Characterization of the Bacterial Cell Associated Calmodulin-Sensitive Adenylate Cyclase from *Bordetella pertussis*†

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ABSTRACT: *Bordetella pertussis* produces a calmodulin-sensitive adenylate cyclase that is associated with the whole bacteria and released into its culture media. Preparations of this enzyme invade animal cells, causing elevations in intracellular cAMP levels. Cell-associated adenylate cyclase accounted for 28% of the total adenylate cyclase activity while 72% was released into the culture supernatant. Over 90% of the cell-associated adenylate cyclase activity was sensitive to trypsin treatment of whole cells, indicating that the catalytic domain of the enzyme is localized on the outer surface of the bacterial cells. Enzyme activity was released from whole cells by treatment with SDS. This activity was resolved as a large form (M_r 215 000) by SDS–polyacrylamide gel electrophoresis. In contrast, the culture supernatant contained only the 45 000-dalton catalytic subunit. Enzyme activity released from spheroplasts by sonication was resolved into a large form (M_r 215 000) and a small form (M_r 45 000). The appearance of the small form with spheroplast formation was probably the result of proteolytic degradation. Antibodies generated against the catalytic subunit purified from culture supernatants cross-reacted with and immunoprecipitated both the large and small forms of adenylate cyclase isolated from bacterial cells. Furthermore, incubation of the cell-associated enzyme with a crude bacterial extract resulted in a time-dependent disappearance of the 215 000-dalton form and a concomitant increase in the amount of the smaller 45 000-dalton form. There was also a parallel increase in the ability of the cell-associated preparation to elevate intracellular cAMP levels in N1E-115 mouse neuroblastoma cells. On the basis of these data, we propose that the adenylate cyclase produced by *B. pertussis* is synthesized as a large precursor molecule (M_r 215 000) and transported to the outer membrane of the bacteria where it is proteolytically processed to a smaller invasive form (M_r 45 000) that is released into the culture supernatant.

Bordetella pertussis releases several factors into its growth media, including a calmodulin (CaM)[1]-sensitive adenylate cyclase, that may contribute to the clinical manifestations of whooping cough [for reviews, see Weiss and Hewlett (1986) and Masure et al. (1987)]. Various preparations of this enzyme have been shown to elevate cAMP levels in several types of eucaryotic cells (Confer & Eaton, 1982; Hanski & Farfel, 1985; Shattuck & Storm, 1985; Selfe et al., 1987). Extracts from both whole bacteria and cell-free culture supernatants have been shown to contain invasive adenylate cyclase (Confer & Eaton, 1982; Hanski & Farfel, 1985; Shattuck & Storm, 1985).

The catalytic subunit of the adenylate cyclase from culture supernatants has been purified to homogeneity (Ladant et al., 1986; Masure et al., 1988). It is a polypeptide with reported apparent molecular weights ranging between 45 000 and 51 000. This catalytic subunit interacts directly with Ca^{2+}, and this interaction may be important for entry of the catalytic subunit into animal cells (Masure et al., 1988). There have been reports of several different forms of the adenylate cyclase associated with whole *B. pertussis* cells (Hanski & Farfel, 1985; Kessin & Franke, 1986; Freidman, 1987). Large forms of the enzyme were reported with apparent molecular weights ranging from 190 000 to 700 000. A small form of the enzyme has been consistently reported with an apparent molecular weight of 45 000–50 000. The relationships between the different forms of the enzyme have not been established, however.

In this study, we report that whole *B. pertussis* cells contains a single form of the adenylate cyclase with a molecular weight of 215 000 in contrast to the 45 000-dalton form found in culture supernatants. We present data which indicate that the smaller catalytic subunit may be derived from the 215 000-dalton form by proteolytic processing and that the 45 000-dalton catalytic subunit is released from bacterial cells into the culture supernatant.

Experimental Procedures

Enzyme Assays. Adenylate cyclase was assayed by the method of Salomon et al. (1974). Each sample contained 20 mM Tris-HCl (pH 7.4), 1 mM [^{32}P]ATP, 5 mM $MgCl_2$, 1 mM EDTA, 0.1% bovine serum albumin, and 2.4 μM CaM. One unit of adenylate cyclase activity catalyzes the synthesis of 1 nmol of cAMP/min. All assays were performed in the presence of 2.4 μM CaM.

Preparation of Calmodulin. CaM was prepared from bovine brain by the procedure of Masure et al. (1984).

