Site-Directed Mutagenesis of Lysine 58 in a Putative ATP-Binding Domain of the Calmodulin-Sensitive Adenylate Cyclase from *Bordetella pertussis* Abolishes Catalytic Activity†

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Received January 4, 1989

ABSTRACT: A 2.7-kb *cya* A gene fragment encoding the amino-terminal end of the calmodulin-sensitive adenylate cyclase from *Bordetella pertussis* has been placed under the control of the *lac* promoter for expression in *Escherichia coli*. Following induction with isopropyl β-D-thiogalactoside, calmodulin-sensitive adenylate cyclase activity was detected in a cell extract from *E. coli*. The expression vector directed the synthesis of a 90-kDa polypeptide that was recognized by rabbit polyclonal antibodies raised against the catalytic subunit of *B. pertussis* adenylate cyclase. Inspection of the deduced amino acid sequence of the *cya* A gene product revealed a sequence with homology to consensus sequences for an ATP-binding domain found in many ATP-binding proteins. On the basis of the analysis of nucleotide binding proteins, a conserved lysine residue has been implicated in the binding of ATP. A putative ATP-binding domain in the *B. pertussis* adenylate cyclase possesses an analogous lysine residue at position 58. To test whether lysine 58 of the *B. pertussis* adenylate cyclase is a crucial residue for enzyme activity, it was replaced with methionine by oligonucleotide-directed mutagenesis. *E. coli* cells were transformed with the mutant *cya* A gene, and the expressed gene product was characterized. The mutant protein exhibited neither basal nor calmodulin-stimulated enzyme activity, indicating that lysine 58 plays a critical role in enzyme catalysis.

Adenylate cyclase catalyzes the formation of 3′,5′-cAMP and inorganic pyrophosphate from ATP. Since cAMP is essential for the modulation of a variety of cellular responses, elucidation of the reaction mechanism for adenylate cyclase has been a subject of great interest. Stereochemical studies of the reactions catalyzed by both the bacterial and the mammalian adenylate cyclase have been conducted (Gerlt et al., 1980; Eckstein et al., 1981). In both cases, the cyclization reaction occurred with inversion of configuration at the α-phosphorus. The enzyme reaction mechanism therefore appears to involve direct nucleophilic attack of the 3′-hydroxyl group on the α-phosphorus, with no formation of an adenylated enzyme intermediate. On the basis of these results, Gerlt et al. (1980) proposed that a basic amino acid at the active site participates in the ionization of the 3′-hydroxyl group of ATP. In addition, previous studies from our laboratory have shown that there is an amino group at the active site of the mammalian adenylate cyclase that forms a Schiff base with 2′,3′-dial ATP (Westcott et al., 1980).

Bordetella pertussis, the etiologic agent of whooping cough, produces several virulence factors implicated in the pathogenesis of the disease (Weiss & Falkow, 1986; Wardlaw & Parton, 1988). One of these factors is an active adenylate cyclase that is released extracellularly into the culture supernatant (Hewlett et al., 1976; Hewlett & Wolff, 1976; Shattuck et al., 1985). This enzyme exhibits two unusual properties: it is strongly activated by calmodulin (CaM),[1] which is not present in bacteria (Wolff et al., 1980), and it can invade animal cells (Confer & Eaton, 1982; Hanski & Farfel, 1985; Shattuck & Storm, 1985).

The gene encoding the *B. pertussis* adenylate cyclase has been cloned and sequenced (Glaser et al., 1988a). Biochemical (Masure & Storm, 1989) and molecular genetic (Glaser et al., 1988a,b) studies have shown that the enzyme is synthesized as a large precursor (177 kDa) that is proteolytically processed to a 45-kDa form which is released into the culture supernatant. It was also demonstrated that the catalytic domain lies within the first 450 amino-terminal amino acids of the precursor molecule (Glaser et al., 1988a). Analysis of the predicted amino acid sequence of the catalytic domain of the enzyme reveals a sequence, IAEGVATKGLGVHA, that has homology with the consensus sequences proposed for an ATP-binding domain derived from many different ATP-binding proteins (Walker et al., 1982; Chin et al., 1988). Homology was observed when the amino terminal of the adenylate cyclase sequence was matched with the carboxy terminal of the consensus sequences. The presence of a conserved lysine residue at position 58 within this putative ATP-binding domain is of particular interest since its amino group may interact electrostatically with the phosphate groups of ATP (Pai et al., 1977; Fry et al., 1985, 1986). Furthermore, this lysine may directly participate in catalysis as proposed by Gerlt et al. (1980). We predicted that specific replacement of lysine 58 could eliminate adenylate cyclase activity of the enzyme. To test this hypothesis, the AAA codon for this lysine was altered to the ATG codon for methionine by oligonucleotide-directed mutagenesis. The mutated gene was inserted into the plasmid pUC19 for expression in *Escherichia coli*, and the effect of the mutation on adenylate cyclase activity was examined. The experimental results indicated that the Met 58 mutant exhibited neither basal nor

