationary phase culture fluids of Fep^- and Fes^- strains contain large quantities of enterochelin Culture fluids of $Fes^+ Fep^+$ strains however, contain equivalent quantities (in terms of 2,3-dihydroxybenzoyl-groups) of 2,3-dihydroxybenzoic acid and barely detectable amounts of enterochelin [7] High levels of enterochelin synthetase activity, associated with low levels of enterochelin in culture fluids, may reflect normal

end-product inhibition of enterochelin synthetase The high levels of enterochelin found in culture fluids of Fep$^-$ strains may result from an uninhibited enterochelin synthetase, since these mutants are unable to transport the inhibitor (ferric-enterochelin) into the cytoplasm Cells harvested from stationary phase cultures of Fes$^-$ strains appear to contain high intracellular concentrations of ferric-enterochelin (red-cell pellet) [16] yet high levels of enterochelin are found in culture fluids The results of the following experiment seem to provide a basis for resolving this apparent paradox The Fes$^-$ strain AN273 was grown in a minimal medium supplemented with 10 μM ferric-enterochelin and 50 μM $FeSO_4$, and samples of culture removed at different stages of growth After centrifugation of these samples, it became apparent that cells had not acquired pink or red colouration until the culture reached stationary phase (unpublished data) This suggests, that at least under these growth conditions, Fes$^-$ cells do not accumulate high intracellular concentrations of ferric-enterochelin until cultures reach stationary phase The intracellular levels of ferric-enterochelin present during logarithmic growth may not be sufficient to inhibit enterochelin synthetase activity Further evidence supporting this proposal is provided by the results of iron-uprake studies conducted by Langman et al [16] These workers have shown, that although the initial rate of ferric-enterochelin uptake by the Fes$^-$ strain is comparable with that of the parent strain, iron uptake virtually ceases after 10 min, whereas it continues for at least an hour in the wild-type strain

Inhibition of the partial reaction catalyzed by F component, by ferric-enterochelin and/or its breakdown products, would thus appear to provide a mechanism by which the activity of existing enzyme complexes may be modified When cells are transporting adequate quantities of ferric-enterochelin, synthesis of the ligand may be inhibited, cells not transporting sufficient ferric-enterochelin may compensate through increased synthesis of the ligand from less inhibited enzymatic apparatus

As mentioned above, synthesis of enzymes concerned with the conversion of chorismate to 2,3-dihydroxybenzoate is markedly less sensitive to the iron status of the cell than is synthesis of enterochelin synthetase components Moreover, we have found that substantial levels of 2,3-dihydroxybenzoate (60–80 μM) are present even in stationary phase culture fluids of strain AN194 grown in media to which 100 μM Fe has been added (unpublished data) These results are consistent with the view that 2,3-dihydroxybenzoate may have a role in the assimilation of iron It may be that this compound is involved in the solubilization and maintenance of iron in solution prior to enterochelin-mediated translocation across the cytoplasmic membrane

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