Bacterial Growth and Isolation of the Invasive Adenylate Cyclase from Culture Supernatants. B. pertussis (Tahoma phase I) was grown from a 5% inoculum in supplemented Stainer–Scholte medium (Stainer & Scholte, 1971). Cells were harvested at an OD_{650} of 0.5. Invasive adenylate cyclase

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[1] Abbreviations: CaM, calmodulin; Chaps, 3-[(3-cholamidopropyl)-dimethylammomio]-1-propanesulfonate; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid; SDS, sodium dodecyl sulfate; TPCK, L-1-(tosylamino)-2-phenylethyl chloromethyl ketone; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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was isolated from culture supernatants by QAE-Sephadex chromatography as described by Masure et al. (1988). The catalytic subunit of the adenylate cyclase used for the generation of polyclonal antibodies was purified by DEAE-Sephadex chromatography as described by Ladant et al. (1986).

Antibody Production and Purification. Antibodies to the catalytic subunit from culture supernatants were raised according to procedures outlined by Ladant et al. (1986). Rabbits were immunized initially with a suspension consisting of 50 μg of antigen in 1 mL of phosphate-buffered saline and 1 mL of Freund's complete adjuvant. Rabbits were boosted on a weekly basis with a suspension consisting of 50 μg of antigen in 1 mL of phosphate-buffered saline and 1 mL of Freund's incomplete adjuvant. The immunoglobulin G fraction of serum was purified by protein A–Sepharose affinity chromatography.

Immunoprecipitation. Adenylate cyclase activity was immunoprecipitated from different enzyme preparations by the procedure described by Rosenberg and Storm (1987).

Cell Fractionation and Proteolysis. To isolate whole cells, bacteria were pelleted by centrifugation in a Beckman J6-3B centrifuge for 1 h at 4200 rpm. The cells were resuspended 5 to 1 (w/v) in 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 2 mM $MgCl_2$. Spheroplasts were formed by resuspension of the bacterial cell pellet 5 to 1 (w/v) in 20 mM Tris-HCl, pH 7.5, 0.25 M sucrose, and 0.01 M EDTA. This suspension was adjusted to 0.2 mg/mL lysozyme and allowed to stir for 1 h at 4 °C. The cells were spun for 1 h at 10000*g*. The decanted supernatant contained the contents of the periplasm. The cell pellet containing the spheroplasts was resuspended in 62.5 mM Tris-HCl, pH 6.8, and 2 mM $MgCl_2$. The cellular contents and membrane fragments were obtained from the spheroplasts by sonication with three to six 3-min pulses from a Branson Sonifier cell disruptor. During sonication, these cells were maintained at a temperature between 4 and 10 °C. The cellular debris containing broken cells were pelleted by centrifugation for 1 h at 25000*g*. The supernatant contained soluble proteins, membrane fragments, and no whole bacteria.

Different cellular preparations were incubated with TPCK-treated trypsin (20 μg/mL) or trypsin–Sepharose (200 μg/mL) for 30 min at 37 °C. The trypsin was neutralized with soybean trypsin inhibitor (40 μg/mL). All solutions added to the bacteria contained 0.2 mM phenylmethanesulfonyl fluoride except in instances where cellular suspensions were treated with trypsin. Some preparations of the enzyme were concentrated with Amicon PM-10 or PM-30 membranes, and all samples were stored at −80 °C.

SDS–Polyacrylamide Gel Electrophoresis and Electrophoretic Elution from Polyacrylamide Gel Slices. SDS–polyacrylamide gel electrophoresis was performed as described by Laemmli (1970). Different forms of the CaM-sensitive adenylate cyclase were further purified by SDS–polyacrylamide gel electrophoresis as described previously (Masure et al., 1988) with some modifications. Samples were equilibrated in SDS sample buffer [62.5 mM Tris-HCl, pH 6.8, 1 mM dithiothreitol, 5% (v/v) glycerol, 1% or 2% (w/v) SDS, and 0.1% bromophenol blue] and electrophoresed into a polyacrylamide gel. The gel lanes were cut into 0.4–0.5-cm slices and soaked in 250–500 μL of a soaking solution that contained 62.5 mM Tris-HCl, pH 6.8. To increase the recovery of enzyme activity, gel slices were frozen at −20 °C. Samples were thawed and aliquots assayed for enzyme activity.