† This work was supported by NIH Grant GM 31708. D.C.A. was supported by an American Diabetes postdoctoral fellowship; H.R.M. was supported by NIH Postdoctoral Fellowship NS-07985.
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[1] Abbreviations: BSA, bovine serum albumin; CaM, calmodulin; EDTA, ethylenediaminetetraacetic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; IPTG, isopropyl β-D-thiogalactoside; PAGE, polyacrylamide gel electrophoresis; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Tween 20, poly(oxyethylene)sorbitan monolaurate.

0006-2960/89/0428-2772$01.50/0

CaM-sensitive adenylate cyclase activity.

Materials and Methods

Materials. *E. coli* strains JG1 (Leib & Gerlt, 1983) and NM522 (Gough & Murray, 1983) have been described previously. The CaM expression vector pVUC-1 (Roberts et al., 1985) was a gift from Dr. D. M. Watterson. Cloning vectors pUC19 and M13mp18 were from Pharmacia. The protoclone λgt11 system was obtained from Promega. The nick-translation system was a product of Du Pont–New England Nuclear Corp. The Muta-Gene in vitro mutagenesis kit was purchased from Bio-Rad, and the gel-purified oligonucleotide primer was obtained from Synthetic Genetics (San Diego, CA).

General Recombinant DNA Techniques. Extraction of chromosomal DNA from the *B. pertussis* Tohama phase I strain, large-scale isolation of plasmid DNA, minipreparation of plasmid and λDNA, restriction enzyme digestions, ligation, transformation, and plaque hybridization were performed as described (Silhavy et al., 1984; Maniatis et al., 1982).

Isolation of the B. pertussis Adenylate Cyclase Gene. The gene encoding the *B. pertussis* adenylate cyclase was isolated by the method of Glaser et al. (1988a) with some modifications. The *E. coli* strain JG1(pVUC-1), an adenylate cyclase mutant that expresses CaM, was used to screen a *B. pertussis* genomic library constructed in λgt11 (Young & Davis, 1983). A recombinant phage carrying a partial but functional *B. pertussis cya* A gene was isolated by complementation of the *E. coli* mutant to ferment lactose on MacConkey agar plates. Restriction enzyme digestion and DNA sequence analyses showed that the 5.3-kb cloned insert (Figure 1, clone 1) contained the promoter region and 85% of the coding sequence of the *cya* A gene as reported by Glaser et al. (1988a). A DNA fragment from this cloned insert was labeled with [^{32}P]dCTP by nick translation, and this radiolabeled probe was used to rescreen the genomic library by plaque hybridization. Several positive clones were obtained and one of them (Figure 1, clone 2) was shown, by restriction enzyme digestion and DNA seuqence analyses, to contain the remaining coding sequence of the *B. pertussis cya* A gene. To construct a full-length *cya* A gene, the 2.7-kb *Bam*HI–*Eco*RI fragment from clone 1 was inserted into the *Bam*HI–*Eco*RI sites of pUC19. The recombinant plasmid was then linearized with *Eco*RI and ligated to the 5.5-kb *Eco*RI fragment from clone 2. After transformation into *E. coli* strain NM522, plasmids from several ampicillin-resistant colonies were analyzed by restriction enzyme digestion. One of them contained the entire *cya* A gene.

Oligonucleotide-Directed Mutagenesis. The 2.7-kb *Bam*HI–*Eco*RI fragment from the cloned *cya* A gene was inserted into the *Bam*HI–*Eco*RI sites of M13mp18. Phage was amplified in a *dut*$^-$*ung*$^-$ *E. coli* strain CJ236 to obtain uridylated single-stranded template DNA (Kunkel, 1985). Mutagenesis was carried out by using a mismatched primer, 5′-GTGGCCACCATGGGATTGGGCG-3′. This primer was designed to substitute methionine for lysine in position 58 of the enzyme. After in vitro synthesis of the complementary strand using T4 DNA polymerase, the double-stranded circular DNA was transformed into *E. coli* strain NM522 (*dut*$^+$*ung*$^+$) and the mutated sequence was confirmed by direct nucleotide sequencing (Sanger et al., 1977).

