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Hydroxamate-Mediated Transport of Iron Controlled by ColV Plasmids

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A new high-affinity system for iron transport, associated with the presence of ColV plasmids, has been detected in *Escherichia coli* and partially characterized. The presence of such "iron-transport plasmids" in *E. coli* cells that are defective in enterochelin-mediated transport of iron enabled them to grow in media to which 2,2′-dipyridyl had been added to reduce availability of iron. In addition, the presence of plasmid deoxyribonucleic acid in a mutant defective in enterochelin biosynthesis was associated with a marked increase in the rate of radioactive-iron uptake. Plasmid-determined uptake of iron was distinct from previously recognized systems for iron transport in *E. coli* K-12, and the colicin V molecule appeared not to be directly involved. Hydroxylamine-nitrogen could be detected in cell pellets of $ColV^+$ cultures, and similar material was detected in supernatant fluids of late log- or stationary-phase cultures. The hydroxamate material was not detected in cell pellets or culture supernatants of strains from which plasmids had been eliminated, and a 95% decrease in hydroxamate synthesis was observed when cells were grown in minimal medium containing 2 μM iron.

All living organisms require iron to maintain life processes. Most bacteria face a significant challenge in their attempts to assimilate this mineral nutrient from environments in which the metal is commonly present either in the form of very insoluble colloidal hydroxides (28), or bound to iron-binding compounds of hosts (31). Bacteria appear to rely on specific, high-affinity transport systems for obtaining their iron under such conditions. Integral parts of these systems are iron-binding compounds or siderophores (22), which are commonly classified as either catechols (compounds containing one or more 2,3-dihydroxybenzoyl groups) or hydroxamates (compounds containing one or more oxidized peptide bonds). Synthesis of siderophores by cells increases when levels of available iron in growth media become barely sufficient to sustain maximal growth; synthesis of enzymes and membrane components of transport systems is correspondingly repressed when cells are grown in the presence of adequate levels of iron (16). Three distinct high-affinity systems for iron transport have been recognized in *Escherichia coli*, and work with mutants has established that each is controlled by chromosomal genes (13, 26, 33).

The most thoroughly studied of the *E. coli* iron transport systems is the enterochelin system. Mutants defective in biosynthesis of enterochelin, or in transport or intracellular hydrolysis of ferric enterochelin, are available (26). In contrast with the enterochelin system, each of the other two known high-affinity iron transport systems involves a ligand which is not produced by the cells themselves. Therefore neither citrate-dependent nor ferrichrome-dependent transport occurs when the required ligand is absent from growth media (6, 13, 19).

E. coli cells also possess a low-affinity iron transport system. Strains defective in enterochelin-mediated iron transport are able to grow in media to which sufficient iron has been added. However, growth is inhibited in the presence of iron-binding compounds such as 2,2′-dipyridyl or nitrilotriacetate, compounds which apparently reduce the availability of iron (15).

The ability of *E. coli* cells to obtain sufficient iron has long been recognized as an important factor determining ability to grow in serum (31). In view of a recent report (27) that ColV plasmids can enhance ability of *E. coli* cells to survive in serum, and considering the known interaction between availability of iron and colicin V action (30), a possible relationship between possession of ColV plasmids and enhanced iron transport was investigated. The present communication contains evidence for the existence of an additional high-affinity system for transport of iron in *E. coli* cells that carry certain ColV plasmids. Such plasmids carry genetic information which controls synthesis of hydroxamate-containing compound(s). This material appears to be cell associated, and synthesis of it is reduced when cells are grown in media containing adequate levels of iron.

MATERIALS AND METHODS

Bacterial strains. *E. coli* strains used are described in Table 1. Stock cultures were maintained on nutrient agar slants containing 30 mM glucose, and, in the case of mutants defective in enterochelin-mediated transport of iron, 10 mM sodium citrate.

Chemicals. Whenever possible, chemicals of analytical reagent quality were used. 2,3-Dihydroxybenzoate (DHB) was obtained from the Aldrich Chemical Company. L-[U-^{14}C]serine and $^{55}FeCl_3$ were supplied by The Radiochemical Centre, Amersham, England. Ferric enterochelin was prepared as described previously (12).

