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Acetylhistidine as Substrate for Acetylornithinase: A New System for the Selection of Arginine Regulation Mutants in *Escherichia coli*

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Summary. The utilisation of acetylhistidine by histidine auxotrophs of *E. coli K*-12 was found to require a functioning acetylornithinase. The growth, on acetylhistidine-containing media, of *his* mutants possessing this enzyme was inhibited by arginine or its precursors acetylornithine, ornithine and citrulline. Mutants able to overcome this inhibition belonged to two classes: those (selected on acetylhistidine + acetylornithine or arginine) in which the arginine biosynthetic enzymes were repressible, as in the parent strains; and those (selected on acetylhistidine + acetylornithine, ornithine, citrulline or arginine) in which these enzymes were formed at high, non-repressible levels. The altered properties of the first class were shown genetically not to result from mutation in the *argR* or *argECBH* regions; the data are consistent with the second class carrying mutations at the *argR* locus.

It is supposed that arginine, ornithine or citrulline, by repressing the formation of acetylornithinase, diminish the rate at which acetylhistidine can be utilised (although an acetylhistidine uptake system under arginine control would equally explain the results); non-repressible mutants would escape this effect. The kinetic properties, in crude extracts, of acetylornithinase from the parent strains and from members of each mutant class, with acetylornithine and acetylhistidine as substrates, were investigated. It was tentatively concluded that, in accord with the genetic results, the first class do not possess an acetylornithinase altered to make it function better with acetylhistidine as substrate. It is suggested that arginine may affect acetylhistidine utilisation by affecting its uptake in a manner not shared with ornithine or citrulline, as well as by repressing proteins of the arginine system, and that this arginine-specific effect is inoperative in the first class of mutants. The nature of the changes leading to ability to grow on acetylhistidine + acetylornithine remains unknown. Possible applications of these findings to the selection of hitherto undiscovered but potentially informative mutant types are discussed.

Introduction

The functional organisation of the *argECBH* cluster in *E. coli* K-12 is still uncertain, especially the relationship of *argE* to the other three genes. Evidence has been obtained (Elseviers, Cunin and Glansdorff, 1969; Cunin, Elseviers, Sand, Freundlich and Glansdorff, in press; Baumberg and Ashcroft, unpublished results) suggesting that *argE* constitutes a single-gene operon unconnected to the *argCBH* operon with which, however, it is probably contiguous (Glansdorff, 1965).

I have investigated the substrate specificity *in vivo* of acetylornithinase, the enzyme coded for by *argE*, in the hope of finding methods for selecting mutants altered in the regulation solely or primarily of *argE*. These studies also relate to others aimed at distinguishing between acetylornithinase and

other *E. coli* enzymes involved in the hydrolysis of N^{α}- formyl- and -acetyl-amino-acids and -peptides (Weissbach and Redfield, 1967; Fry and Lamborg, 1967; Adams, 1968).

Materials and Methods

Strains. The bacterial strains used are listed in Table 1. Genotype symbols are taken from Taylor and Trotter (1967).

Table 1. *Partial description of E. coli K-12 strains used*

Strain	Nutritional markers[a]										
	his	*ile*	*metF*	*pro*	*thi*	*ppc*	*asp*	*argC*	*argE*	*argG*	*argH*
6P	−	−	−	−	+	+	+	+	+	+	+
34X	−	+	+	+	−	+	−	+	+	+	+
6G	−	−	−	+	+	−	+	+	+	+	+
6E69	−	−	−	+	+	+	+	+	−	+	+
6PC56	−	−	−	−	+	+	+	−	+	+	+
6H50	−	−	−	+	+	+	+	+	+	+	−
HfrH	+	+	+	+	+	+	+	+	+	+	+
34XH14	−	+	+	+	−	+	−	+	+	+	−
34G7	−	+	+	+	−	+	−	+	+	−	+

[a] Mutant phenotypes corresponding to "−" alleles in this table are: *his*, *ile*, *metF*, *pro*, *thi*, *asp* — requirement for histidine, isoleucine, methionine, proline, thiamine and aspartate, respectively; *ppc* — absence of phosphoenolpyruvate carboxylase and requirement for glutamate, aspartate or succinate; *argC*, *E*, *G* and *H* — absence of *N*-acetylglutamic γ-semialdehyde dehydrogenase, acetylornithinase, argininosuccinate synthetase, and argininosuccinase respectively, and requirement for arginine (*argC* strains also respond to acetylornithine, ornithine or citrulline, and *argE* strains to ornithine or citrulline).