Expression of the B. pertussis Adenylate Cyclase Gene in E. coli. The 2.7-kb *Bam*HI–*Eco*RI *cya A* gene fragment was placed under the control of the *lac* promoter on the plasmid pUC19. The recombinant plasmid was transformed into *E. coli* strain NM522 for expression. The plasmid-harboring clone was grown at 37 °C in 50 mL of LB medium (Miller, 1972) containing 100 μg/mL ampicillin. When the OD_{650} of the culture reached 0.7, IPTG was added to a final concentration of 1 mM, and incubation was continued for an additional 2 h. The cells were collected by centrifugation at 8000*g* for 10 min, and the cell pellet was suspended in 2 mL of buffer containing 50 mM Tris-HCl (pH 7.5), 2 mM EDTA, and 0.2 mM PMSF. The cells were disrupted with a Heat System W140 sonicator for 90 s with the power setting at 30% of maximum. The bacterial extract was assayed for adenylate cyclase activity in the presence and absence of 2.4 μM CaM.

Adenylate Cyclase Assay. The production of cAMP was measured by the method of Salomon et al. (1974). Each assay contained 20 mM Tris-HCl (pH 7.4), 1 mM [α-^{32}P]ATP, 5 mM $MgCl_2$, 1 mM EDTA, and 0.1% BSA. Some samples were assayed in the presence of 2.4 μM CaM. CaM was prepared from bovine brain by the procedure of Masure et al. (1984). One unit of adenylate cyclase catalyzed the synthesis of 1 nmol of cAMP/min.

Immunotransfer. Rabbit polyclonal antibodies raised against the catalytic subunit of *B. pertussis* adenylate cyclase have been described previously (Masure & Storm, 1989). One milliliter of IPTG-induced *E. coli* culture was centrifuged in a microfuge for 2 min, and the cell pellet was lysed in 100 μL of sample buffer according to the method of Laemmli (1970). Samples were boiled for 5 min, and 20 μL per well was loaded onto a 10% SDS–polyacrylamide gel. Proteins separated by SDS–PAGE were electrophoretically transferred onto nitrocellulose paper with the ABN Polyblot Transfer System according to manufacturer's instructions. The electrophoretic transfer was carried out for 1 h at 280 mA. To prevent nonspecific protein binding in subsequent steps, the nitrocellulose paper was blocked by incubation for 1 h at room temperature with TPBS (Tween 20, 5 g/L; KH_2PO_4, 61.25 g/L; NaOH, 10.25 g/L; NaCl, 87.75 g/L) containing 4% (w/v) BSA. The nitrocellulose paper was rinsed three times with H_2O and once with TPBS and then incubated overnight at 4 °C with 50 mL of a 1:500 dilution of immune or preimmune IgG in TPBS. After the nitrocellulose paper was washed three times with TPBS, it was incubated with horseradish peroxidase linked goat anti-rabbit IgG for 1 h at room temperature. The nitrocellulose paper was then washed twice with TPBS and once with 50 mM Tris-HCl, pH 7.5, and 150 mM NaCl (buffer A), and the antigen–antibody complexes were visualized by incubating the nitrocellulose paper with buffer A containing 20% methanol, 5 mg/mL 4-chloro-1-naphthol, and 0.03% H_2O_2. Staining was terminated by washing the paper several times with buffer A.

Results

Cloning and Expression of the B. pertussis cya A Gene in E. coli. The gene encoding the *B. pertussis* adenylate cyclase was first isolated by selecting for the restoration of maltose fermentation in a *cya*$^-$ *E. coli* strain that harbored a CaM expression vector (Glaser et al., 1988a). To study the structure–function relationships of this enzyme, we cloned the entire *cya* A gene using a similar method (Figure 1) and placed it under the control of the *lac* promoter as described under Materials and Methods. This allowed efficient expression of the gene product in *E. coli* in the absence of any transregulatory elements as proposed by Weiss and Falkow (1984). Because the calmodulin-sensitive adenylate cyclase activity is localized in the 450 amino-terminal amino acids of the precursor molecule (Glaser et al., 1988a), the plasmid (pDA1) which contains the 2.7-kb *Bam*HI–*Eco*RI *cya* A gene fragment that encodes the amino-terminal end of the enzyme was used in subsequent expression and mutagenesis experiments.