Media. The mineral salts mixture used in all minimal media was the medium 56 described by Monod et al. (21), except that ferrous sulfate was normally omitted. When required, 100 μM 2,2'-dipyridyl was included to further reduce the availability of iron. Low-iron minimal medium, which contains about 0.5 μM Fe, was prepared using a previously described Al_2O_3 adsorption method (9). Levels of iron in media were measured by atomic absorption spectroscopy using a Varian Techtron model 63 carbon-rod atomizer. In other cases, either 50 μM ferrous sulfate or 10 mM sodium citrate was included in growth media to increase availability of iron. Thiamine hydrochloride (1 μM) was included in all media, and 30 mM glucose was used as carbon source. When necessary, other supplements were added to the minimal medium: 700 μM L-arginine; 210 μM L-histidine hydrochloride; 320 μM L-leucine; 200 μM L-phenylalanine; 1.45 mM L-proline; 200 μM L-tryptophan; 200 μM L-tyrosine; 100 μM DHB; 10 μM ferric enterochelin; 20 μg of nalidixic acid per ml; 3,600 BAEE units of trypsin (Sigma) per ml. Amino acids, ferrous sulfate, sodium citrate, and 2,2'-dipyridyl were autoclaved with the medium 56, but glucose, thiamine hydrochloride, DHB, ferric enterochelin, and trypsin were sterilized separately and added to media before inoculation (liquid media) or pouring (solid media). Oxoid no. 1 agar (15 g/liter) was used to prepare solid media. Unless otherwise stated, 10 mM citrate was included in the nutrient broth (Oxoid no. 2) used for growth of cells during genetic experiments. When colicin V was required in selective media, ColV$^+$ organisms were spread on the agar surface and incubated overnight at 37°C before being killed with chloroform and washed off the surface.

Growth of cells and preparation of cell extracts. Unless otherwise stated, cells were grown as described previously (11) in appropriately supplemented minimal medium containing 2,2'-dipyridyl. Growth was followed with a Klett-Summerson colorimeter (blue filter). Cells were harvested in early stationary phase (approximately 300 Klett units), and cell extracts were prepared as described previously (11).

Growth responses. The presence of various lesions in the enterochelin system was monitored by examining growth responses to DHB, ferric enterochelin, or citrate, as described previously (20).

TABLE 1. *Strains of E. coli*

Strain	Relevant genetic loci[a]	Plasmid[c]	Plasmid traits[b]	Source or reference
AN194	*thi proC leu trp tonA*			K-12 derivative (15)
AN193	*thi proC leu trp tonA entA*			K-12 derivative (obtained from I. G. Young)
AN90	*thi proC leu trp tonA entD*			K-12 derivative (6)
AN273	*thi proC leu trp tonA fes*			K-12 derivative (15)
AN102	*thi proC leu trp tonA fepB*			K-12 derivative (6)
AN49	*thi his proA argE pheA tyrA trp entF*			K-12 derivative (20)
AN53	*thi entA tonA*$^+$			K-12 derivative (this laboratory)
KH1086	*thi proC leu trp tonA entD*	ColV, I-K94	Tra$^+$ Cva$^+$	ColV$^+$ form of AN90 obtained from K. G. Hardy
711 ColV$^+$	*thi lac pro his trp* Nal^r	pRJ1000	Tra$^+$ Cva$^+$ Hyd$^+$	*E. coli* K-12 obtained from H. Williams Smith
RJ79	*thi proC leu trp tonA*	pRJ1000	Tra$^+$ Cva$^+$ Hyd$^+$	ColV$^+$ form of AN194
RJ80	*thi proC leu trp tonA entA*	pRJ1000	Tra$^+$ Cva$^+$ Hyd$^+$	ColV$^+$ form of AN193
RJ81	*thi proC leu trp tonA entD*	pRJ1000	Tra$^+$ Cva$^+$ Hyd$^+$	ColV$^+$ form of AN90
RJ82	*thi proC leu trp tonA fes*	pRJ1000	Tra$^+$ Cva$^+$ Hyd$^+$	ColV$^+$ form of AN273
RJ83	*thi proC leu trp tonA fepB*	pRJ1000	Tra$^+$ Cva$^+$ Hyd$^+$	ColV$^+$ form of AN102
RJ84	*thi proC leu trp tonA*			ColV$^-$ form of RJ79
RJ85	*thi proC leu trp tonA entA*			ColV$^-$ form of RJ80
RJ86	*thi proC leu trp tonA entD*			ColV$^-$ form of RJ81
RJ87	*thi entA tonA*$^+$ ColV^r			ColV^r form of AN53
RJ88	*thi proC leu trp tonA entD* ColV^r			ColV^r form of AN90
RJ89		pRJ1001	Cva$^+$ Hyd$^+$	Clinical isolates; obtained from V. Petrocheilou
RJ90		pRJ1002	Cva$^+$ Hyd$^+$	
RJ91		pRJ1003	Cva$^+$ Hyd$^+$	