6P is a *proA* or *B* mutant isolated from strain 619 (Baumberg, Bacon and Vogel, 1965); 6PC56 is an *argC* mutant of 6P. 34X is a T6-resistant mutant of strain PA3402, kindly provided by N. Glansdorff; 34XH14 and 34XG7 are respectively *argH* and *argG* mutants of 34X. 6G was isolated among *arg*E$^+$ transductants of strain 6196 (Baumberg *et al.*, 1965) with Plkc grown on PA214 (Glansdorff, 1965). 6E69 and 6H50 were isolated among *ppc*$^+$ transductants of 6G with Plkc grown respectively on AB1469 (Pittard, Loutit and Adelberg, 1963) and a *met*$^+$ transductant of AB1450 (Pittard *et al.*, 1963). Hfr H was kindly provided by W. Hayes.

Media. The minimal salts medium used was medium A of Davis and Mingioli (1950). The carbon source was glucose at a concentration of 0.5%, except for *ppc* strains, where sodium succinate at 0.5% was used. The final concentration of amino-acids, arginine precursors, and acetylhistidine was 100 µg/ml (of the L-form), of thiamine 10 µg/ml, and of aspartate (added as aspartic acid adjusted to pH 7 with NaOH) 200 µg/ml. Required supplements other than histidine may be assumed to have been always present unless otherwise noted. Media contained either histidine or acetylhistidine, as indicated.

L-broth (Lennox, 1955) was used in transduction experiments.

Maintenance and Growth of Cultures. Colonies from platings of the parent strains 6P and 34X on acetylhistidine media, and mutant colonies from platings of these strains on acetylhistidine + arginine or a precursor, were purified by streaking twice on non-selective media. Single colonies were then streaked on various media to test growth responses, and were also transferred to L-broth and to liquid histidine media. A loop from an L-broth culture served as inoculum for growth of cultures (usually 10 ml) for enzyme assays; the liquid histidine media cultures were centrifuged, washed with and finally resuspended in medium A, and these suspensions inoculated into liquid media for determination of growth

curves. Bacterial growth was followed as optical density (OD) at 660 mμ in a Unicam SP500 spectrophotometer, a correction being applied for the non-linear relation between OD and bacterial mass for OD's above about 0.3.

Enzyme Assays. In general, assays were performed on cells made permeable with toluene. The harvested cells were suspended in 1 ml potassium phosphate buffer, pH 7, containing reduced glutathione at 10^{-3} M and EDTA at 10^{-4} M (KPGE buffer); 0.1 ml toluene was added, and the mixture incubated at 37° for 30 min with occasional agitation. It was then centrifuged and the bacterial pellet resuspended in 1 ml KPGE buffer. This, or suitable dilutions, was used in the enzyme assays; the OD at 660 mμ of a suitable dilution (reading less than 0.2) was taken as a measure of bacterial mass.

In certain experiments, cells were broken by means of an MSE 100 watt ultrasonic disintegrator operating at maximum amplitude for 15 sec. The extracts were centrifuged at 38,000 g for 15 min, and the supernatants dialysed overnight against a 50-fold excess of KPGE buffer.

Acetylornithinase was assayed by the method of Vogel and Bonner (1956), with slight modifications. Acetylornithine δ-transaminase was assayed by the method of Albrecht and Vogel (1964). The ability of cell extracts to hydrolyze acetylhistidine to histidine was measured in an assay mixture identical to that for acetylornithinase assay except in that acetylornithine was replaced by acetylhistidine (in amounts indicated, adjusted to pH 7). The reaction was stopped by placing the tubes (4 in. by $^1/_2$ in. medium-walled) in a boiling-water bath. The ensuing colorimetry followed the method of Satake, Okuyama, Ohashi and Shinoda (1960): 1 ml 4% $NaHCO_3$ solution and 1 ml 0.1% aqueous picryl sulphonic acid (2,4,6-trinitrobenzene-1-sulphonic acid) were added to each tube, and the tubes were incubated in the dark at 37° for 2 hrs. 1 ml 1 N HCl was then added, the solutions clarified by centrifugation if necessary, and their OD's at 400 mμ read.

Enzyme activities of toluenised suspensions were calculated as μmole product formed/ml cell suspension/hr. This was divided by the OD of the cell suspension to give a value (referred to as 'specific activity') measuring activity per unit cell mass.

Transduction. This was carried out with phage Plkc according to Siegel and Bryson (1967). Transductants were streaked on the same medium as used for their selection, and unselected markers scored by replica plating.

