FL 1060 Binding Protein of *Escherichia coli* is Probably under the Control of Adenosine 3′,5′-Cyclic Monophosphate

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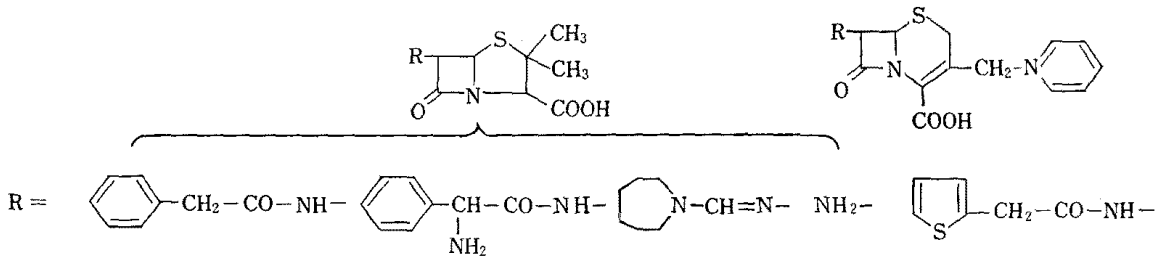
FL 1060 (Mecillinam) is a unique β-lactam antibiotic which induces spherical shaped cells in *Escherichia coli* at low concentrations without inhibiting cell division or causing cell lysis.[1~5] Only one of six penicillin binding proteins which reside in the inner membrane of *E. coli* has been revealed to be bound to FL 1060.[6,7] A mutant, whose membrane proteins lost the binding activity to FL 1060, was selected from FL 1060-resistant mutants. The mutant took spherical shape in the absence of FL 1060.[7] Rounding of *E. coli* cells in the presence of FL 1060, therefore, is attributed to the alteration or lacking of FL 1060 binding protein in the inner membrane.

On the other hand, adenyl cyclase deficient mutants (*cya*$^-$) of *E. coli* take spherical shape in the absence of exogeneous adenosine 3′, 5′-cyclic monophosphate (cAMP).[8~10] In view of the similarity of the morphological changes, we tested the sensitivity of *cya*$^-$ mutants of *E. coli* to FL 1060 in the presence and absence of cAMP. This communication reports the possibility that the function or biosynthesis of FL 1060 binding protein is under the control of cAMP.

Strains AO-33 and CA7902 were used as *cya*$^-$ mutants. AO-33 was obtained from *E.*

TABLE I. SENSITIVITY OF *E. coli* AO-33 AND CA7902 AND THEIR REVERTANTS TO β-LACTAM ANTIBIOTICS IN THE PRESENCE AND ABSENCE OF cAMP



Strains	cAMP 1 mM	MIC[a] (μg/ml)	Ratio	MIC[a] (μg/ml)	Ratio	MIC[a] (μg/ml)	Ratio	MIC[a] (μg/ml)	Ratio	MIC[a] (μg/ml)	Ratio
AO-33	−	25	1	0.63	2	63	800	31	0.5	2.5	1
	+	25		0.31		0.078		63		2.5	
AO-33 Rev-1	−	25	1	1.3	2	0.039	1	31	1	2.5	1
	+	25		0.63		0.039		31		2.5	
AO-33 Rev-2	−	13	1	0.63	2	0.078	2	31	1	2.5	1
	+	13		0.31		0.039		31		2.5	
CA7902	−	50	1	13	4	125	800	31	1	2.5	1
	+	50		3.1		0.16		31		2.5	
CA7902 Rev-1	−	50	2	6.3	4	0.16	1	31	1	2.5	1
	+	25		1.6		0.16		31		2.5	
CA7902 Rev-2	−	50	1	3.1	2	0.31	2	31	1	2.5	1
	+	50		1.6		0.16		31		2.5	

[a] Nutrient broth was used as an assay medium.

coli K-12 W2252 (Hfr C, *met*B$^-$, *rel*$^-$) *thy*$^-$*, as a lactose and galactose non-fermentative mutant in the absence of exogeneous cAMP according to the method described previously.[12] To determine that AO-33 is an exact *cya*$^-$ mutant, genetic analysis by Pl transduction was performed. According to Taylor's genetic map of *E. coli* K-12,[11] *cya* and *ilv* are located at 83.5 and 83 min, respectively. *E. coli* K-12 AB2277 (*ilv*$^-$, *cya*$^+$, *gal*$^-$, *lac*$^-$, *mal*$^-$, *ara*$^-$)** was transduced to *ilv*$^+$ with Plvir lysate of AO-33. 46% of 76 *ilv*$^+$ transductants could not ferment mannose and glycerol in the absence of exogeneous cAMP, but could ferment in its presence. AO-33, therefore, was confirmed to be a *cya*$^-$ mutant. *E. coli* K-12 CA7902 (Hfr H, *cya*$^-$, *rel*$^-$) was originally obtained by Beckwith[12] and was kindly supplied by Prof. T. Yokota of Juntendo University. FL 1060 was the product of Leo Laboratories (Ballerup, Denmark) and cAMP was kindly supplied by Yamasa Shoyu Co. (Choshi, Japan). Benzylpenicillin and Ampicillin were the products of Meiji Seika Co. (Japan) and Takeda Pharmacheutical Industries Inc. (Japan), respectively. 6-Aminopenicillanic acid and Cephaloridine were purchased from Sigma Chemical Co. (U.S.A.) and Eli Lilly Co. (U.S.A.), respectively. The minimum inhibitory concentration (MIC) was determined by the broth dilution method after 24 hr' incubation at 37°C without shaking in the presence or absence of 1 mM cAMP. To exclude the revertants (*cya*$^-$), AO-33 or CA7902 was streaked on a MacConkey-lactose (1%) agar plate in every experiment. Lactose non-fermentative white small colonies were collected and used as *cya*$^-$ cells. *cya*$^+$ revertants listed in Table I were obtained from fermentative large red colonies on the plate and were purified.

Table I shows the MIC of several β-lactam antibiotics to *cya*$^-$ mutants and their revertants in the presence and absence of exogeneous cAMP. Only *cya*$^-$ mutants were highly and selectively resistant to FL 1060 in the absence of cAMP. Their revertants were sensitive to FL 1060 either in the presence or absence of cAMP. Among other β-lactam antibiotics than FL 1060 listed in Table I, 6-aminopenicillanic acid is also known to induce spherical shaped cells in *E. coli* at low concentrations.[7] At higher concentrations, however, 6-aminopenicillanic acid but not FL 1060 induces cell lysis, suggesting a little difference in mode of action between them.[7] As shown in Table I, MIC of 6-aminopenicillanic acid was much higher than that of FL 1060 and was not influenced by the presence or absence of cAMP. This result also suggests the difference in mode of action between FL 1060 and 6-aminopenicillanic acid.

The high and selective resistance to FL 1060 and morphological change of *cya*$^-$ mutants in the absence of exogeneous cAMP can be well explained by the assumption that FL 1060 binding protein cannot function normally without cAMP or that the binding protein is not synthesized or built in membrane under the condition.

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