[a] Genetic symbols of strains are as described by Bachmann et al. (3), except for *fepB* (34).

[b] Symbols denoting plasmid traits are those recommended by Novick et al. (23), except for Hyd, which refers to production of hydroxamate.

Measurement of iron uptake. The procedure used to measure uptake of radioactive iron was based on that described by Langman et al. (15), except that strains to be tested were grown overnight in minimal medium containing 10 mM sodium citrate. Cells were collected by filtration (Amicon, pore size 0.22 μm) and washed with 5 ml of sterile phosphate-buffered saline before being suspended in low-iron minimal medium. Inocula were adjusted to ensure that cells, when harvested at 60 to 70 Klett units, had either passed through six mass doublings or ceased to grow owing to iron limitation. Radioactivity was counted in a Packard model 460C liquid scintillation counter using a Triton-xylene scintillation cocktail (1).

Detection of strains able to produce colicin V. The method used to detect production of colicin V was based on a double-layer technique described previously (17). Organisms to be tested were stabbed into diagnostic sensitivity test agar, and after overnight incubation they were chloroformed and overlaid with indicator strain (RJ79 or RJ84). Colicin V production was detected by observing which test organisms inhibited growth of overlaid strain RJ84 but not that of strain RJ79. The nature of the colicin being produced by strain 711 ColV$^+$ and the three clinical isolates was confirmed using the *cvt* indicator strain of Davies and Reeves (8).

Transfer of ColV plasmids. The method used to transfer ColV plasmids was based on a precedure described previously (27). Nutrient broth containing citrate was seeded with prospective donor and recipient strains and incubated at 37°C for 24 h without shaking to allow mating to occur. Samples of the resulting mixed cultures were spread onto minimal medium plates containing colicin V. When strain 711 ColV$^+$ was used as donor, histidine was omitted from selective media as a contraselection. When wild-type isolates of *E. coli* able to produce colicin V were used as donors, strains resistant to nalidixic acid were used as recipients, and mixed cultures were inoculated onto plates containing colicin V and nalidixic acid. Colonies that grew after incubation were checked to confirm that they were able to produce colicin V and exhibited the amino acid requirements of the recipient organism.

Elimination of ColV plasmids from *E. coli* strains. The sodium dodecyl sulfate method of Tomoeda et al. (29) was employed, except that all media contained citrate. Strains from which ColV plasmids have been eliminated are referred to below as ColV$^-$ strains.

Isolation of colicin V-resistant (ColV^r) derivatives of strains AN53 and AN90. Solid minimal media containing enterochelin, 2,2′-dipyridyl, and colicin V were inoculated with 0.1-ml samples of stationary-phase broth cultures of the strains from which ColV^r mutants were sought. Colonies that grew after incubation were purified and checked for resistance to colicin V and for growth response to enterochelin. Organisms acquiring resistance to colicin V through a mutation of the *tonB* gene would not grow on such a selective medium.

Isolation of nalidixic acid-resistant (Nal^r) derivatives of strains. Nutrient agar containing 20 μg of nalidixic acid per ml plus citrate was inoculated with 0.1-ml samples of stationary-phase broth cultures of strains from which Nal^r mutants were sought. Colonies that grew after incubation were purified and checked for resistance to nalidixic acid.