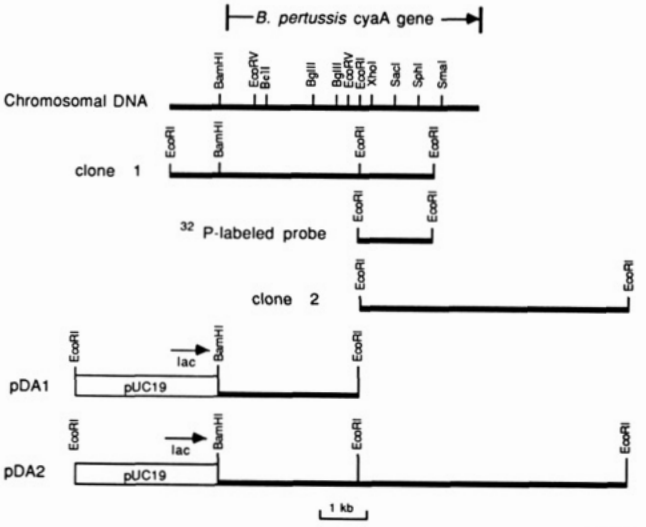

FIGURE 1: Construction of expression vectors carrying the *B. pertussis cya* A gene. A λgt11 recombinant phage carrying the cloned insert 1 was isolated as described under Materials and Methods. The 2-kb *Eco*RI fragment from this cloned insert was labeled with [^{32}P]dCTP by nick translation and used to isolate clone 2 by plaque hybridization. pDA1 is a pUC19 derivative carrying the 2.7-kb *Bam*HI–*Eco*RI fragment from clone 1. The 5.5-kb *Eco*RI fragment from clone 2 was inserted into the *Eco*RI site of pDA1 to generate pDA2.

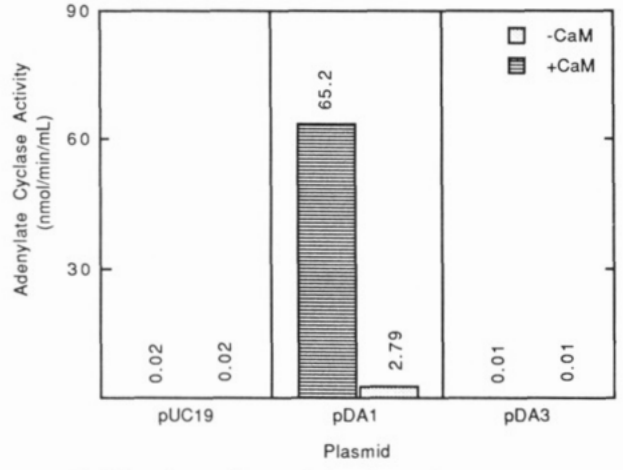

FIGURE 2: Adenylate cyclase activity of *E. coli* strain NM522 harboring plasmids pUC19, pDA1, and pDA3. Bacteria were induced with IPTG and disrupted by sonication as described under Materials and Methods. Sonicated cell extracts were assayed for adenylate cyclase activity in the presence and absence of 2.4 μM CaM. pUC19 is the cloning vector without the adenylate cyclase gene; pDA1 is the plasmid harboring the gene encoding the wild-type structure of adenylate cyclase; pDA3 is the plasmid carrying the mutant adenylate cyclase gene with Met in place of Lys at position 58. The results are an average of triplicate assays with a standard deviation of less than 5%.

After transforming the plasmid pDA1 (Figure 1) into *E. coli* strain NM522, CaM-sensitive adenylate cyclase activity was readily detectable in the sonicated cell extract (Figure 2). Although the *E. coli* host strain used in this experiment was *cya*$^+$, its endogenous enzymatic activity was too low to be detected under the described assay conditions. Therefore, the adenylate cyclase activity measured in this experiment was contributed entirely by the cloned *B. pertussis* enzyme.

