Journal of General Microbiology (1980), **119**, 31–34. *Printed in Great Britain*

Isolation of *Escherichia coli* Mutants (*cpdB*) Deficient in Periplasmic 2′:3′-Cyclic Phosphodiesterase and Genetic Mapping of the *cpdB* Locus

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(*Received* 12 *October* 1979)

Mutants of *Escherichia coli* deficient in the periplasmic enzyme 2′:3′-cyclic phosphodiesterase have been obtained. The gene, designated *cpdB*, was mapped by conjugation and transduction and found to be located about 0·11 min to the right of the *cycA* locus on the *E. coli* genetic map.

INTRODUCTION

We have previously reported (Beacham *et al.*, 1973) a selection technique for mutants deficient in periplasmic enzymes (Heppel, 1971; Beacham, 1979) which degrade nucleotides. The procedure is based on sensitization of a uridine monophosphate pyrophosphorylase-deficient strain to 5-fluorouracil by the nucleotide in question; the degradation of the nucleotide allows the lethal synthesis of fluorouridine monophosphate. This method successfully yielded uridine diphosphoglucose hydrolase-deficient, and also porin-deficient, mutants (Beacham *et al.*, 1973; Beacham *et al.*, 1977). Surprisingly, however, 2′:3′-cyclic phosphodiesterase mutants were not obtained. In this paper we remedy this situation and report the phenotypic properties of such mutants and genetic mapping of the mutation.

METHODS

Organisms. These are listed in Table 1. Growth conditions were as previously described (Beacham *et al.*, 1973; Beacham & Yagil, 1976).

Mutagenesis. *N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine was used as described by Miller (1972).

Conjugation. This was done by established procedures (see Miller, 1972).

Transduction. The heat inducible bacteriophage P1cm *clr*100 (Rosner, 1972) was used. Lysates were prepared by selecting lysogens as chloramphenicol-resistant clones at 32 °C, followed by heat induction. Infection of cells by transducing lysates was performed at 32 °C, at a multiplicity of infection of approximately 1·0, and transductants were selected at 32 °C; the exception was the selection of ValS$^+$ recombinants at 38 °C where a low (approximately 0·1) multiplicity of infection was used. Selection or scoring of CpdB$^+$ transductants was performed in a *pyr* background on media containing 100 μM-2′:3′-cyclic uridine monophosphate (cyclic UMP) as a sole pyrimidine source; 3′-uridine monophosphate (3′-UMP) could also be used, but some background growth of the *cpdB* cells resulted. Since *pyrE*$^+$ transductants are also selected on media containing cyclic UMP, all putative CpdB$^+$ transductants were first scored for retention of the Pyr$^-$ phenotype. Three-factor crosses were interpreted on the basis that recombinants resulting from four recombinational events are least frequent.

Cyclic phosphodiesterase (EC 3.1.4.16). This enzyme was assayed by measuring the release of inorganic phosphate from cyclic UMP or 3′-UMP (Beacham *et al.*, 1973; Anraku, 1964 *a*), using toluene-treated cell suspensions.

Genetic symbols. The symbols used are those described by Bachmann *et al.* (1976) except *cpdB* for the gene specifying 2′:3′-cyclic phosphodiesterase. *Cpd* has been previously assigned, in *Salmonella*, to the gene specifying 3′:5′-cyclic phosphodiesterase and we suggest that it, and its homologue in *E. coli*, be designated *cpdA*.