Estimation of total 2,3-dihydroxybenzoyl group concentration in supernatant fluid. Supernatant fluid (4 ml) was acidified to approximately pH 1 with 0.8 ml of 1 M HCl and extracted with an equal volume of diethyl ether. The organic phase was dried over anhydrous sodium sulfate, and the concentration of 2,3-dihydroxybenzoyl groups in the diethyl ether was determined spectrophotometrically ($\epsilon_{mM}^{315\ nm}$ = 3.05). All spectra were measured from 230 nm to 350 nm on a Perkin-Elmer 402 UV-visible spectrophotometer.

Identification of compounds containing 2,3-dihydroxybenzoyl groups. DHB and its seryl derivatives were identified by means of a two-dimensional chromatography system described previously (24).

Biochemical assays. Assay conditions for enterochelin synthetase have been described previously (11). Protein was estimated by the method of Lowry et al. (18) with bovine serum albumin (fraction V) as the standard. The method used to estimate bound hydroxamic acid was that described by Csáky (7) using a modification (10) of the hydrolysis conditions. The concentration of hydroxylamine-nitrogen groups was estimated using a standard curve prepared from known quantities of hydroxylamine-hydrochloride. Sodium nitrite (15 mM) did not interfere with this assay for hydroxamate.

RESULTS

Growth of ColV$^+$ and ColV$^-$ forms of *E. coli* iron transport mutants. ColV$^+$ transconjugants of four strains defective in enterochelin-mediated transport of iron (Table 1) and of an otherwise isogenic strain able to transport iron via the enterochelin-dependent system were obtained by matings of these strains with strain 711 ColV$^+$. Ten putative transconjugant colonies from each transfer experiment were purified and tested for ability to produce colicin V and for ability to grow in the presence of 2,2′-dipyridyl. All (40/40) ColV$^+$ transconjugants were found to grow in the presence of 2,2′-dipyridyl. These results indicated that determinants of a high-affinity system for transport of iron may be associated with the ColV plasmid pRJ1000. Two ColV$^+$ transconjugants from each of the above transfer experiments were treated with sodium dodecyl sulfate. Three colonies from each elimination experiment were purified and tested for ability to produce colicin V and for ability to grow in the presence of 2,2′-dipyridyl. Growth of all (24/24) ColV$^-$ isolates tested was inhibited in the presence of 2,2′-dipyridyl unless growth factors required by the original recipients were supplied. Thus it appears that the ability of the ColV$^+$ derivatives of mutants affected in synthesis or utilization of enterochelin to grow under conditions of iron limitation (presence of 2,2′-

dipyridyl) is dependent on the presence of the ColV plasmid. Such growth responses suggest that pRJ1000 carries one or more genes which specify a high-affinity system for transport of iron.

Iron uptake by ColV$^+$ and ColV$^-$ forms of the Ent$^-$ strain AN193. Direct evidence for a plasmid-determined system for iron transport was obtained when the rates of iron uptake by the Ent$^-$ strains RJ80 and RJ85 were compared (Fig. 1). Before testing, both strains were grown in low-iron minimal media to ensure that synthesis of components of the high-affinity systems for iron transport was derepressed. Nitrilotriacetate was included in the uptake mixture to reduce the low-affinity uptake of iron to an acceptable background level (15), as shown by strain RJ85. The presence of pRJ1000 in strain RJ80 is associated with a rapid uptake of radioactive iron under the same growth and uptake conditions.

Relationship between the plasmid-controlled iron transport system and previously recognized transport systems. ColV$^+$ transconjugants of strains AN193 and AN90, and ColV$^-$ derivatives of these, were studied further. Concentrations of 2,3-dihydroxybenzoyl groups in supernatant fluids were estimated after strains were grown in minimal medium containing 2,2′-dipyridyl (Table 2). Neither the ColV$^+$ form of strain AN193, nor its ColV$^-$ derivative, produced DHB or enterochelin. Although both the ColV$^+$ form of strain AN90 and its ColV$^-$ derivative were able to synthesize DHB, neither produced detectable levels of enterochelin or its breakdown products. Levels of enterochelin synthetase activity in cell extracts prepared from ColV$^+$ and ColV$^-$ strain AN90 were found to be negligible unless active D component (present in cell extract prepared from strain AN49) was added to reaction mixtures (data not shown). These results indicate that pRJ1000 does not promote transport of iron via the enterochelin system.