Slices containing peak adenylate cyclase activity were eluted from gel slices by electrophoresis in the following fashion. A nondenaturing discontinuous polyacrylamide gel was prepared in the absence of SDS (Laemmli, 1970). The gel slice containing the peak fraction of adenylate cyclase activity was suspended in an empty gel well, 1 cm from the bottom. The solution in which the gel slice was soaked was adjusted to 5% (v/v) glycerol and 0.005% bromophenol blue and layered above and below the suspended gel slice. Current (20 mA) was applied to the gel for approximately 20 min and the protein thus eluted into the solution below the gel. This material was collected in a syringe and assayed for adenylate cyclase activity. The glycerol, bromophenol blue, and excess SDS were removed by pressure dialysis with an Amicon Micro-Ultrafiltration System. We estimate that 70–80% of the protein was recovered by this procedure.

Table I: Distribution of Adenylate Cyclase Activity Produced by *Bordetella pertussis*

sample[a]	act.[b] (nmol min^{-1} mL^{-1})	volume (mL)	total act. (nmol min^{-1})	distribution of act.[c] (%)
culture supernatant	13.0 (±3.3)	16500	214500	72
whole bacteria	395 (±34)	200	79000	26
spheroplasts	1330 (±96)	60.0	79600	
periplasm	5.20 (±.1)	165	858	0.3
sonicated spheroplasts	1410 (±73.5)	60.0	84600	28

[a] Tohoma phase I strain of *B. pertussis* was grown in 16.5 L of defined media (Stainer & Scholte, 1971) to an OD_{650} of 0.5. The different samples were prepared as described under Experimental Procedures. [b] Adenylate cyclase activity was measured according to Salmon et al. (1974) in the presence of 2.4 μM CaM. [c] The distribution of the CaM-sensitive adenylate cyclase activity is based on total activity assuming that the assay of whole bacteria represents enzyme accessible on the outer surface of the bacteria and the assay of sonicated spheroplasts represents the entire activity associated with the bacterial cells.

Determination of Intracellular cAMP Levels in Neuroblastoma Cells with B. pertussis Adenylate Cyclase. Elevation of intracellular cAMP in mouse neuroblastoma cells (N1E-115) exposed to different preparations of the bacterial adenylate cyclase was measured as described previously (Shattuck & Storm, 1985; Masure et al., 1988). cAMP was measured by a competitive binding assay with the regulatory subunit of cAMP-dependent protein kinase (Gilman, 1970).

Results

Localization of the Cell-Associated Adenylate Cyclase Activity. CaM-stimulated adenylate cyclase activity was detected in both culture supernatants (72% of the total activity) and whole bacteria (26% of the total activity, Table I). Liberation of the contents in the periplasm with spheroplast formation did not release a significant amount of enzyme activity. Assay of intact spheroplasts revealed that there was still a considerable amount of enzyme activity associated with the spheroplast vesicles, presumably through attachment of the enzyme to the inner membrane. Disruption of the spheroplasts by osmotic shock and sonication liberated the cell-associated adenylate cyclase activity.

CaM stimulation of adenylate cyclase activity with whole cells suggested that the majority of cell-associated catalytic activity may be accessible on the outside surface of the cell, assuming that CaM cannot cross the outer membrane. Therefore, various preparations of bacterial cells were treated with trypsin in order to determine what percentage of total cell-associated enzyme activity was accessible on the surface of bacterial cells. Whole bacteria (1470 ± 140 nmol min^{-1} mL^{-1}) and lysed spheroplasts (1600 ± 120 nmol min^{-1} mL^{-1}) were assayed for adenylate cyclase activity. Spheroplast