Chemicals. Acetylornithine (N^{α}-acetyl-L-ornithine) was obtained from Cyclo Chemical Corp., Los Angeles, or was prepared by the technique of Thompson and Gering (1962). Acetylhistidine (N^{α}-acetyl-L-histine monohydrate) was obtained from Cyclo Chemical Corp. Picryl sulphonic acid was obtained from Sigma London Chemical Co. Ltd.

Results

I. Growth of Histidine Auxotrophs by Use of Acetylhistidine

The growth of the histidine auxotroph 6P in liquid medium containing histidine or acetylhistidine is demonstrated in Fig. 1. It is seen that 6P grew as well on acetylhistidine media as on histidine media in the absence of arginine or one of its precursors acetylornithine, ornithine and citrulline, but that arginine (most pronouncedly) and ornithine and citrulline (slightly less so) caused at first slowing-down, then cessation, of growth. The effect of acetylornithine is variable; in the experiment shown, it acted comparably to ornithine or citrulline, but in some other experiments, inhibition was slight or absent.

The inhibiting effect could be decreased or increased by raising or lowering respectively the concentration of acethylhistidine.

On solid media, 6P cells formed colonies with 100% efficiency on acetyl-histidine medium in the absence of arginine or a precursor. In the presence of ornithine or citrulline, one cell in $5\times10^5-1\times10^8$ gave rise to a colony, which usually grew at the same rate as on histidine; in the presence of arginine, one

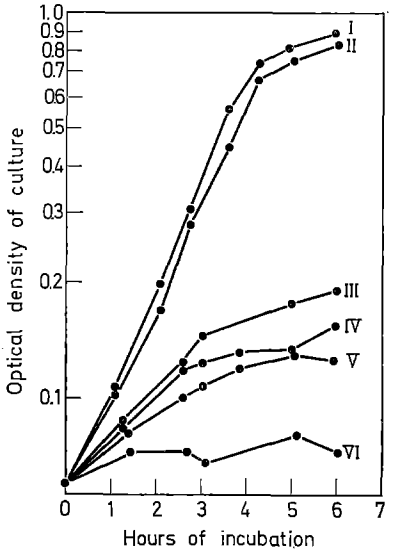

Fig. 1. Growth curves of strain 6P in various histidine and acetylhistidine media. (*i*) Histidine medium — curves obtained for media with or without arginine or a precursor are almost superimposable; (*ii*) acetylhistidine without arginine or a precursor; (*iii*), (*iv*), (*v*), (*vi*) acetylhistidine + acetylornithine, ornithine, citrulline, and arginine respectively

cell in $2\times10^6-1\times10^9$ gave rise to a colony, but this took one or two days longer than on acetylhistidine + ornithine to reach a comparable size. In the presence of acetylornithine, the fraction of cells giving rise to colonies was one in 10^5 or more, and was highly variable but seemed to increase with inoculum size; the colonies grew at widely differing rates, some of them at the same rate as on histidine.

Similar results, both in liquid and on solid media, were obtained with strain 34X.

II. Regulation Characteristics of Mutants Able to Use Acetylhistidine in the Presence of Arginine or its Precursors

A number of independent spontaneous isolates from platings of 6P and 34X on acetylhistidine + arginine or a precursor, were examined for regulation of synthesis of two of the arginine biosynthetic enzymes, acetylornithinase (coded for by *argE*) and acetylornithine δ-transaminase (coded for by *argD*, which is not linked to the *argECBH* cluster). The isolates were grown up in histidine media with and without arginine, and toluenised suspensions of the cells were assayed for these two enzymes (see Materials and Methods). Table 2 shows specific activities and ratios of these between cultures grown without and with arginine from a particular isolate, for the various isolate classes found (isolates are divided into these classes on the basis firstly of the selecting medium and secondly — where two types with different regulation characteristics could be isolated on the same medium — on the basis of these characteristics).

Table 2. *Specific activities of acetylornithinase and acetylornithine δ-transaminase in cultures of isolates, selected on acetylhistidine + arginine or a precursor, grown in histidine media without and with arginine*