The synthesis of the *B. pertussis* enzyme in *E. coli* was also analyzed by immunoblotting with polyclonal antibodies raised against the catalytic subunit of the *B. pertussis* enzyme. *E. coli* harboring the plasmid pDA1 produced two immunoreactive polypeptides with molecular masses of approximately 90 kDa which were absent in the whole cell lysate of the

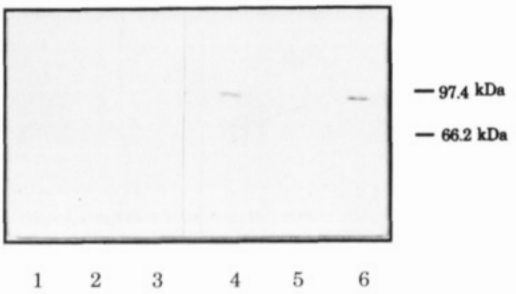

FIGURE 3: Immunoblot analysis of *B. pertussis* adenylate cyclase expressed in *E. coli*. A total of 20 μL of lysed cell extract from the indicated strains of *E. coli* was run on a 10% SDS–polyacrylamide gel. The proteins were then electrophoretically transferred onto nitrocellulose paper and examined for the presence of immunoreactive polypeptides by using polyclonal antibodies raised against the catalytic subunit of the *B. pertussis* adenylate cyclase. Lanes 1 and 4, NM522(pDA3); lanes 2 and 5, NM522(pUC19); lanes 3 and 6, NM522(pDA1). Lanes 1–3, incubated with preimmune IgG; lanes 4–6, incubated with immune IgG. pUC19 is the cloning vector without an adenylate cyclase gene; pDA1 is the plasmid harboring the gene encoding the wild-type structure of adenylate cyclase; pDA3 is the plasmid containing the gene for mutant adenylate cyclase containing methionine in place of lysine at position 58. The migration of molecular weight standards (phosphorylase B and bovine serum albumin) is indicated in the margin.

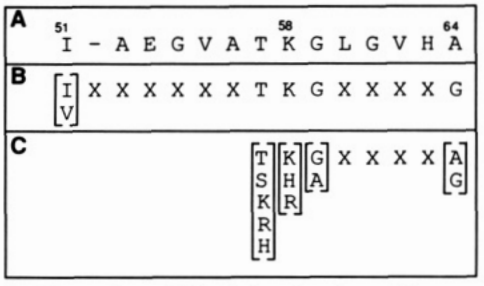

FIGURE 4: Comparison of the deduced amino acid sequence of *B. pertussis* adenylate cyclase with A-type ATP-binding domain consensus sequences. Panel A, the deduced amino acid sequence for the *B. pertussis* adenylate cyclase from amino acid 51 to amino acid 64 (Glaser et al., 1988a); panel B, the A-type consensus sequence for an ATP-binding domain (Walker et al., 1982); panel C, the A-type consensus sequence for an ATP-binding domain as modified by Chin et al. (1988). The sequence in panel A is presented from amino to carboxy terminal; the consensus sequences in panels B and C are presented from carboxy to amino terminal.

control strain (Figure 3). The lower molecular weight polypeptide was probably a proteolytic fragment derived from the upper band. The antibodies used in this experiment appeared to be specific since preimmune IgG gave no other detectable bands.

Site-Directed Mutagenesis of the B. pertussis cya A Gene. Analysis of the deduced amino acid sequence of the enzyme (Figure 4) revealed the presence of an inverted sequence that is analogous to a consensus sequence conserved among many ATP-binding proteins (Walker et al., 1982; Chin et al., 1988). Moreover, the sequence IAEGVATKGLGVHA in the *B. pertussis* adenylate cyclase contains a lysine residue at position 58 that may play a direct role in ATP binding and/or catalysis. To evaluate the importance of this amino acid residue for adenylate cyclase activity, a Lys 58 to Met 58 substitution mutation was generated by oligonucleotide-directed mutagenesis. In addition to incorporating base substitutions required for changing the wild-type Lys-encoding triplet into one designating Met, the oligonucleotide was designed to generate a restriction map that is different between the mutant and the wild type. Substitution of the codon specifying Lys (AAA) with that for Met (ATG) created an *Nco*I site in the

mutant. The vector used for mutagenesis was M13mp18 containing the 2.7-kb *Bam*HI–*Eco*RI *cya* A gene fragment. After amplification of the phage in a *dut*$^-$*ung*$^-$ *E. coli* strain, the synthetic 22-base oligonucleotide corresponding to the coding strand of the *cya* A gene was used to prime DNA synthesis and convert the single-stranded template into a double-stranded circular molecule. Introduction of this heteroduplex molecule into *E. coli* strain NM522 (*dut*$^+$*ung*$^+$) allowed for efficient recovery of mutant DNA. The presence of the desired mutation was verified by the appearance of the *Nco*I site in the DNA as well as by direct nucleotide sequencing (data not shown).

