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In Vivo Formation of Hybrid Aspartate Transcarbamoylases from Native Subunits of Divergent Members of the Family *Enterobacteriaceae*

K. F. FOLTERMANN, D. A. BECK, AND J. R. WILD*

Department of Biochemistry and Biophysics, Texas A&M University System, College Station, Texas 77843

Received 24 February 1986/Accepted 14 April 1986

The genes encoding the catalytic (*pyrB*) and regulatory (*pyrI*) polypeptides of aspartate transcarbamoylase (ATCase, EC 2.1.3.2) from several members of the family *Enterobacteriaceae* appear to be organized as bicistronic operons. The *pyrBI* gene regions from several enteric sources were cloned into selected plasmid vectors and expressed in *Escherichia coli*. Subsequently, the catalytic cistrons were subcloned and expressed independently from the regulatory cistrons from several of these sources. The regulatory cistron of *E. coli* was cloned separately and expressed from *lac* promoter-operator vectors. By utilizing plasmids from different incompatibility groups, it was possible to express catalytic and regulatory cistrons from different bacterial sources in the same cell. In all cases examined, the regulatory and catalytic polypeptides spontaneously assembled to form stable functional hybrid holoenzymes. This hybrid enzyme formation indicates that the r:c domains of interaction, as well as the dodecameric architecture, are conserved within the *Enterobacteriaceae*. The catalytic subunits of the hybrid ATCases originated from native enzymes possessing varied responses to allosteric effectors (CTP inhibition, CTP activation, or very slight responses; and ATP activation or no ATP response). However, each of the hybrid ATCases formed with regulatory subunits from *E. coli* demonstrated ATP activation and CTP inhibition, which suggests that the allosteric control characteristics are determined by the regulatory subunits.

Aspartate transcarbamoylase (carbamoylphosphate:L-aspartate carbamoyl-transferase; EC 2.1.3.2. [ATCase]) catalyzes the first unique step of pyrimidine biosynthesis in *Escherichia coli*. The enzyme from *E. coli* is the most studied representative of the class B ATCase enzymes (3, 14, 29, 39). It is a dodecamer with a molecular weight of 310,000 composed of six identical catalytic polypeptides organized as enzymatically functional catalytic trimers (c_3) and six identical regulatory polypeptides organized as regulatory dimers (r_2) (17, 37). These subunits are associated as a zinc-dependent $2(c_3):3(r_2)$ holoenzyme possessing a variety of intricate protein-protein interactions (9, 17, 32, 33, 37) which determine heterotropic allosteric effects and homotropic substrate characteristics (4, 8, 19, 22, 25). ATCase from *E. coli* is allosterically inhibited by CTP and activated by ATP. The holoenzyme exhibits cooperative homotropic interactions in response to the substrates aspartate (16) and carbamoyl phosphate (4). The dodecamer can be reversibly dissociated into its constituent subunits (c_3 and r_2) by treatment with mercurials such as *p*-chloromercuribenzoate or neohydrin (15, 41). The catalytic trimer possesses catalytic activity but has no regulatory properties (17). The regulatory dimer has no catalytic activity but binds the nucleotide effectors CTP and ATP with stoichiometries consistent with those of the holoenzyme (37). In the presence of zinc acetate and dithiothreitol, these two subunits can be reassociated in vitro to form a holoenzyme that is identical to the native holoenzyme in allosteric, physical, and catalytic characteristics (15).

In *E. coli*, adjacent cistrons encode the two polypeptide chains of ATCase and are expressed in the order: promoter, leader, catalytic polypeptide, regulatory polypeptide (20, 30, 34, 36). The *pyrB* cistron codes for the catalytic polypeptide, and *pyrI* codes for the regulatory polypeptide (34, 40). The two cistrons are organized as a bicistronic operon possessing a common control region characterized by a leader polypeptide and a UTP-dependent attenuator (27, 31, 36). When *pyrB* and *pyrI* are expressed from transcriptionally independent cistrons in the same cell, all detectable catalytic polypeptides are incorporated into a functional ATCase holoenzyme (13).