Class	Supplement to acetylhistidine medium on which class was selected	No. of isolates tested	Enzyme	Specific activity in culture grown on histidine medium a) without arginine	b) with arginine	Lowest and highest values of individual ratios of activities, (a)/(b)	Ratio of mean activities
			(i) Isolates obtained from strain 34X				
A (control)	None	5	AcOase[a]	4.9 (4.4—5.8)[b]	1.0 (0.95—1.3)	3.9—6.0[c]	4.7
			Tase	0.97 (0.82—1.3)	0.22 (0.17—0.25)	4.1—6.1	4.4
B	Acetylornithine	6	AcOase	5.8 (3.8—8.0)	2.3 (1.4—3.0)	2.2—3.8	2.5
			Tase	0.95 (0.81—1.3)	0.37 (0.24—0.52)	1.8—5.3	2.6
C	Acetylornithine	3	AcOase	18 (10—24)	20 (12—24)	0.8—1.0	0.9
			Tase	1.9 (1.3—2.3)	2.0 (1.8—2.3)	0.5—1.3	1.0
D	Ornithine	8	AcOase	21 (7.6—34)	19 (6.0—26)	0.5—1.5	1.1
			Tase	2.0 (1.5—3.1)	2.7 (0.84—4.9)	0.5—1.8	0.8
E	Citrulline	3	AcOase	24 (22—28)	22 (19—24)	1.1—1.2	1.1
			Tase	1.8 (1.5—2.3)	2.0 (1.6—2.5)	0.6—1.3	0.9
F	Arginine	10	AcOase	5.6 (4.7—7.1)	1.1 (0.69—1.4)	3.3—9.5	5.2
			Tase	1.1 (0.67—2.1)	0.23 (0.15—0.35)	3.7—10.2	4.8
			(ii) isolates obtained from strain 6P)				
A (control)	None	10	AcOase	15 (13—16)	2.5 (1.7—3.1)	5.5—7.8	6.0
			Tase	2.1 (1.5—2.4)	0.16 (0.10—0.22)	11—18	13
B	Acetylornithine	5	AcOase	15 (11—18)	2.2 (1.2—2.7)	6.3—9.0	6.8
			Tase	2.1 (1.5—2.3)	0.18 (0.10—0.34)	4.4—23	12
D	Ornithine	10	AcOase	32 (26—41)	30 (22—35)	0.7—1.4	1.1
			Tase	2.8 (2.0—3.3)	3.1 (2.4—4.1)	0.8—1.4	0.9
E	Citrulline	5	AcOase	30 (24—37)	28 (15—45)	0.6—2.1	1.1
			Tase	2.9 (2.4—3.4)	2.5 (2.2—2.8)	0.9—1.2	1.2
F	Arginine	26	AcOase	13 (9—16)	1.9 (0.74—2.2)	7.3—13	6.8
			Tase	1.7 (1.1—2.0)	0.19 (0.14—0.33)	7.8—14	8.9
G	Arginine	5	AcOase	39 (36—47)	23 (16—27)	1.4—2.3	1.7
			Tase	2.5 (1.9—3.2)	2.8 (2.1—3.4)	0.9—1.1	0.9

[a] Abbreviations used: AcOase = acetylornithinase; Tase = acetylornithine δ-transaminase.

[b] The mean of the activities of the whole class is given first, followed (in brackets) by the lowest and highest individual activities.

[c] The ratios of each pair of activities (corresponding to growth without and with arginine) was calculated for a given isolate, and the lowest and highest ratios for the class are shown in this table.

It is seen that there are two groups of classes: those in which acetylornithinase and acetylornithine δ-transaminase activities are reduced by a factor of more than two when the isolate was grown in histidine medium with arginine (class A, parental type, and mutant classes B and F); and those in which the reduction in these activities is always less than two-fold — they are then almost the same whether the cultures were grown with arginine or without it (mutant classes C, D, E and G). The latter behaviour closely resembles that of non-repressible mutants, with mutations mapping at the *argR* Locus, found among canavanine-resistant mutants (Maas, 1961) and among isolates growing without proline in the presence of arginine, from *argD proA* or *B* strains (Bacon and Vogel, 1963; Itikawa, Baumberg and Vogel, 1968). In two cases, the regulation pattern may possibly be intermediate. Class B isolates derived from strain 34X (but not from 6P) show reproducibly higher levels of acetylornithinase and acetylornithine δ-transaminase in arginine-grown cultures than do class A or F isolates; and class G isolates show a slight reduction in acetylornithinase level in arginine-grown cultures. In addition, no class C isolates were obtained from 6P, nor class G isolates from 34X. In view of the variation in enzyme activities found in different experiments with the same isolate, and of the small numbers of isolates tested, the significance of these deviations is uncertain.

It may be noted that the results shown in Table 2 make it unlikely that any of the mutants are defective in the uptake of arginine, ornithine or citrulline; such mutations would lead to enzyme levels in cultures grown without arginine being of the order of those in class A (parental type) isolates, and in cultures grown with arginine, lying between those in class A isolates grown with arginine and those in class A isolates grown without arginine. Such a pattern is nowhere apparent.