Expression of Mutant cya A Gene in E. coli. The 2.7-kb *Bam*HI–*Eco*RI fragment containing the mutant *cya* A gene was inserted into the *Bam*HI–*Eco*RI sites of pUC19 to generate pDA3. Transformation of *E. coli* strain NM522 with the recombinant plasmid pDA3 allowed the selection of clones resistant to ampicillin. Immunoblotting analysis of cell lysates prepared from mutant clones showed that they contained two immunoreactive polypeptides with electrophoretic mobilities and quantities identical with those found in the lysate of the wild-type strain (Figure 3). To examine the effect of the Lys to Met mutation on enzyme activity, the sonicated cell extract prepared from the mutant strain was assayed for adenylate cyclase activity in the presence and absence of calmodulin. The results indicated that replacement of lysine residue 58 with methionine abolished both the basal and CaM-stimulated activities of the enzyme (Figure 2).

Discussion

The CaM-sensitive adenylate cyclase is one of the major virulence factors produced by *B. pertussis*. This enzyme is one of the most active adenylate cyclases yet identified, and it has the ability to invade and elevate cAMP levels in animal cells (Masure et al., 1987). Weiss et al. (1984) reported that *B. pertussis* mutants lacking adenylate cyclase are avirulent, indicating that this enzyme plays an important role in the pathogenesis of the disease. It is therefore of fundamental importance to understand the structure–function relationships of this enzyme. Glaser et al. (1988a) recently cloned the gene encoding the enzyme and determined its nucleotide sequence. Analysis of the nucleotide sequence revealed an open reading frame that can code for a polypeptide of 1706 amino acids. These authors demonstrated that a 2.1-kb *Bam*HI–*Bgl*II fragment at the 5′ end of the gene was sufficient to encode the CaM-sensitive catalytic domain of the enzyme. Biochemical studies have demonstrated that the *B. pertussis* adenylate cyclase is synthesized as a large precursor that is proteolytically processed to the 45-kDa form found in culture supernatants (Masure & Storm, 1989).

The isolation of the *cya* A gene and its expression in *E. coli* made it possible to modify specific amino acid residues that may be crucial for catalytic activity. Walker et al. (1982) proposed two consensus sequences ("A-type" and "B-type") for ATP-binding domains on the basis of analysis of several enzymes that utilize ATP in catalysis. Chin et al. (1988) modified these two consensus sequences by comparing 50 nonhomologous ATP-binding proteins of both procaryotic and eucaryotic origins. Comparison of the predicted amino acid sequence of the catalytic domain of the *B. pertussis* adenylate cyclase with the A-type consensus sequences proposed by Walker et al. (1982) and by Chin et al. (1988) showed a region of striking homology if the amino terminal of the putative ATP-binding domain from the bacterial adenylate cyclase was aligned with the carboxy terminal of the consensus sequences (Figure 4). Two other lysines, also part of putative ATP-binding domains, were found at residues 65 and 355. The prescence of a conserved lysine residue within this sequence was of particular interest since it has been proposed that a lysine residue may be directly involved in the catalytic mechanism of bacterial and mammalian adenylate cyclases (Gerlt et al., 1980; Westcott et al., 1980). In addition, X-ray diffraction and NMR studies have shown that the A type sequence is part of the ATP-binding domain of adenylate kinase and that the conserved lysine may interact with the triphosphate moiety of the nucleotide (Pai et al., 1977; Fry et al., 1985, 1986). Therefore, we replaced Lys 58 with methionine, by means of site-directed mutagenesis, and expressed the mutant enzyme in *E. coli*. We chose methionine to replace lysine because the side-chain bond lengths and angles of these amino acids are similar, thus maintaining the overall structure of the enzyme. Although the mutant enzyme was an appropriate molecular size and was synthesized at a level equivalent to that of the wild-type *B. pertussis* enzyme expressed in *E. coli*, the mutant enzyme displayed no enzymatic activities in either the presence or absence of CaM. These results are thus consistent with the hypothesis that Lys 58 of the *B. pertussis* adenylate cyclase plays a critical role in catalysis. Furthermore, the availability of this adenylate cyclase mutant will clarify the role of the invasive enzyme in the pathogenesis of *B. pertussis* and may facilitate the development of a safe pertussis vaccine.