The regulatory characteristics of ATCases from various members of the family *Enterobacteriaceae* have been compared previously (3, 14, 23, 28, 38, 39). All of the enzymes appeared to be class B ATCases as determined by molecular weight (300,000), sigmoidal saturation by aspartate, and dissociation into a smaller catalytic form (approximately 100,000) (14, 39). Despite the apparent structural similarities, these enzymes differ from that of *E. coli* in homotropic kinetic properties and in heterotropic effector responses (Table 1). For example, ATP and CTP are both positive effectors of the ATCases from *Proteus vulgaris*, *Serratia marcescens* and *Aeromonas hydrophila*, while some *Yersinia* strains are not effected by either nucleotide (14, 39). *Erwinia herbicola* and *Erwinia carnegieana* are not affected by ATP and are only minimally inhibited by CTP. Aspartate concentrations required for half-maximal velocity range from 3 mM for *E. herbicola* to 30 mM for *P. vulgaris*. The divergent catalytic and regulatory characteristics appear to be conserved within the general tribal classifications of the *Enterobacteriaceae* (39).

In this paper we report the formation of hybrid ATCases created by the in vivo assembly of native catalytic and regulatory subunits from divergent bacterial sources. These

* Corresponding author.

TABLE 1. Characteristics of ATCases from selected members of the *Enterobacteriaceae*[a]

Enterobacteriaceae	Tribal classification	ATCase characteristics
Citrobacter diversus *Citrobacter freundii* *Edwardsiella tarda* *Enterobacter aerogenes* *Escherichia coli* *Salmonella typhimurium* *Shigella flexneri*	Tribe I	ATP activation CTP inhibition $[S]_{0.5}$ aspartate = 5–8 mM
Aeromonas hydrophila *Enterobacter liquefaciens* *Serratia marcescens*	Tribe II	ATP activation CTP activation $[S]_{0.5}$ aspartate = 17–20 mM
Proteus vulgaris	Tribe III	ATP activation CTP activation $[S]_{0.5}$ aspartate = 30–35 mM
Yersinia enterocolitica *Yersinia kristensenii* *Yersinia frederiksenii*	Tribe IV	No ATP effect No CTP effect $[S]_{0.5}$ aspartate = 4 mM[b]
Yersinia enterocolitica (different DNA relatedness group)[c]		Activation by ATP and CTP $[S]_{0.5}$ undetermined
Yersinia intermedia		ATP activation CTP and UTP inhibition $[S]_{0.5}$ undetermined
Erwinia carnegieana *Erwinia herbicola*	Tribe V	No ATP effect CTP slight inhibition $[S]_{0.5}$ aspartate = 3 mM

[a] Summarized from Foltermann et al. (14), Wild et al. (38), Wild et al. (39), and unpublished observations.
[b] $[S]_{0.5}$ determined for *Y. enterocolitica*.
[c] Various *Y. enterocolitica* strains showed different enzymatic characteristics and corresponded to different DNA relatedness groups (14).

hybrid enzymes were evaluated for catalytic characteristics and regulatory control.

MATERIALS AND METHODS

Bacterial strains. Bacterial strains used as sources of DNA for cloning *pyrBI* from various enteric bacteria are listed in Table 2. The host strain for all plasmid constructions was *E. coli* HB101-4442 (13) created by the insertion and subsequent excision of Mu *cts* into *E. coli* HB101 (5). A *pyrBI* auxotroph of JM103 (24) was used as the host system when *pyrI* was expressed on M13mp8. This strain was made *pyrBI*$^-$ by P1 phage transduction with a lysate grown on a heat-stable (43°C) Ap^r *pyrBI* strain generated by Mu d(*lac* Ap^r) insertion (7).

Plasmid and bacteriophage vectors. Expression vectors (5, 7, 10, 21, 24), recombinant DNA plasmids, and bacteriophage (24) used in these experiments are described in Table 3.