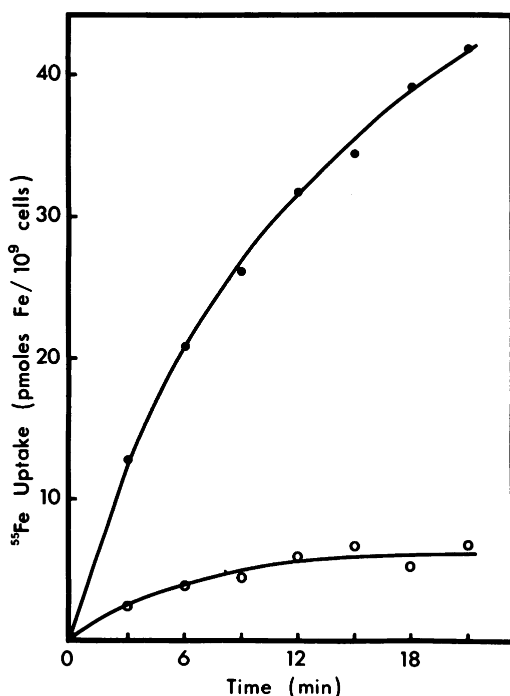

FIG. 1. *Iron uptake by ColV$^+$ and ColV$^-$ forms of the Ent$^-$ strain AN193. Strain RJ80 (Cva$^+$, ●) and strain RJ85 (Cva$^-$, ○) were grown in low-iron minimal media. Uptake of 1 μM ^{55}Fe by nongrowing cells was measured in the presence of 100 μM sodium nitrilotriacetate. Each point represents the average of three independent determinations.*

The possibility that pRJ1000 promotes iron transport via some other chromosome-determined system, such as the citrate-dependent system or the ferrichrome-dependent system, was investigated in the following way. Two stains blocked in the conversion of chorismate to DHB, the ColV$^+$ EntA$^-$ strain RJ80 and the ColV^r EntA$^-$ TonA$^+$ strain RJ87, were streaked approximately 2 mm apart on solid minimal medium containing 2,2′-dipyridyl. Although strain RJ80 grew, it failed to cross-feed strain RJ87. In a control experiment, strain RJ80 successfully cross-fed the ColV^r strain RJ88 after incubation for 32 h. Presumably DHB produced by strain RJ88 (*entD*) was converted by strain RJ80 to enterochelin, which was then excreted. These results suggest that pRJ1000 does not promote iron transport by specifying the synthesis of a diffusible iron transport compound that

TABLE 2. *Presence of DHB and its seryl derivatives in supernatant fluids*[a]

Strain	Concn of DHB groups in supernatant fluid (μM)[b]	Compound detected[c]	
		DHB	Enterochelin
RJ80	<0.1	−	−
RJ85	<0.1	−	−
RJ81	30.3	+	−
RJ86	18.3	+	−

[a] Cells were grown in 50-ml volumes in 250-ml sidearm flasks to stationary phase (approximately 300 Klett units) in minimal medium containing 2,2′-dipyridyl. Media for strains RJ85 and RJ86 were supplemented with 10 μM enterochelin.

[b] Estimated as described in the text.

[c] The presence (+) of DHB, its seryl derivatives, or both was detected by two-dimensional chromatography as described in the text.

can be utilized via some chromosome-determined system in *E. coli* K-12. It therefore appears that pRJ1000 promotes transport of iron via a novel high-affinity system.

Colicin V production and iron transport. The relationship between colicin V production and transport of iron was investigated by growing ColV$^+$ transconjugants of *ent, fes, or fep* strains on solid minimal medium containing 2,2′-dipyridyl and trypsin. Even though colicin V appeared to be inactivated by the trypsin (as judged from the uninhibited growth of indicator strains subsequently overlaid in soft agar), the ColV$^+$ transconjugants grew well. This result suggests that the colicin V molecule itself is not required for growth of such strains on solid medium containing 2,2′-dipyridyl.