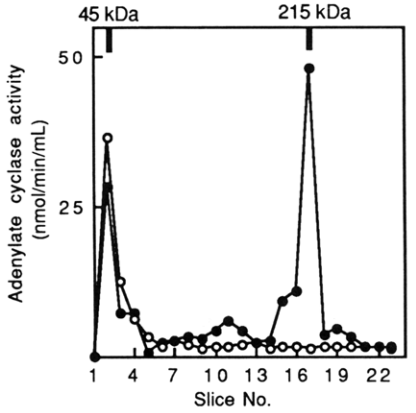

FIGURE 1: Migration profile from SDS–polyacrylamide gel electrophoresis of the cell-associated and culture supernatant forms of the CaM-stimulated adenylate cyclase from *B. pertussis*. 200 μL of cell-associated adenylate cyclase from sonicated spheroplasts (●) and invasive adenylate cyclase from culture supernatants (○) were equilibrated with SDS sample buffer [62.5 mM Tris-HCl, pH 6.8, 1 mM dithiothreitol, 5% (v/v) glycerol, 1% (w/v) SDS, and 0.1% bromophenol blue] and applied to a 6% polyacrylamide–SDS gel. Each sample contained 100 units (units = nanomoles per minute) of adenylate cyclase activity. Gel lanes were cut into 0.45-cm slices, and each slice was placed into 400 μL of a solution containing 20 mM Tris-HCl, pH 7.5, 40 mM NaCl, 2 mM $MgCl_2$, and 2% Chaps. Samples were frozen for 12 h at −20 °C, thawed, and assayed in the presence of 2.4 μM CaM and 0.08% Chaps. The apparent molecular weights of the peaks of adenylate cyclase activity were determined on the basis of the relative migration of the standards myosin (200K), β-galactosidase (116K), phosphorylase *b* (92K), bovine serum albumin (66K), and ovalbumin (45K). The cell-associated adenylate cyclase from spheroplasts and the cell-invasive adenylate cyclase from culture supernatants were prepared as described under Experimental Procedures.

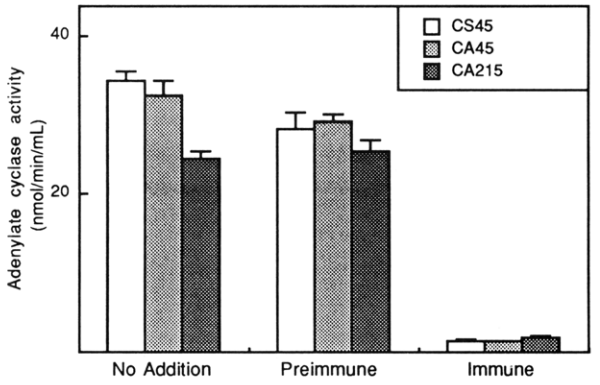

FIGURE 2: Immunoprecipitation of the three forms of the adenylate cyclase from *B. pertussis*. The catalytic subunit from an invasive preparation of the adenylate cyclase from culture supernatants (CS45) and the large (CA215) and small (CA45) forms of the cell-associated adenylate cyclase were incubated with either no addition, preimmune (20 μg/mL), or immune (20 μg/mL) IgG specific for CS45. The samples were incubated for 24 h at 4 °C in 50 mM Tris-HCl, pH 7.4, 250 mM sucrose, 5 mM $MgCl_2$, and 1 mM EDTA. Samples were next incubated with 100 μL of a suspension of *Staphylococcus aureus* cells for 4 h at 4 °C. The supernatants were collected following centrifugation and assayed for adenylate cyclase activity. The results are the average of triplicate points. Samples for immunoprecipitation were purified by electrophoretic elution from preparative SDS–polyacrylamide gel slices as described under Experimental Procedures.

formation, disruption by osmotic shock, and sonication only slightly increased the total amount of adenylate cyclase activity detectable. Trypsin treatment of these whole bacteria destroyed most of the adenylate cyclase activity detectable with whole cells (22.0 ± 3.0 nmol min^{-1} mL^{-1}). In addition, subsequent spheroplast formation and sonication of these trypsin-treated cells liberated only a small pool of covert adenylate cyclase activity (140 ± 40 nmol min^{-1} mL^{-1}). Identical results were obtained when whole cells were treated with trypsin–Sepharose. Therefore, loss of adenylate cyclase activity did not result from the passage of free trypsin through the outer cell membrane. We estimate that 92% of the adenylate cyclase activity associated with whole cells is found in the outer membrane with the catalytic domain accessible on the outside surface.

Identification of a Large (CA215) and a Small (CA45) Form of the Cell-Associated Adenylate Cyclase. B. pertussis adenylate cyclase can be renatured from SDS gels by the procedure described under Experimental Procedures. This property of the enzyme made it possible to determine the molecular weights for different forms of the enzyme. Cell-associated adenylate cyclase activity released from spheroplasts and invasive enzyme activity isolated from culture supernatants were submitted to SDS gel electrophoresis and enzyme activity recovered from individual gel slices (Figure 1). The adenylate cyclase activity released from spheroplasts ran as two peaks with apparent molecular weights of 215 000 and 45 000. In contrast, the invasive form of the enzyme, obtained from culture supernatants, ran as a single peak on SDS–polyacrylamide gels with an apparent molecular weight of 45 000. There was no evidence that the large form of the enzyme was released into the culture supernatant. The large (CA215) form of the cell-associated enzyme migrated with the same apparent molecular weight when extracted from SDS gels and rerun on another SDS gel, even in sample buffer containing 2% SDS (data not shown). This indicated that CA215 was not an aggregate of CA45 since it was not converted to the smaller form after boiling in 2% SDS.