Media. Bacteria were routinely grown on tryptone-yeast extract (TYE) medium. Cultures used for ATCase assays were grown in the modified M56 medium as described earlier (39). Cultures used for large-scale plasmid preparations were grown in M9 medium (24) supplemented with 0.2% glucose, 0.1% Casamino Acids (Difco Laboratories, Detroit, Mich.), 2 μg of thiamine per ml, and 30 μg of uracil per ml (as required). Selective platings were performed on TYE plates and on minimal plates supplemented as above containing the appropriate antibiotics in the following concentrations: 40 μg of ampicillin per ml, 25 μg of chloramphenicol per ml, and 25 μg of tetracycline per ml.

Bacterial DNA isolation. Total genomic DNA from the various enteric bacteria (except *E. coli*) was isolated by pronase-sodium dodecyl sulfate treatment (2). The isolates were subjected to purification by a standard CsCl density ultracentrifugation technique (24) or were extracted four times with an equal volume of buffer-saturated phenol followed by two extractions with chloroform-isoamyl alcohol (24:1, vol/vol) and dialyzed against several changes of DNA buffer (10 mM Tris, 1 mM EDTA, pH 8).

F'-plasmid DNA was purified from *E. coli* K-12 (KLF17/KL132) carrying F'117 (F' *pyrB*$^+$ *argI*$^+$; *E. coli* Genetic Stock Center no. 4255) by the procedures of Deonier and Mirels (12).

Plasmid DNA isolation. Plasmid DNA was isolated as previously described (31) by using a cleared lysate method.

Restriction and ligation. Restriction endonucleases and T4 DNA ligase were purchased from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.) and used as recommended by the manufacturer. Ligation mixtures were incubated overnight at 4°C. T4 DNA polymerase and *Bal* 31 were obtained from Bethesda Research Laboratories and were used by the adapted methods of Maniatis et al. (24).

Transformations. Competent cells of *E. coli* HB101-4442 were prepared and used by the procedures of Dagert and Ehrlich (11) in the initial cloning of the *pyrBI* regions. Isolates from the TYE-antibiotic plates were screened to estimate the frequency of insertion, and all *pyrBI* transformants were isolated from the minimal plates containing antibiotics. After the initial cloning, frozen competent cells were used for transformation with plasmid DNA by the method of Morrison (26).

Plasmid screening. Plasmids containing inserts were identified by the rapid plasmid isolation procedure of Barnes (1) for quick screening application and by the Holmes and Quigley technique (18) when restriction digests or transformations were to be performed with the DNA.

ATCase characterization. Cell extracts were prepared by sonication, ATCase was assayed, and molecular weights were determined by ascending Sephadex G-200 chromatography as described previously (39). The assay was performed at 28°C in the presence of 5 mM aspartate and 4 mM carbamoyl phosphate except when noted differently. The concentration of the nucleotide effectors, ATP and CTP, was 2 mM.

Activity stain. Nondenaturing 6% polyacrylamide slab gels were used to analyze the subunit structure of ATCase. These

TABLE 2. Bacterial strains

Enterobacteriacae	Description	Source
Aeromonas hydrophila	ATCC 7966	ATCC[a]
Edwardsiella tarda	ATCC 15947	ATCC
Erwinia herbicola	TAMU 177[b]	B. G. Foster[b]
Escherichia coli	K-12, ECGSC 4255[c]	B. J. Bachmann[c]
Proteus vulgaris	ATCC 13315	ATCC
Salmonella typhimurium	LT-2, SGSC[d]	K. E. Sanderson[d]
Serratia marcescens	HY, ATCC 8195	W. L. Belser[e]

[a] Strains originated from American Type Culture Collection.
[b] Texas A&M University.
[c] *E. coli* Genetic Stock Center (Yale University).
[d] *Salmonella* Genetic Stock Center (University of Calgary).
[e] University of California, Riverside.

gels were specifically stained for ATCase activity by precipitating the enzymatically released P_i as an insoluble lead salt, followed by conversion to lead sulfide (M. A. Bothwell, Ph.D. thesis, University of California, Berkeley, 1975). Histidine was used instead of imidazole to avoid nonspecific staining and background problems encountered when using aged imidazole and crude cell extracts. Since enteric ATCases have been shown to vary in substrate requirements, the reaction mix for the gel system contained high levels of aspartate (100 to 150 mM), 1 to 2 mM ATP, and 3.5 mM carbamoyl phosphate. The reaction was allowed to proceed for 15 min at room temperature with substrate present.