Production of a hydroxamate compound by strains harboring pRJ1000. Since no production of phenolic iron transport compounds attributable to the presence of pRJ1000 could be detected, supernatant fluids obtained from cultures of ColV$^+$ transconjugants were tested for the presence of hydroxamate. Hydroxylamine-nitrogen was detected in both supernatant fluid and cell pellets of the ColV$^+$ strain RJ79. In contrast, detectable hydroxamate was not produced by the ColV$^-$ strain RJ84 (Table 3). When other ColV$^+$ transconjugants (and their ColV$^-$ forms) were tested similarly, the same pattern was observed. These data suggest that pRJ1000 carries on it information specifying production of a hydroxamate compound which may be involved in iron transport.

Relationship between production of hydroxamate and iron transport. The iron-binding sites of many siderophores contain one or more hydroxamate groups (22). The possible involvement of the hydroxamate material in iron transport was investigated by observing the effects on its synthesis of including either iron or citrate in low-iron growth media. In the presence of 2 μM Fe or 10 mM added citrate, strain RJ79 produced less than 0.1 μM hydroxylamine-nitrogen; in the absence of such additions, 52 μM hydroxylamine-nitrogen was produced. Thus the inclusion of either of these substances in growth media led to a complete inhibition of the production of detectable hydroxamate.

TABLE 3. *Presence of hydroxamate in cell pellets and supernatant fluids*

Strain[a]	Hydroxylamine-nitrogen (μM)[b]	
	Supernatant fluid	Cell pellet
RJ79	27.1	40.4
RJ84	<0.1	<0.1

[a] Strains RJ79 and RJ84 were grown to stationary phase (approximately 300 Klett units) in minimal medium containing 2,2′-dipyridyl.

[b] Cell pellets obtained after centrifugation of 5 ml of stationary-phase culture were made up to 1 ml with distilled water; 1-ml volumes of supernatant fluid were assayed. The amount of hydroxamate was determined as described in the text.

Preliminary characterization of the hydroxamate material. The detection of hydroxamate material associated with cells of strains carrying pRJ1000 (Table 3) suggested that, unlike enterochelin, this material may be cell associated rather than soluble material excreted by cells into growth media. Further support for such a view was obtained when the ColV$^+$ strain RJ79 was grown in minimal medium containing 2,2′-dipyridyl, and the production of phenolic compounds and hydroxamate in both cell pellets and supernatant fluid was monitored (Fig. 2). Cell-associated hydroxamate and supernatant phenolic compounds (shown by thin-layer chromatography to be enterochelin and its breakdown products) were detected early in the logarithmic phase of growth. In contrast, hydroxamate was not detected in supernatant fluid until late logarithmic phase. It is of interest that whereas the level of enterochelin (and its breakdown products) in supernatant fluids remained constant throughout stationary phase, the amount of cell-associated hydroxamate decreased and the amount of hydroxamate in supernatant fluid increased. A similar pattern of hydroxamate production relative to the growth curve was observed when the Ent$^-$ Hyd$^+$ strain RJ80 was tested (results not shown).

Hydroxamate production and colicin V production. The possibility that a more widespread association exists between ability to synthesize hydroxamate iron transport material, and production of colicin V, was investigated as follows. Three ColV plasmids (pR1001, pRJ 1002, and pRJ1003), originally detected in independent clinical isolates from blood cultures of patients with septicemia, were transferred into a Nal^r derivative of strain AN193. ColV$^+$ transconjugants obtained from each transfer experiment were found to grow on minimal medium containing 2,2′-dipyridyl, to produce hydroxamate material (synthesis of which was repressed in cells grown in medium where iron was readily available), and to be unable to cross-feed the ColV^r strain RJ87. In control experiments, ColV$^+$ transconjugants of a Nal^r derivative of strain AN90 were able to cross-feed strain RJ87, as described above. In contrast, a ColV$^+$ transconjugant obtained after transfer of plasmid ColV, I-K94 (4) into AN90 was unable to grow