Immunoprecipitation of Three Forms of Adenylate Cyclase. The presence of a large form of the enzyme associated with the bacterial cell and only a small form found in culture supernatants suggested that the adenylate cyclase may be synthesized as a large precursor polypeptide which is processed to a smaller invasive form of the enzyme. To test this hypothesis, antibodies specific to the 45 000-dalton form present in culture supernatants were isolated and examined for their ability to immunoprecipitate the three forms of adenylate cyclase described above (Figure 2). The catalytic subunit of the cell-invasive adenylate cyclase from culture supernatant (CS45), CA215, and CA45 were purified by electrophoretic elution from preparative SDS–polyacrylamide gels as described under Experimental Procedures. All three forms of the adenylate cyclase were immunoprecipitated by antibodies prepared against the catalytic subunit from culture supernatants. Preimmune immunoglobulin did not precipitate any of the adenylate cyclase preparations. These data illustrate that CA215 and CA45 are immunologically related to the catalytic subunit released into culture media.

Degradation of the Large (CA215) Form of the Cell-Associated Adenylate Cyclase and Production of an Invasive Adenylate Cyclase. To test the hypothesis that CA215 is converted to the smaller invasive form of the enzyme, an initial isolate from whole cells was compared to a mixture of CA215 and CA45 isolated from spheroplasts. In addition, the mixture obtained from spheroplasts was allowed to incubate at 4 °C, and samples were removed and frozen at −20 °C at timed intervals up to 20 h. These samples were analyzed by SDS–polyacrylamide gel electrophoresis (Figure 3) and assayed for their ability to elevate intracellular cAMP levels in neuroblastoma cells (Figure 4). *B. pertussis* was grown to an OD_{650} of 0.5, and cells were harvested by centrifugation for 30 min at 8000*g*. Cells obtained at this point were considered time zero. Whole cells lysed with SDS at zero time contained only