Samples for activity gels were prepared by sonication as described above for the enzymatic assay or by the following procedure for screening a large number of transformants. Single-colony isolates were inoculated into 5 ml of TYE containing the appropriate antibiotic and incubated in a tube roller overnight at 37°C. This culture was harvested by centrifugation, washed once in 40 mM potassium phosphate buffer (pH 7.0), and resuspended in 200 to 300 μl of the same buffer containing 0.02 mM zinc acetate and 1 mM dithiothreitol. Samples were subjected to sonication in disposable plastic transfer pipettes with a cup horn sonicator (intensity 6 for five 30-s pulses). Cell extracts were prepared by centrifugation in a microcentrifuge for 5 min at 8°C. Supernatants were decanted, and 20 to 30 μl was run on an activity gel.

TABLE 3. Plasmids, bacteriophage, and recombinant molecules

Plasmid[a]	Marker[b]	Enzyme[c]	Size[d] (kb)	ATP[e]	CTP[e]
pPBh101-Ah	Ap^r *pyrBI*	*Bam*HI	11	+	+
pPBh401-Et	Ap^r *pyrBI*	*Bam*HI	17	+	−
pPBh103-Eh	Tc^r *pyrBI*	*Pst*I(p)[f]	11	0	s−
pPBc110-Eh	Tc^r *pyrB*	*Pst*I	10	0	0
pPBh104-Ec	Tc^r *pyrBI*	*Pst*I	10.4	+	−
pPBc201-Ec	Tc^r *pyrB*	*Bgl*II[g]	9.7	0	0
pPBh103-Pv	Ap^r *pyrBI*	*Hin*dIII-*Nru*I[h]	11.5	+	+
pPBc401-Pv	Ap^r *pyrB*	*Sst*I[i]	6.5	0	0
pPBh101-St[j]	Ap^r Tc^r *pyrBI*	*Eco*RI-*Ava*I	7.1	+	−
pPBc101-St	Tc^r *pyrB*	*Pst*I	>20	0	0
pPBh101-Sm	Ap^r *pyrBI*	*Hin*dIII	>20	+	+
pPBc101-Sm	Tc^r *pyrB*	*Pst*I	5.0	0	0
pPBr301-Ec	Cm^r *pyrI*	*Pst*I-*Hin*dIII[k]	6.8	No catalysis	
M13-PI5101-Ec	*pyrI*	*Ava*II[l]	8.0	No catalysis	
pACYC184	Tc^r Cm^r		3.9	Cloning vector	
pBH20	Ap^r Tc^r		4.5	Cloning vector	
pBR322	Ap^r Tc^r		4.3	Cloning vector	
pUC8	Ap^r		2.7	Cloning vector	
M13mp8	None		7.3	Phage	

[a] Plasmid nomenclature is defined for a series of plasmids constructed to contain specific cistrons from the *pyrBI* operon. All are designated pPB (plasmid containing the *pyrB* operon). The polypeptides produced from the construction are identified as holoenzyme (h, both catalytic and regulatory chains produced), catalytic subunit (c, produces catalytic chain only), and regulatory subunit (r, produces regulatory chain only). If the cloned cistrons are carried by a pBR322 origin vector, the plasmids are designated by 100 or 200 series numbers. If the cloned cistrons are carried by a P15A vector (e.g., pACYC184), the plasmids are designated as 300 series. Recombinant plasmids involving pUC series plasmids are 400 series, and phage borne fragments are designated as 500 series. The bacterial origins of the cloned cistrons are designated by the generic description, such that Ah indicates *A. hydrophila*; Et, *E. tarda*; Ec, *E. coli*; Eh, *E. herbicola*; Pv, *P. vulgaris*; St, *S. typhimurium*; and Sm, *S. marcescens*.