III. Growth Characteristics of Mutants Utilising Acetylhistidine in the Presence of Arginine or its Precursors

The growth responses shown by the various isolate classes when streaked on acetylhistidine media are shown in Table 3. Growth in corresponding liquid media was in good agreement with these data. As might be expected, the isolates were better able than wild-type to grow on the medium on which they had been selected; additional interesting features are the increased ability of class F (and, very occasionally, class B) mutants to utilise acetylhistidine in the presence of ornithine or citrulline, and of class C, D, E and G mutants to utilise it in the presence of arginine; I shall discuss these points later.

IV. Requirement of Functioning Acetylornithinase for Growth on Acetylhistidine

The above results suggest that the utilisation of acetylhistidine by a histidine auxotroph involves some component whose effectiveness is lessened by arginine and its precursors. The growth of *argD proA* or *B* strains in the absence of proline depends on a functioning acetylornithinase, and is also inhibited by arginine if the strain is $argR^+$, but not if it is *argR* (Itikawa *et al.*, 1968); the similarity to the observations on acetylhistidine utilisation suggests that acetylhistidine may be de-acetylated by acetylornithinase.

Table 3. *Growth of mutants utilising acetylhistidine in the presence of arginine or its precursors, when streaked on solid acetylhistidine media*

Isolate class	Acetylhistidine medium supplemented with			
	Nothing	Acetyl-ornithine	Ornithine or citrulline	Arginine
A (control)	++[a]	++ to very slight, not increasing after 1 day[b]	−	−
B	++	++	± to −	−
C	++	++	++ to ±	± to −
D	++	++	++ to ±	± to −
E	++	++	++ to ±	± to −
F	++	++ to +	+ to ±	+ to ±
G	++	++	++ to ±	± to −

[a] ++ = indicates good growth in 1 day; + = indicates good growth in 2 days; ± = indicates good growth in 3 days or more.

[b] In all cases where the growth response was variable, considerable dependence on inoculum size was noted.

To test this hypothesis, strains were constructed that carried *argE*, *argC* or *argH* mutations (the latter two as controls to ensure that any effect observed was specific to an *argE* mutation and not dependent on a general block in arginine synthesis), in a background permitting utilisation of acetylhistidine in the presence of ornithine or, to a lesser extent, arginine. From the plating of strain 6G on acetylhistidine + ornithine medium, a typical class D isolate (as regards regulation and growth characteristics) was obtained. This strain was then transduced to succinate independence with Plkc grown on 6E69 (*argE*), 6PC56 (*argC*), or 6H50 (*argH*), and *ppc*$^+$ *argE*, *C* or *H* recombinants isolated. The *argC* recombinant grew well on acetylhistidine + ornithine, and it and the *argH* recombinant grew on acetylhistidine + arginine with the typical slow growth of a class D isolate; whereas the *argE* recombinant showed no growth on either medium (the results were the same for liquid and solid media).

Since the mutation associated with class D isolates that permits growth on acetylhistidine + ornithine is located in the *asp-argG* region, far from the *ppc-argECBH* region (see next section), the inability of the *argE* recombinant to utilise acetylhistidine in the presence of ornithine cannot be due to the re-introduction through transduction of sensitivity to ornithine, but must result from the loss of acetylornithinase activity. The latter is therefore probably involved in the hydrolysis of acetylhistidine; direct evidence for this will be given later.

V. Location of Mutations Conferring Ability to Utilise Acetylhistidine in the Presence of Arginine or its Precursors

Transductions with phage Plkc have been carried out with members of the various isolate classes to ascertain whether the mutations involved map in the *argR* or *argECBH* regions. These loci are implicated because of the physiological resemblance of isolate classes C, D, E and G to known *argR* mutants, and

Table 4. *Plkc transductions used to find approximate positions of mutations conferring ability to utilise acetylhistidine in the presence of arginine or its precursors*

Donor	Recipient	No. of isolates tested	Transductant class selected	Unselected donor marker scored	Frequency of unselected donor marker among transductants
HfrH	C(34X)[a]	2	*asp*$^+$	Inability to utilise acetylhistidine in the presence of ornithine	3—6%
HfrH	D(34X)	3	*asp*$^+$		1—9%
HfrH	E(34X)	2	*asp*$^+$		2—10%
HfrH	F(34X)	5	*asp*$^+$		None
F(34X)	34XH14	5	*argH*$^+$	Ability to utilise acetylhistidine in the presence of arginine	None
D(6P)	34XG7	3	*argG*$^+$[b]	Ability to utilise acetylhistidine in the presence of ornithine	2—9%
E(6P)	34XG7	2	*argG*$^+$[b]		6—8%
F(6P)	34XG7	5	*argG*$^+$[b]		None
F(6P)	34XH14	5	*argH*$^+$		None
G(6P)	34XG7	3	*argG*$^+$[b]		3—12%