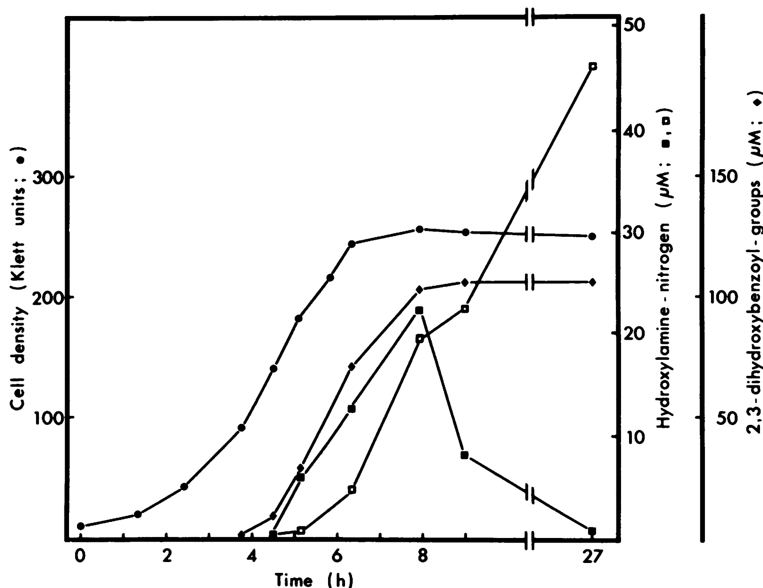

FIG. 2. *Relationship between growth, production of hydroxamate, and producton of compounds containing DHB by strain RJ79. Cells were grown in minimal medium containing 2,2′-dipyridyl. Culture growth (●) was measured by means of a Klett-Summerson colorimeter. Samples (5 ml) of culture were centrifuged, and the amount of hydroxamate (□) and the total 2,3-dihydroxybenzoyl group concentration (◆) in supernatant fluid were measured. Cell pellets were suspended in distilled water (1 ml) and assayed for hydroxamate (■). Growth of cells and assay conditions were as described in the text.*

on media containing 2,2′-dipyridyl or to produce hydroxylamine material.

DISCUSSION

A new high-affinity system for transport of iron by *E. coli* cells has been recognized upon transfer of ColV plasmids into strains defective in synthesis or utilization of enterochelin. Before receipt of plasmids, such strains were unable to grow on minimal medium containing 2,2′-dipyridyl, since the enterochelin-dependent system had been made inoperative by mutation, and neither the citrate-dependent system nor the ferrichrome-dependent system was functional because the respective ligands were not provided. The presence of 2,2′-dipyridyl prevented transport of adequate quantities of iron via the low-affinity uptake system. Transfer of ColV plasmids into these strains enabled growth to occur, and, in the case of pRJ1000, elimination of the plasmid was found to result in loss of this capability. The ability of certain ColV plasmids to stimulate growth of host organisms under such conditions is attributed to a plasmid-controlled high-affinity system for transport of iron. Further evidence for such an iron transport system was obtained from measurements of radioactive iron uptake by $ColV^+$ and $ColV^-$ forms of strain AN193. In contrast with the slow rate of iron uptake by cells of the Ent^- $ColV^-$ strain RJ85, cells of the Ent^- $ColV^+$ strain RJ80 took up iron rapidly.

The plasmid-determined system for iron utilization does not appear to involve any of the previously recognized chromosome-determined systems. Neither synthesis nor utilization of enterochelin was affected by the presence of plasmid DNA in the $ColV^+$ transconjugants studied. Failure to detect a diffusible growth factor is evidence that plasmid genes are not merely enabling synthesis of ligands, such as ferrichrome or citrate, required for the operation of other (chromosome-determined) systems for iron transport. It seems that genetic information carried by pRJ1000 codes for a completely distinct high-affinity system for iron transport.