Acknowledgments

We thank Xia Zhengui and Dr. Mary Gross for helpful discussions and critical reading of the manuscript.

Registry No. 5′-ATP, 56-65-5; adenylate cyclase, 9012-42-4.

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Articles

Photoreceptor Channel Activation by Nucleotide Derivatives†

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Received July 28, 1988; Revised Manuscript Received November 4, 1988

ABSTRACT: Cyclic nucleotide activated sodium currents were recorded from photoreceptor outer segment membrane patches. The concentration of cGMP and structurally similar nucleotide derivatives was varied at the cytoplasmic membrane face; currents were generated at each concentration by the application of a voltage ramp. Nucleotide-activated currents were analyzed as a function of both concentration and membrane potential. For cGMP, the average $K_{0.5}$ at 0 mV was 24 μM, and the activation was cooperative with an average Hill coefficient of 2.3. Of the nucleotide derivatives examined, only 8-[[(fluorescein-5-ylcarbamoyl)methyl]thio]-cGMP (8-Fl-cGMP) activated the channel at lower concentrations than cGMP with a $K_{0.5}$ of 0.85 μM. The next most active derivative was 2-amino-6-mercaptopurine riboside 3',5'-monophosphate (6-SH-cGMP) which had a $K_{0.5}$ of 81 μM. cIMP and cAMP had very high $K_{0.5}$ values of $\sim$1.2 mM and >1.5 mM, respectively. All nucleotides displayed cooperativity in their response and were rapidly reversible. Maximal current for each derivative was compared to the current produced at 200 μM cGMP; only 8-Fl-cGMP produced an identical current. The partial agonists 6-SH-cGMP, cIMP, and cAMP activated currents which were $\sim$90%, 80%, and 25% of the cGMP response, respectively. 5'-GMP, 2-aminopurine riboside 3',5'-monophosphate, and 2'-deoxy-cGMP produced no detectable current. The $K_{0.5}$ values for cGMP activation, examined from -90 to +90 mV, displayed a weak voltage dependence of $\sim$400 mV/*e*-fold; the index of cooperativity was independent of the applied field. The current-voltage relationship at saturating cGMP concentrations was fitted by a simple voltage-dependent closed to open conformational change. The voltage dependence of this conformational change did not, however, account entirely for the voltage-dependent shift in $K_{0.5}$. Similarly, the voltage dependence could not be explained by a charged ligand moving into a binding site within the membrane field.

In vertebrate photoreceptor outer segments, light energy absorbed by rhodopsin regulates the flow of an electrical current through a cation channel in the plasmalemma. The cell's response to light results in a decrease of 3',5'-cGMP, which closes the channel and reduces the transmembrane current. For the photoreceptor channel, cGMP is a rapidly reversible, direct agonist of channel gating with a $K_{0.5}$[1] from

† This work was supported by NIH Grants EY-06640, BRSG 07083-21, and 05415-25 to J.C.T., University Research Fund and NIH Grants NS 00865 and BRSG S07-RR-05415-26 to R.E.F., NIH Grant GM 25256 to Paul Mueller, and the U.K. Medical Research Council (J.F.E.).
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[1] Abbreviations: 8-Br-cGMP, 8-bromoguanosine 3',5'-monophosphate; 8-Fl-cGMP, 8-[[(fluorescein-5-ylcarbamoyl)methyl]thio]-guanosine 3',5'-monophosphate; 6-SH-cGMP, 2-amino-6-mercaptopurine riboside 3',5'-monophosphate; 2-amino-cPMP, 2-aminopurine riboside 3',5'-monophosphate; *I*, transmembrane current; I_{max}, current produced by saturating ligand concentrations; *V*, transmembrane potential; V_0, potential for an *e*-fold change; N_h, Hill index of cooperativity; $K_{0.5}$, ligand concentration for half-maximal current activation; K_o, apparent equilibrium dissociation constant for channel opening; K_b, apparent equilibrium dissociation constant for ligand binding; *L*, ligand concentration, *IV*, plot of membrane current versus membrane voltage.

0006-2960/89/0428-2776$01.50/0