Transductants were selected for a nutritional marker, and were scored (see Materials and Methods) for ability to utilise acetylhistidine in the presence of ornithine or arginine, as indicated. The final column shows the lowest and highest proportions, among the isolates tested, of transductants inheriting the acetylhistidine-utilisation characteristics of the donor. At least 100 transductants from each cross were tested.

[a] This denotes: class C isolates obtained from strain 34X, and similarly in other cases.

[b] It was noted that only a very small proportion of *asp*$^+$ recombinants were recovered among the *argG*$^+$ recombinants in these crosses. This would be consistent with strain 6P itself carrying a mutation at the *asp* locus, together with a suppressor at some other site, as suggested by Reiner (1969) for certain of his K-12 strains. It is possible that the *asp* allele present in 6P is in part responsible for the Mg^{2+} sensitivity described by Urm, Leisinger, Vogel and Vogel (1969) for strain 619, from which it is derived.

because some mutant phenotypes might result from an altered acetylornithinase due to mutation in *argE*. *ArgR* is not linked to *argECBH*, but is known to be co-transducible with *asp* and *argG* by phage Plkc (Taylor and Trotter, 1967).

The crosses employed are shown in Table 4. Location in the *argR* region was tested by looking for linkage to *asp* or *argG*, and in the *argECBH* region by looking for linkage to *argH*. It is seen that the mutations carried by isolate classes C, D, E and G are linked to *asp* or *argG*, this being consistent with their lying at the *argR* locus. Mutations carried by isolate class F are not linked to *asp* or *argG*, nor to *argH*, this latter suggesting that the properties of isolates in this class do not derive from an altered acetylornithinase. Their positions remain unknown.

It has not been possible to devise a procedure capable of distinguishing unambiguously between the class B and class A (parent strain) phenotypes. Nothing has been discovered, therefore, about the location of mutations carried by class B isolates.

VI. Acetylornithinase-dependent De-acetylation of Acetylhistidine by Crude Extracts

The parent strains 34X and 6P, one class D and one class F isolate derived from each of them (referred to as 34XAHD1, 34XAHF2, 6PAHD1, and

Table 5. *Kinetic characteristics of extracts with acetylornithinase activity in acetylornithinase and acetylhistidine de-acetylating reactions*

Extract	Substrate				Ratio of (V_{max}) acetylornithine to (V_{max}) acetylhistidine
	acetylornithine		acetylhistidine		
	V_{max} (μmole/hr)	K_m (mM)	V_{max} (μmole/hr)	K_m (mM)	
34X	21	10	1.2	210	17
6P	37	14	1.6	150	24
34XAHD1	159	19	5.1	540	31
34XAHF1	11	11	0.6	86	20
6PAHD1	148	17	4.0	450	37
6PAHF1	77	13	2.5	290	31

Assays were carried out as described in Materials and Methods. Amounts of protein in the assay mixtures varied from 0.5 to 4 mg; concentrations of acetylornithine and acetylhistidine were 2 to 100 mM, and 70 to 400 mM, respectively.

6PAHF1 respectively) were grown up in histidine medium without arginine or its precursors, and the 6P-derived class D isolate carrying an *argE* mutation, whose preparation is described in section IV above (referred to as 6PAHDE69) in histidine + ornithine; extracts of the cultures were prepared by ultrasonic disintegration and assayed for acetylornithinase and ability to hydrolyze acetylhistidine, as described in Materials and Methods. In preliminary tests, with assay mixtures containing 0.5—4 mg protein and acetylornithine or acetylhistidine at 6 and 80 mM respectively, the 6PAHDE69 extract showed no detectable acetylornithinase activity nor any ability to de-acetylate acetylhistidine, while the other extracts showed both activities, suggesting that acetylornithinase is primarily responsible for acetylhistidine hydrolysis.

Further experiments permitted the calculation, for each extract, of a K_m for acetylornithine and acetylhistidine, and the ratio of V_{max} for acetylornithine to V_{max} for acetylhistidine. The results are given in Table 5.