Production of hydroxamate material by cells harboring a ColV plasmid suggests an involvement of this material in transport of iron. The finding that the presence of 2 μM Fe leads to a greater than 95% reduction in the synthesis of hydroxamate provides further evidence that this material is involved in iron transport.

We propose that plasmid-determined hydroxamate normally associates with the cell envelope and that it may constitute part of a cell-bound system for transport of iron similar to that reported for schizokinen (2). The detection of hydroxamate in cell pellets of *E. coli* is consistent with such a model. In contrast, the secreted

siderophore, enterochelin, could not be detected in cell pellets of *E. coli* K-12 (K. T. Greenwood, unpublished data). The appearance of cell-associated hydroxamate early in the growth cycle and the similar rates at which this material and supernatant enterochelin are formed suggest that each substance constitutes the physiologically active component of its respective iron transport system. It is of interest that levels of cell-associated hydroxamate decrease during stationary phase, and that such loss coincides with an increase in levels of hydroxamate detectable in culture supernatants obtained after centrifugation. This latter material may represent material lost from the surface of cells. The molecular structure(s) of the hydroxamate compound(s) and its functions(s) in iron transport are being investigated in our laboratory.

The recent demonstration by Smith (27) that enhanced virulence is commonly associated with $ColV^+$ forms of *E. coli* strains has stimulated interest in ColV plasmids. The nature and activity of the virulence factor(s) associated with ColV plasmids have not yet been fully characterized, although it is now known that colicin V itself is not necessary for virulence (4, 25).

We feel it is significant that genetic information controlling production of hydroxamate has been found to be associated with the presence of four of the five ColV plasmids studied, and that the Hyd and Cva determinants both appear to be located on pRJ1000. Visual inspection of at least 200 open circular DNA molecules on electron microscope grids did not reveal any that were significantly different in size from the 14 that were measured (molecular weight of pRJ1000 DNA = $101.3 \times 10^6 \pm 1.6 \times 10^6$ [standard deviation]; R. K. J. Luke and K. G. Hardy, unpublished data). We therefore propose that hydroxamate-mediated acquisition of iron contributes to the virulence of $ColV^+$ bacteria by enabling cells to overcome the bacteriostatic effect of serum associated with severe deprivation of iron (5, 14, 31). The cell-bound system for iron acquisition may provide a more efficient mechanism for extracting iron from ligands present in animals than does the enterochelin system.

A previously recognized ColV virulence factor (4) enhances the survival of host bacteria in fresh serum. The mechanism for increased virulence of such colicinogenic strains is presumed to result from resistance to a bactericidal effect of serum dependent on antibody and complement. The determinant for serum resistance (designated *iss*) is closely linked to genes specifying production of colicin V on plasmid ColV, I-K94 (4).

It is now apparent that ColV plasmids may possess determinants for more than one type of virulence factor. In this context, it is of interest that whereas ColV, I-K94 does not produce hydroxamate, genes specifying both *iss* and Hyd are present on pRJ1000 (D. Sugden, K. G. Hardy, and R. K. J. Luke, unpublished data). It seems likely that each determinant contributes separately to the pathogenic potential of strains harboring them.

Since the completion of this work, Williams (32) has reported evidence for enhanced iron uptake in *E. coli* strains carrying other ColV plasmids. The plasmid-determined uptake of iron was shown to operate independently of the enterochelin-dependent system for iron transport. Colicinogenic *E. coli* were able to grow in vivo and to extract iron from transferrin molecules in vitro (32). It seems likely that the plasmid-associated acquisition of iron reported by Williams is mediated via the hydroxamate-dependent system for iron transport now described.

Finally, it is now apparent that hydroxamate production in *E. coli* is not restricted to cells that harbor ColV plasmids. In a recent survey, it was found that a very high proportion of virulent *E. coli* strains (including both $ColV^+$ and $ColV^s$ organisms) were able to produce hydroxamate, and that this iron transport system is widely distributed among *E. coli* isolated from various sources (S. J. Stuart, K. T. Greenwood, and R. K. J. Luke, manuscript in preparation). Whether or not a functional relationship exists between ability to produce colicin V and ability to carry out hydroxamate-mediated transport of iron is currently under investigation in our laboratory.

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