0022-1287/80/0000-9008 $02.00

Table 1. *Strains of Escherichia coli* K12 *used*

Strain	Genotype	Derivation, source or reference
AB1157-1	*thr leu thi lacY galK ara xyl met proA his argE rpsL tsx sup upp*	Beacham *et al.* (1973)
GU13	As for AB1157-1, but *cpdB*	From AB1157-1 (see text)
GU26	As for AB1157-1, but *cpdB*	From AB1157-1 (see text)
GU1328*	As for GU13, but *xyl*$^+$ *pyrE*	From AT2243 × GU13
GU13283	As for GU1328, but *ampA purA*	From Hlt3 × GU1328 by transduction
Hlt3	*ampA purA proA thr leu lacY gal malA xyl mtl rpsL tsx* $\lambda^-\lambda^R$ *supE?*	B. J. Bachmann
AB4141	*valS thi metC lct lacY galK xyl ara rpsL tfr tsx supE*	B. J. Bachmann
AB4141-12	As for AB4141, but *xyl*$^+$ *pyrE*	From AT2243 × AB4141
x^{316}	*cycA supE* λ^-	B. J. Bachmann (Curtiss *et al.*, 1965; Russell, 1972)
AT2243	HfrC *pyrE metB tonA rel* T2^R	B. J. Bachmann
CSH62	HfrH *thi*	Miller (1972)
CSH60	HfrRa-2 *sup*	Miller (1972)
CSH68	Hfr6 *mtl met malB*	Miller (1972)

* Owing to the *upp* mutation, this strain and its derivatives require uridine (1 mM) rather than uracil.

Table 2. *Cyclic phosphodiesterase and 3′-nucleotidase activity in strains of Escherichia coli*

Strain	Substrate ...	Specific activity (nmol min^{-1} mg^{-1}) Cyclic UMP	3′-UMP
AB1157-1		12·5	22·5
GU13		0·2	1·8
GU26		0·7	3·8
GU1328		0·4	1·8

RESULTS AND DISCUSSION

2′:3′-Cyclic phosphodiesterase (Anraku, 1964*a*, *b*) catalyses the following two-step reaction: 2′:3′-cyclic UMP → 3′-UMP → uridine+phosphate. Other nucleotides, e.g. 2′:3′-cyclic AMP, are also hydrolysed (Anraku, 1964*a*). Following mutagenesis of strain AB1157-1 with nitrosoguanidine, *cpdB* mutants were selected as resistant to a mixture of 5-fluorouracil (1 μg ml^{-1}) and 3′-AMP (1 mM) (see Beacham *et al.*, 1973, for further details). Two mutant clones, GU13 and GU26, were eventually obtained which were deficient in 2′:3′-cyclic phosphodiesterase and 3′-nucleotidase activity as shown in Table 2. The 3′-nucleotidase deficiency is particularly incomplete presumably due to other periplasmic enzymes capable of hydrolysing 3′-nucleotides (Dvorak *et al.*, 1967). Anraku (1964*b*) has provided evidence that the hydrolysis of cyclic- and 3′-nucleotides by cyclic phosphodiesterase occurs at different sites on the enzyme. The two mutants reported here show loss of both activities indicating that an active site is not specifically altered, but rather a complete loss of function of the protein. However, it might nevertheless be possible, on the basis of two active sites, to isolate mutants affected in either cyclic- or 3′-nucleotide hydrolysis.

Though resistant to 5-fluorouracil plus 3′-AMP, this phenotype was not clear enough to be used for mapping studies, and an alternative phenotype was sought. A uracil, or uridine, requirement was conferred on GU13 by the introduction of a *pyrE* mutation as outlined in Table 1. Whereas *cpdB*$^+$ *pyrE* strains will utilize cyclic UMP or 3′-UMP as a uracil source, *cpdB pyrE* strains will not. This provides a very clear phenotypic distinction between *cpdB*$^+$ and *cpdB* strains, but only in a *pyr* background.

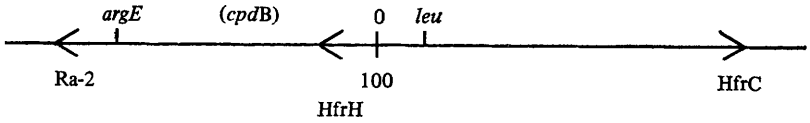

Fig. 1. Preliminary mapping of the *cpdB* locus using conjugation. The origins of the Hfr strains used are shown.