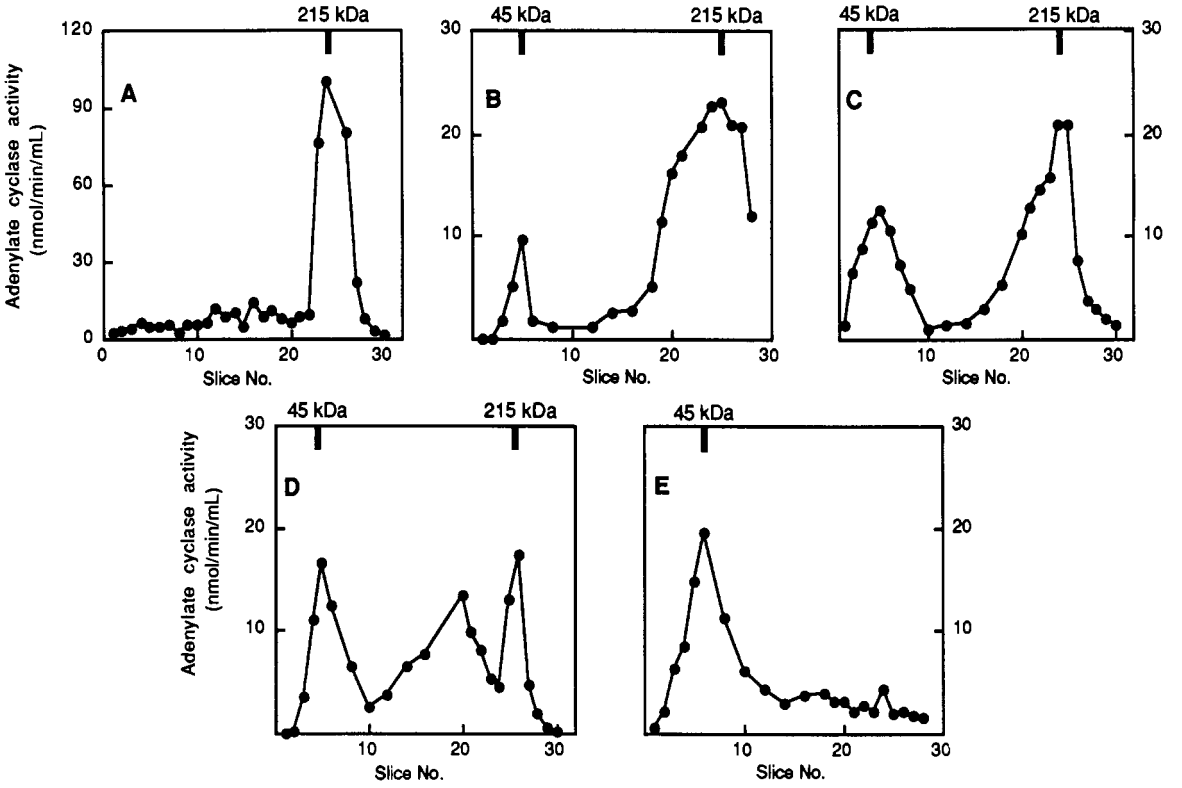

FIGURE 3: Degradation of the large (CA215) and production of the small (CA45) forms of the cell-associated adenylate cyclase from *B. pertussis*. (A) 5 g of fresh, packed cells was resuspended 5 to 1 (w/v) in SDS sample buffer (defined in Figure 3) and sonicated 2 × 60 s to shear DNA. A 200-μL aliquot was applied to a 6% polyacrylamide–SDS gel. A preparation containing cell-associated adenylate cyclase obtained from sonicated spheroplasts was allowed to incubate at 4 °C. Samples (200 μL) were removed at time intervals [(B) 2 h; (C) 4 h; (D) 12 h; and (E) 20 h], equilibrated with SDS sample buffer, and electrophoresed into a 6% polyacrylamide gel. Samples (A–E) in each gel lane were treated in a manner described in Figure 3 and assayed for adenylate cyclase activity. Whole cells were converted into spheroplasts as described under Experimental Procedures. Spheroplasts were resuspended (5 to 1, v/v) in 62.5 mM Tris-HCl, pH 6.8, and 2 mM $MgCl_2$ and sonicated 3 × 60 s with a 1-min interval between pulses. Liberated adenylate cyclase activity was separated from cell fragments by centrifugation at 25000*g* for 30 min. Time intervals were based from the initial isolation of bacterial cells. The adenylate cyclase activity (nanomoles per minute per milliliter) of this material at each time point (B–E) was 1280 (±180) at 2 h, 1437 (±50) at 4 h, 1421 (±70) at 12 h, and 1350 (±40) at 20 h.

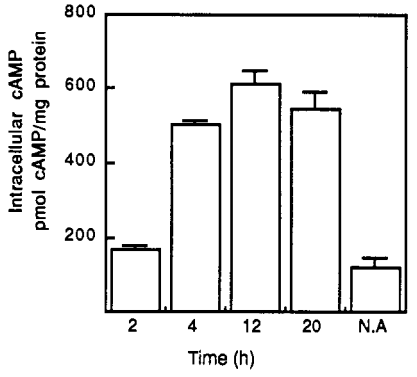

FIGURE 4: Production of an invasive form of the cell-associated adenylate cyclase from *B. pertussis*. 500 units of adenylate cyclase activity from the time points (2, 4, 12, and 20 h) of the material described in Figure 3 (panels B, C, D, and E) were incubated with mouse neuroblastoma cells (N1E-115) cells for 20 min at 37 °C as described in Masure et al. (1988). N.A. represents no addition and the basal level of cAMP in nontreated cells. The results are an average of duplicate assay points.