[b] Antibiotic resistance markers and expressed *pyrBI* genes only.

[c] Primary restriction endonuclease used in cloning procedures.

[d] Size of cloned fragment plus plasmid vector.

[e] Allosteric effector response to 2 mM ATP or CTP (+, activation; −, inhibition; s−, slight inhibition; 0, no effect).

[f] *Pst*I(p) indicates partial restriction cleavage.

[g] An internal deletion of approximately 0.7 kilobases of pPBh104-Ec.

[h] The preliminary fragment was produced by partial *Hin*dIII digestion, and the subclone resulted from an internal *Nru*I deletion.

[i] The preliminary fragment was produced by *Sst*I digestion; however, *Bal* 31 was used to reduce the fragment size before use of these studies. The smaller fragment was inserted into an *Sma*I site.

[j] pPBh101-St was obtained from Rod Kelln, University of Regina (pGM1).

[k] The subcloned fragment was isolated from pPBr101-Ec (13) by double restriction and inserted into the cloning vector by blunt-end ligation into a *Bam*HI site.

[l] The restriction fragment from pPHh104-Ec was inserted into a *Hin*cII site.

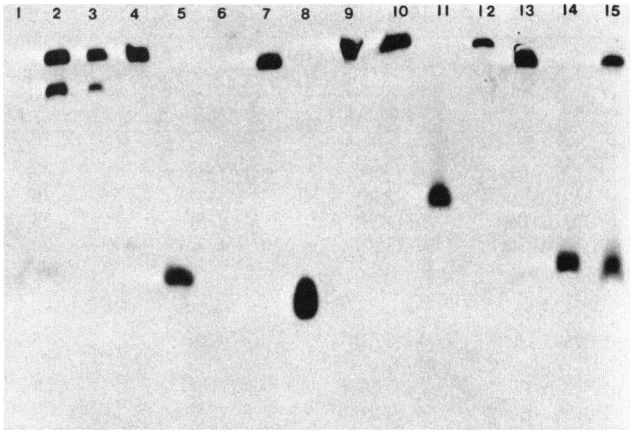

FIG. 1. ATCase activity gel for detection of hybrid enzyme formation. The holoenzyme migrates much more slowly through the native 6% acrylamide gel and is represented by higher bands. The catalytically active trimers (c_3) migrate more rapidly and can be detected in lanes 5, 8, 11, 14, and 15. The lanes contain extracts from the following genetic constructions: 1, *E. coli* HB101-4442, a *pyrBI*$^-$ derivative of HB101; 2, *E. coli* K-12, chromosomal *pyrBI*; 3, *E. coli* holoenzyme expressed from pPBh104-Ec; 4, *E. coli* "hybrid" holoenzyme produced from pPBc201-Ec and pPBr301-Ec; 5, *E. coli* catalytic subunit produced from pPBc201-Ec; 6, *E. coli* regulatory subunit from pPBr301-Ec; 7, *P. vulgaris-E. coli* hybrid holoenzyme produced from pPBc401-Pv and pPBr301-Ec; 8, *P. vulgaris* catalytic subunit from pPBc401-Pv; 9, *P. vulgaris* holoenzyme from pPBh103-Pv; 10, *E. herbicola-E. coli* hybrid holoenzyme produced from pPBc110-Eh and pPBr301-Ec; 11, *E. herbicola* catalytic subunit from pPBc110-Eh; 12, *E. herbicola* holoenzyme from pPBh103-Eh; 13, *S. marcescens-E. coli* hybrid holoenzyme produced from pPBc101-Sm and pPBr301-Ec; 14, *S. marcescens* catalytic subunit from pPBc101-Sm; and 15, *S. marcescens* holoenzyme from pPBh101-Sm.