Table 3. P1(cm *clr*100)-*mediated transductional crosses involving the ampA, purA, cpdB and valS loci*

Cross	Donor	Recipient	Selected marker	No. scored	Unselected markers: Type	Unselected markers: No.	Frequency of cotransduction	Gene order indicated
1	CSH68	GU13283 (*ampA purA cpdB*)	Pur$^+$	144	$Amp^S Cpd^+$	10	*amp–purA* (29)	*ampA–purA–cpdB*
					$Amp^R Cpd^+$	47		
					$Amp^S Cpd^-$	32	*purA–cpdB* (39·5)	
					$Amp^R Cpd^-$	55		
2	x^{316} (*cycA*)	GU13283 (*purA cpdB*)	Pur$^+$	150	$Cpd^- Cyc^-$	9	*cycA–purA* (44)	*purA–cycA–cpdB*
					$Cpd^+ Cyc^-$	57		
					$Cpd^+ Cyc^+$	0	*cpdB–purA* (38)	
					$Cpd^- Cyc^+$	84		
3	x^{316} (*cycA*)	GU13283 (*purA cpdB*)	Cpd$^+$	67	$Cyc^- Pur^-$	26	*purA–cpdB* (46)	*purA–cycA–cpdB*
					$Cyc^+ Pur^+$	1		
					$Cyc^- Pur^+$	30	*cycA–cpdB* (83·6)	
					$Cyc^+ Pur^-$	10		
4	GU1328 (*cpdB*)	AB4141-12 (*valS*)	ValS$^+$	150	Cpd$^+$	10	*cpdB–valS* (6·6)	

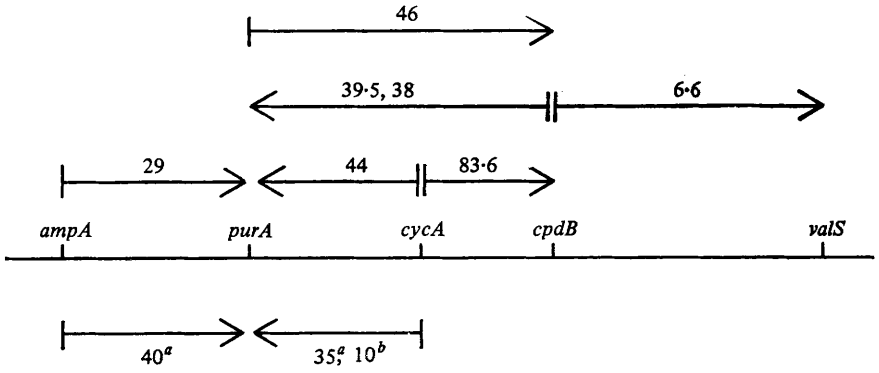

Fig. 2. Genetic mapping of the *cpdB* locus using transduction. Values are the percentage cotransduction; the arrows point to the selected marker. Values below the map are from: *a*, Russell (1972), and *b*, Isono & Kitakawa (1978).

Genetic mapping

Preliminary mapping was performed using conjugation. Selection for Arg$^+$, Leu$^+$ and Cpd$^+$ recombinants using HfrRa-2 and HfrC indicated that *cpdB* was linked to *argE* and *leu* to the extent of 20 to 50%. However, *cpdB* did not enter with HfrH when Leu$^+$ was selected. This placed *cpdB* between the origins of HfrRa-2 and HfrH (Fig. 1). Subsequent studies (Table 3) have shown the transductional relationships depicted in Fig. 2. The three-factor data from cross 1 (Table 3) is inconsistent with *ampA* or *cpdB* as the middle marker, placing *cpdB* to the right of *purA*, in the order *ampA–purA–cpdB*. Crosses 2 and 3 show that *cpdB* is closely linked to *cycA* and concur that it lies to the right of *cycA*. The linkage values of *cpdB* and *cycA* to *purA*, when the latter is selected (cross 2), agree with the gene

order deduced from the three-factor data. The higher value for *purA–cpdB* obtained in cross 3 is less reliable to compare with the *purA–cycA* distance since it is obtained in a different cross, selecting a different marker, and lower numbers were scored.