It is seen that the K_m's for acetylornithine and acetylhistidine vary from 10 to 19 and 90 to 540 mM respectively, while the V_{max} ratios vary from 17 to 37. These variations are probably not significant, particularly in view of the low rates of acetylhistidine hydrolysis being measured. It would appear, then, that acetylhistidine is hydrolyzed to histidine by acetylornithinase in crude extracts, that the K_m's of acetylornithinase for acetylornithine and acetylhistidine are about 14 and 290 mM respectively, and that the ratio of V_{max} for acetylornithine to V_{max} for acetylhistidine is about 30. It also seems that class D and class F mutants do not contain an acetylornithinase with kinetic properties different (in being, for instance, more favourable for hydrolysis of acetylhistidine) from that of the parent strain.

The kinetic characteristics of acetylornithinase from class B mutants are still being studied.

Discussion

The results presented above are consistent with the supposition that histidine auxotrophs of *E. coli* K-12 can use acetylhistidine only by splitting off its

acetyl group through the agency of acetylornithinase. If this enzyme is lacking, as in an *argE* mutant, acetylhistidine cannot be utilised. In addition, the presence of arginine or a precursor interferes with this utilisation, and I shall now consider how this inhibition comes about.

Arginine and its precursors can act at the level of acetylhistidine uptake, or of its hydrolysis within the cell, or both. It may be pointed out that an effect on the synthesis or function of an acetylhistidine uptake system would be not unexpected if this were the same as (or shared components with) the arginine-repressible acetylornithine uptake system described by Vogel (1960). At either level, the action may be to inhibit the function, or to repress synthesis of the protein(s) required for it (exogenous ornithine and citrulline here being effective by increasing the intracellular arginine concentration). The properties of the mutant classes can then be explained as follows.

Classes C, D, E and G: these non-repressible mutants are insensitive to interference with acetylhistidine utilisation arising via repression, whether this affects primarily synthesis of acetylornithinase or of the uptake system. However, these mutants, though not affected by ornithine or citrulline, retain some sensitivity to arginine. The levels of arginine enzymes in arginine-grown cultures are about half those in ornithine- or citrulline-grown cultures, and this difference, considered with respect to acetylornithinase, seems insufficient to explain the residual arginine sensitivity. Therefore (since arginine does not inhibit acetylornithinase) the specific effect of arginine most probably involves acetylhistidine uptake, by either its inhibition or its repression. If repression is involved, the synthesis of uptake protein(s) must be repressed by arginine far more than by ornithine, i.e. its synthesis is highly non-co-ordinate with that of the biosynthetic enzymes in the region of complete to near-complete repression. The suggestion of an inhibition of acetylhistidine uptake by arginine, on the other hand, requires no such assumptions.

Class F: these mutants may possess an altered uptake mechanism lacking the specific arginine sensitivity. They therefore remain slow-growing on acetylhistidine + arginine, ornithine or citrulline due to repression of synthesis of acetylornithinase and/or the acetylhistidine uptake system.

Class B: as acetylornithine (under the conditions used) does not repress, these mutants might be characterised by, for instance (i) a deficient permeation system for acetylornithine; (ii) an altered uptake mechanism for acetylhistidine that is no longer inhibited by acetylornithine; or (iii) an acetylornithinase with altered kinetic properties, so that acetylornithine no longer acts as an inhibitor of acetylhistidine hydrolysis.

The explanations put forward above suggest that the release of non-repressible mutants from inhibition of acetylhistidine utilisation by arginine (partial release only), ornithine or citrulline, may be due to non-repressibility primarily either of acetylornithinase or of the acetylhistidine uptake system. If the former applies (but not the latter, unless the gene(s) controlling uptake are part of the same operon as *argE*), it should be possible to isolate mutants, among classes C, D, E and G, with operator mutations affecting *argE* expression. The regulation characteristics, in such mutants, of the enzymes coded for by *argECBH* should help to clarify the nature of the functional organization of this cluster.

The acetylornithinase of *E. coli* has now been shown to hydrolyze, apart from its "natural substrate" acetylornithine: acetyl-DL-methionine *in vitro* (Vogel and Bonner, 1956); formyl-L-methionine *in vitro* (Fry and Lamborg, 1967; Adams, 1968); acetylglutamic γ-semialdehyde *in vitro* and *in vivo* (Itikawa *et al.*, 1968); and acetylarginine *in vivo* (Bollon, Leisinger and Vogel, 1969). Inhibition by arginine of acetylglutamic γ-semialdehyde utilisation, and by acetylornithine of acetylarginine utilisation — comparable to phenomena discussed in this paper — are described in the relevant references. Evidence has also been obtained for hydrolysis by acetylornithinase *in vivo* of acetyl-DL-methionine and *in vitro* of acetyl-L-alanine, acetyl-L-arginine, acetyl-L-glutamine, acetyl-DL-norleucine, acetyl-DL-norvaline, and possibly acetyl-DL-serine (Baumberg, unpublished work). It is possible that this wide specificity of acetylornithinase is advantageous to *E. coli*, and that it explains (teleologically) why this enzyme is less repressible, in the K-12 strain, than the other biosynthetic enzymes (Baumberg *et al.*, 1965; Glansdorff and Sand, 1965).