RESULTS

Characterization of native ATCases from plasmids carrying *pyrBI* cistrons. The *pyrBI* cistrons were cloned from preparations of total chromosomal DNA from each enteric source except *E. coli* (cloned from F′ DNA [13]). The ATCase produced from each cloned fragment was compared with the enzyme already described from the parental source (Tables 1 and 3). The subunit architecture of each enzyme was determined by native activity gel and molecular sieve chromatography (Sephadex G-200). In all cases, the intact *pyrBI* operons were cloned from the chromosomal preparation, and the expressed ATCase holoenzymes were observed to be stable (Fig. 1) (unpublished observation). Table 3 summarizes the following characteristics of the plasmids carry-

TABLE 4. Effector response of hybrid ATCases

ATCase holoenzyme[a]	Effector response[b] at $[S]_{0.5}$ Asp		[Aspartate] (mM)[c]
	2 mM ATP	2 mM CTP	
Native			
pPBh104-Ec	+ (140)	− (25)	5
pPBh103-Pv	+ (210)	+ (130)	30
pPBh103-Eh	0 (100)	s− (75)	3
pPBh101-Sm	+ (150)	+ (135)	20
Hybrid			
pPBc201-Ec			
pPBr301-Ec	+ (120)	− (20)	5
pPBc401-Pv			
pPBr301-Ec	+ (110)[d]	− (25)	4
pPBc110-Eh			
pPBr301-Ec	+ (110)[d]	− (50)	4
pPBc101-Sm			
pPBr301-Ec	+ (170)	− (25)	125

[a] Plasmid characterization described in Table 3. The bacterial species from which the recombinant plasmids were formed is indicated by the alphabetical descriptor: Ec, *E. coli*; Pv, *P. vulgaris*; Eh, *E. herbicola*; Sm, *S. marcescens*. Basic nomenclature: pPBh, plasmid, *pyrB* operon holoenzyme; pPBc, plasmid *pyrB* operon, catalytic chain production only; pPBr, plasmid *pyrB* operon, regulatory chain production only.

[b] Percent activity of ATCase determined under standard conditions in the absence of nucleotide allosteric ligands (+, activation; −, inhibition; s−, slight inhibition).

[c] Aspartate concentrations approximating $[S]_{0.5}$ for each of the holoenzymes. These concentrations were used to determine allosteric response.

[d] Activation is more pronounced at lower aspartate concentrations (e.g., 130% at 2 mM).

ing *pyrBI* regions from various enteric bacteria: the enzyme used to clone the cistrons, the size of the plasmid, and the nature of the responses of the enzymes to ATP and CTP. In all cases, these responses are the same as those determined for enzyme preparations from the native bacterial sources (data not shown).

Determination of subunit architecture of native and hybrid enzymes. Figure 1 shows the subunit architecture of ATCases produced from selected native *pyrBI* and *pyrB* plasmids and hybrid constructions. Separate catalytic trimers were observed to move readily through the native acrylamide gel, while holoenzymes migrated at a much slower rate. Stable holoenzyme was detected in each combination of separately expressed catalytic and regulatory cistrons from different enteric bacteria. Virtually all the catalytic activity was recovered in an aggregated structure, and no catalytic trimer activity was noted. These gels measure differences owing to both protein charge and molecular size and permit the identification of various molecular conformations of active enzyme. On occasion, discrete bands might be observed immediately below that of the holoenzyme (Fig. 1, lanes 2 and 3). These enzymes appear to comigrate with the regulatory-deficient species of ATCase, $2(c_3):2(r_2)$ (6, 33). Specific mobility differences were observed for the catalytic subunits of *P. vulgaris* and *E. herbicola* as compared with the *E. coli* catalytic subunit.

Characterization of hybrid ATCase holoenzymes. Table 4 summarizes the allosteric responses of the hybrid holoenzymes. In each case, the hybrid holoenzymes possess a regulatory response (CTP inhibition, ATP activation) characteristic of the *E. coli* holoenzyme, independent of the source of the catalytic subunit. In addition, most of the enzymes possessed an $[S]_{0.5}$ of 4 to 5 mM aspartate; the only exception was the *S. marcescens-E. coli* hybrid which required 125 mM aspartate for half-maximal saturation. The unusually high requirement of this hybrid for aspartate is identical to that reported for the enzyme assembled from purified subunits (35).