Two different values for the distance between *purA* and *cycA* exist in the literature (Fig. 2; Russell, 1972; Isono & Kitakawa, 1978); our data are consistently in agreement with the higher value. Cross 4 shows the expected low degree of cotransduction to *valS*.

By converting the cotransduction frequencies to minutes using the Wu formula (Wu, 1966), the total genetic distance between *ampA* and *valS* based on additivity of cotransduction frequency is 2·4 min; this is about 0·3 min greater than indicated in the current edition of the *E. coli* genetic map (Bachmann *et al.*, 1976).

In summary, the *cpdB* gene is placed about 0·11 min, or approximately 4·5 kilobases, to the right of the *cycA* locus.

We thank the University Grants Committee for their support.

REFERENCES

ANRAKU, Y. (1964*a*). A new cyclic phosphodiesterase having 3′-nucleotidase activity from *Escherichia coli* B. I. Purification and some properties of the enzyme. *Journal of Biological Chemistry* **239**, 3412–3419.

ANRAKU, Y. (1964*b*). A new cyclic phosphodiesterase having a 3′-nucleotidase activity from *Escherichia coli* B. II. Further studies on substrate specificity and mode of action of the enzyme. *Journal of Biological Chemistry* **239**, 3420–3424.

BACHMANN, B. J., LOW, K. B. & TAYLOR, A. L. (1976). Recalibrated linkage map of *Escherichia coli* K-12. *Bacteriological Reviews* **40**, 116–167.

BEACHAM, I. R. (1979). Periplasmic enzymes in gram-negative bacteria. *International Journal of Biochemistry* **10**, 877–883.

BEACHAM, I. R. & YAGIL, E. (1976). Genetic location of the gene (*ush*) specifying periplasmic uridine 5′-diphosphate glucose hydrolase (5′-nucleotidase) in *Escherichia coli* K-12. *Journal of Bacteriology* **128**, 487–489.

BEACHAM, I. R., KAHANA, R., LEVY, L. & YAGIL, E. (1973). Mutants of *Escherichia coli* K-12 'cryptic', or deficient in 5′-nucleotidase (uridine diphosphate-sugar hydrolase) and 3′-nucleotidase (cyclic phosphodiesterase) activity. *Journal of Bacteriology* **116**, 957–964.

BEACHAM, I. R., HAAS, D. & YAGIL, E. (1977). Mutants of *Escherichia coli* 'cryptic' for certain periplasmic enzymes: evidence for an alteration of the outer membrane. *Journal of Bacteriology* **129**, 1034–1044.

CURTISS, R., CHAROMELLA, L. J., BERG, C. M. & HARRIS, P. E. (1965). Kinetic and genetic analyses of D-cycloserine inhibition and resistance in *Escherichia coli*. *Journal of Bacteriology* **90**, 1238–1250.

DVORAK, H. F., BROCKMAN, R. W. & HEPPEL, L. A. (1967). Purification and properties of two acid phosphatase fractions isolated from osmotic shock fluid of *Escherichia coli*. *Biochemistry* **6**, 1743–1751.

HEPPEL, L. A. (1971). The concept of periplasmic enzymes. In *Structure and Function of Biological Membranes*, pp. 224–247. Edited by L. I. Rothfield. New York: Academic Press.

ISONO, K. & KITAKAWA, U. (1978). Cluster of ribosomal protein genes in *Escherichia coli* containing genes for proteins S6, S18 and L9. *Proceedings of the National Academy of Sciences of the United States of America* **75**, 6163–6167.

MILLER, J. H. (1972). *Experiments in Molecular Genetics*. New York: Cold Spring Harbor Laboratory.

ROSNER, J. L. (1972). Formation, induction and curing of bacteriophage P1 lysogens. *Virology* **48**, 679–689.

RUSSELL, R. R. B. (1972). Mapping of a D-cycloserine resistance locus in *Escherichia coli* K-12. *Journal of Bacteriology* **111**, 622–624.

WU, T. T. (1966). A model for three-point analysis of random general transduction. *Genetics* **54**, 405–410.