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References

Adams, J. M.: On the release of the formyl group from nascent protein. J. molec. Biol. **33**, 571—589 (1968).

Albrecht, A. M., Vogel, H. J.: Acetylornithine δ-transaminase: partial purification and repression behaviour. J. biol. Chem. **239**, 1872—1876 (1964).

Bacon, D. F., Vogel, H. J.: "Internal cross-feeding" and repressive control. Fed. Proc. **22**, 476 (1963).

Baumberg, S., Bacon, D. F., Vogel, H. J.: Individually repressible enzymes specified by clustered genes of arginine synthesis. Proc. nat. Acad. Sci. (Wash.) **53**, 1029—1032 (1965).

Bollon, A. P., Leisinger, T., Vogel, H. J.: Differential repressor effectiveness in *Escherichia coli* under adjustable arginine restriction in batch cultures. Genetics **61**, s6 (1969).

Davis, B. D., Mingioli, E. S.: Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bact. **60**, 17—28, (1950).

Elseviers, D., Cunin, R., Glansdorff, N.: Reactivation of arginine genes under the influence of polar mutations. FEBS Letters **3**, 18—20 (1969).

Fry, K. T., Lamborg, M. R.: Amidohydrolase activity of *Escherichia coli* extracts with formylated amino acids and dipeptides as substrates. J. molec. Biol. **28**, 423—433 (1967).

Glansdorff, N.: Topography of cotransducible arginine mutations in *Escherichia coli* K-12. Genetics **5**, 167—179 (1965).

— Sand, G.: Coordination of enzyme synthesis in the arginine pathway of *Escherichia coli* K-12. Biochim. biophys. Acta (Amst.) **108**, 308—311 (1965).

Itikawa, H., Baumberg, S., Vogel, H. J.: Enzymic basis for a genetic suppression: accumulation and deacylation of *N*-acetylglutamic γ-semialdehyde in enterobacterial mutants. Biochim. biophys. Acta (Amst.) **159**, 547—550 (1968).

Lennox, E. S.: Transduction of linked genetic characters of the host by bacteriophage Pl. Virology **1**, 190—206 (1955).

Maas, W. K.: Studies on repression of arginine biosynthesis in *Escherichia coli*. Cold Spr. Harb. Symp. quant. Biol. **26**, 183—191 (1961).

Pittard, J., Loutit, J. S., Adelberg, E. S.: Gene transfer by F' strains of *Escherichia coli* K-12. I. Delay in initiation of chromosome transfer. J. Bact. **85**, 1394—1401 (1963).

Reiner, A. M.: Isolation and mapping of polynucleotide phosphorylase mutants of *Escherichia coli*. J. Bact. **97**, 1431—1436 (1969).

Satake, K., Okuyama, T., Ohashi, M., Shinoda, T.: The spectrophotometric determination of amine, amino acid and peptide with 2,4,6-trinitrobenzene 1-sulfonic acid. J. Biochem. (Tokyo) **47**, 654—660 (1960).

Siegel, E. C,, Bryson, V.: Mutator gene of *Escherichia coli* B. J. Bact. **94**, 38—47 (1967).

Taylor, A. L., Trotter, C. D.: Revised linkage map of *Escherichia coli*. Bact. Rev. **31**, 332—353 (1967).

Thompson, J. F., Gering, R. K.: Preparation of N^{α}-acetylornithine. Arch. Biochem. **99**, 326—327 (1962).

Urm, E., Leisinger, T., Vogel, R. H., Vogel, H. J.: Gene governing magnesium sensitivity in *Escherichia coli* K-12. Genetics **61**, s59 (1969).

Vogel, H. J.: Repression of an acetylornithine permeation system. Proc. nat. Acad. Sci. (Wash.) **46**, 488—494 (1960).

— Bonner, D. M.: Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. biol. Chem. **218**, 97—106 (1956).

Weissbach, H., Redfield, B.: Deformylation of *N*-formylmethionine by *Escherichia coli* extracts. Biochem. biophys. Res. Commun. **27**, 7—11 (1967).

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