CA215. At 2 h, SDS–polyacrylamide gel analysis of the cell-associated adenylate cyclase from sonicated spheroplasts showed both the 45000- and 215000-dalton forms of the enzyme, with the latter predominating. With increasing time, the relative amount of the larger subunit decreased with a parallel increase in the amount of the 45000-dalton catalytic subunit. On the basis of the integrated areas for CA45 at 2 and at 20 h, the amount of CA45 had increased 8-fold. An intermediate form of the enzyme with a molecular weight of 110000 was observed at 12 h, but it was not present at 20 h. Although there may be intermediate forms of the enzyme between CA45 and CA215 which are produced by proteolysis, they were not characterized. The relative amounts of the two forms associated with whole cells varied from one preparation to another, and this probably reflects varying amounts of proteolysis from sample to sample. The enzyme preparations described above were tested for their ability to elevate cAMP levels in mouse neuroblastoma cells (Figure 4). The enzyme preparation at 2 h, which contained predominately CA215, had little or no effect on the intracellular cAMP levels of neuroblastoma cells. Preparations from the 4-, 12-, and 20-h time points did elevate intracellular cAMP levels when incubated with neuroblastoma cells. These data are consistent with the proposal that the bacteria produce a large cell-associated form of the enzyme, CA215, that is proteolytically processed to a smaller invasive form of the enzyme.

Discussion

The calmodulin-sensitive adenylate cyclase produced by *B. pertussis* was originally characterized as an extracellular enzyme (Hewlett & Wolff, 1976; Hewlett et al., 1976). There have been various reports for the molecular weight of this enzyme associated with whole cells or released into the culture media ranging from 45000 to 700000 (Hanski & Farfel, 1985; Shattuck et al., 1985; Kessin & Franke, 1986; Friedman, 1987). This study provides a clearer understanding of the relationships between the different forms of the enzyme. We

have identified a large form of the bacterial cell associated adenylate cyclase from *B. pertussis* with an apparent molecular weight of 215 000 (CA215) by SDS–polyacrylamide gel electrophoresis. CA215 is apparently degraded to a smaller 45 000-dalton form (CA45). The majority of the cell-associated catalytic activity was accessible on the outside surface of the bacterial cell. Most of the enzyme activity remained associated with the cells with the formation of spheroplasts, suggesting that there may be attachment sites associated with the inner membrane. In contrast to the large cell-associated form, only the small (M_r 45 000) catalytic subunit was detected in bacterial culture supernatants which contains the invasive form of the enzyme (Shattuck et al., 1985; Masure et al., 1988). The localization of the precursor of the adenylate cyclase on the outer membrane of the bacteria and presumed subsequent processing on this surface are different from the assembly and processing of other Gram-negative bacterial toxins. Pertussis toxin, cholera toxin, and the *Escherichia coli* heat-labile enterotoxin are synthesized as separate subunits, assembled in the periplasm, and transported across the outer membrane into the culture supernatant (Hirst & Holmgren, 1987a,b; Nicosia & Rappuoli, 1987; Hofstra & Witholt, 1984, 1985; Hirst et al., 1984).

The enzyme present in the culture supernatant and those forms associated with bacteria cells were recognized by an antibody raised against the invasive catalytic subunit. Furthermore, incubation of the cell-associated adenylate cyclase preparation for various periods of time resulted in a progressive increase in the smaller form of the enzyme, with the disappearance of the larger form. Paralleling this apparent conversion of CA215 to CA45 was an increase in the invasive properties of the enzyme preparation. In fact, the enzyme directly extracted from whole cells had no effect on intracellular cAMP levels of target cells. On the basis of these data, we propose that *B. pertussis* adenylate cyclase is synthesized in the bacteria as a large precursor molecule, CA215, that is proteolytically processed to form the smaller catalytic subunit. It is also possible that proteolytic processing of CA215 or other distinct proteins may release other polypeptides into the culture media that are important for invasion of animal cells by the catalytic subunit of the adenylate cyclase. The data presented in this study, however, do not address this issue.

The recent data describing the cloning and sequencing of the gene for the *B. pertussis* adenylate cyclase are consistent with the major conclusions of this study (Glaser et al., 1988). These investigators succeeded in isolating the entire gene for the adenylate cyclase which would code for a polypeptide of approximately 172 000 daltons. The catalytic domain was localized toward the N-terminal end of the polypeptide. When the cDNA was expressed in *E. coli*, a large molecular weight form of the enzyme was synthesized, but there was no formation of the 45 000-dalton catalytic subunit, or release of the enzyme into the culture media. Presumably, *E. coli* is unable to correctly process the precursor polypeptide normally for secretion. Although the apparent molecular weight of CA215 is greater than that predicted from the sequence data, molecular weights determined from SDS gels are sometimes misleading. The availability of the full-length clone for the enzyme should be a useful experimental tool for defining the mechanism of transport and processing of the adenylate cyclase to an active toxin.

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Registry No. Adenylate cyclase, 9012-42-4.

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