Characterization of hybrid ATCase formed from phage carrying the *pyrI* cistron. All hybrids produced with the *pyrI* gene product expressed from the *lac* promoter-operator of the M13 system also seemed to be stable and showed the same kinetic and effector responses as hybrids generated with plasmid-expressed *pyrI* gene product.

DISCUSSION

Previous studies have demonstrated that the genes expressing ATCase from *E. coli* are organized as a bicistronic operon (20, 30, 36). The *pyrBI* operon organization has been conserved among several additional members of the *Enterobacteriaceae* including *Salmonella typhimurium*, *S. marcescens*, *P. vulgaris*, and *E. herbicola*. Unpublished DNA sequencing studies in this laboratory (*S. marcescens*, *P. vulgaris*, and *E. herbicola*) and elsewhere (*S. typhimurium*; R. Kelln, University of Regina, Regina, Saskatchewan, Canada) have verified a similar bicistronic organization for several of the cloned genetic systems described in this study. The native holoenzymes produced from these cloned enteric operons appear identical to those observed from the native bacterial sources (14, 39) as shown by molecular weight determination and by enzymatic characteristics.

It has been shown that stable ATCase holoenzyme is formed in vivo when *E. coli pyrB* and *pyrI* cistrons are expressed separately from plasmids of different incompatibility groups simultaneously present in the cell (13). This technique was used to construct hybrid holoenzymes in vivo, utilizing the native subunits from various members of the *Enterobacteriaceae*. Figure 1 reveals that, in each case, stable hybrid holoenzymes were formed with catalytic subunits from one bacterium and regulatory subunits from *E. coli*. Earlier studies have suggested that ATCase holoenzymes from various members of the *Enterobacteriaceae* have the same molecular architecture because they have a similar molecular weight (300,000 ± 15,000), sigmoidal aspartate saturation curves, and the ability to dissociate into functional catalytic subunits (100,000 molecular weight) (14, 39). The ability to form in vivo hybrid holoenzymes among various members of the *Enterobacteriaceae* confirms that there is a common overall architectural structure among the ATCases.

Each catalytic site is formed at the interface of two monomers of a catalytic trimer (19, 22), and it has been proposed that each participates in a complex communication network with a catalytic site in the other trimer through several domains of bonding (c::r:r::c) (8, 9). The assembly of the holoenzyme, $2(c_3):3(r_2)$, provides six c:c bonding domains within the catalytic trimers, three r:r bonding domains within the regulatory dimers, and six r:c domains linking the regulatory and catalytic subunits. Furthermore, there may be some additional contact between the catalytic chains in separate trimers despite a large aqueous center in the holoenzyme (19, 22, 25). In hybrid holoenzyme formation, r:c domains linking the regulatory and catalytic subunits are stably formed and define heterologous protein-protein interfaces which establish functional allosteric control and communication between active sites. All these hybrid holoenzymes exhibited a substrate requirement of 4 to 5 mM

aspartate, except for the *S. marcescens* c_3-*E. coli* r_2 hybrid. The unusually high substrate requirement of 125 mM aspartate for this hybrid is consistent with the earlier in vitro work of Shanley et al. (35) and appears to be correlated with differences in the amino acid sequence of the *Serratia* catalytic chain (K. M. Kedzie and J. R. Wild, unpublished observation). The same communication network that signals a homotropic response to substrate binding may also provide a means by which heterotropic effector response is relayed between subunits. Hybrid holoenzymes constructed with the various enteric catalytic subunits and *E. coli* regulatory subunits possessed an effector response that is characteristic of the *E. coli* ATCase. These heterotropic responses are often vastly different from the native effector response (Table 4) and suggest that the regulatory subunit directly imposes its allosteric control over various catalytic subunits.

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