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Developmentally Regulated Compartmentalization of Adenylate Cyclase in *Dictyostelium discoideum*

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Adenylate cyclase of aggregation phase *Dictoystelium discoideum* is activated by extracellular adenosine 3′, 5′-cyclic monophosphate (cAMP), and the cAMP synthesized is secreted. The distribution of the enzyme was determined in sucrose gradients loaded with whole cell lysates. Cell lysates prepared after 4.5 hr of starvation revealed membranes containing adenylate cyclase at 44% and 33% sucrose. The activity of the latter peak was detected in the presence of the detergent (CHAPS), 3-(3-cholamidopropyl) dimethylammonio-3-propanesulfonate, which inhibited the activity of the former to some extent. Adenylate cyclase activity of the 2 peaks differed with respect to solubility in CHAPS and their kinetics. The 44% sucrose region of the gradient contained the bulk of the plasma membranes, as judged by a cell surface glycoprotein marker (contact site A). The 33% peak is composed of small vesicular structures, as determined by electron microscopy. The distribution of adenylate cyclase activity detected in sucrose gradients shifted from the 33% to the 44% sucrose peak during development. In addition, the 44% peak became increasingly resistant to the inhibitory effect of CHAPS. Both changes were accelerated by extracellular cAMP, but only the latter was abolished when the production of endogeneous cAMP was inhibited by caffeine. Pulsing cells with cAMP overcame the inhibitory effect of caffeine.

Key words: caffeine, intracellular compartments, development

The early developmental stages of *Dictyostelium discoideum* are characterized by the appearance of activatable adenylate cyclase and the production of adenosine 3′, 5′ -cyclic monophosphate (cAMP). The cAMP is secreted and, after binding to cell surface receptors, initiates a new cycle of activation. Since extracellular cyclic nucleotide phosphodiesterase restricts the duration of the stimulus and the response is subject to desensitization, spontaneous oscillations result during the aggregation phase, reviewed by Devreotes [1]. These features of the *D. discoideum* adenylate cyclase differ from the better known systems of higher eukaryotes in which cAMP is not secreted and extra-

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cellular cAMP does not stimulate the enzyme. A study of the adenylate cyclase of *D. discoideum* may, therefore, expose interesting functional and structural differences.

In higher eukaryotes, agonist-occupied receptors are linked to the adenylate cyclase by GTP-binding proteins. So far, attempts to demonstrate a similar organisation in *D. discoideum* have failed: the enzyme could not be activated by cAMP in vitro, and agents acting on GTP-binding proteins (i.e. fluoride, cholera toxin) have no effect on adenylate cyclase activity. However, it has been demonstrated recently that, in the presence of the detergent 3-(3-cholamidopropyl) dimethylammonio-3-propanesulfonate (CHAPS), Mg^{2+}-dependent adenylate cyclase activity was inhibited, suggesting that, as in other systems, *D. discoideum* cyclase occurs in a coupled (Mg^{2+} – or Mn^{2+} – dependent) and uncoupled (Mn^{2+} – dependent) state [2]. Moreover, the affinity of cell surface receptors for cAMP is lowered by GTP [3], and enzyme activity is enhanced by GTP, GppNHp and GTPγs in lysates of cAMP pretreated and control cells [4].

On the other hand, some unusual aspects of the system have been described. Hintermann and Parish [5] have reported that, in *D. discoideum*, adenylate cyclase is localized in an intracellular compartment. Similarly, Padh and Brenner [6] have suggested that the enzyme is incorporated into the membranes of intracellular vesicles which, on stimulation of the cell by cAMP, become transiently permeable to the substrate ATP otherwise sequestered from its target. I investigated the distribution of adenylate cyclase in sucrose gradients loaded with whole lysates of *D. discoideum*. Adenylate cyclase activity is detected at a high sucruse density, which corresponds to the bulk of the plasma membranes. A second peak of activity is detected at a lower sucrose concentration when assayed in the presence of the detergent CHAPS. The relative distribution between the 2 peaks of activity is developmentally regulated and affected by the presence of extracellular cAMP. The implications of these findings for the mechanism of activation of adenylate cyclase and for secretion of cAMP are discussed.

MATERIALS AND METHODS

Materials

cAMP, ATP, creatine kinase, phosphocreatine, DTT and caffeine were from Sigma. [α – ^{32}P]ATP was from New England Nuclear (NEG 003), CHAPS was purchased from Serva, and benzamidine hydrochloride from Janssen Chimica, Beerse, Belgium. Adenosine 3′, 5′-cyclic phosphorothioate (cAMPS) and [^{35}S]PAPS were gifts from Dr. F. Eckstein, Tuebingen, and Dr. R.W.H. Lee, EMBL, Heidelberg, respectively.

Cell Culture

Axenically grown strain AX2-214 cells were starved and harvested at the times indicated as described in [7].

Cell Lysis and Sucrose Gradients

Amoebae were lysed after resuspension in a lysis solution of pH 10 (10 mM Tris, 1 mM EDTA, 2 mM DTT, 5 mM benzamidine) at 5×10^7 cells per milliliter by applying 6 high voltage electric impulses (9.3 kV/cm) at intervals of 3 sec (for a description of the apparatus for generating high voltage electrical impulses of short duration, see [8]). The Plexiglas chamber containing the cell suspension had a diameter of 8 mm, and the electrodes were 14 mm apart. The resistance across the filled chamber amounted to 770Ω, and a 50 μF capacitor was used. After vortexing for 30 sec, more

than 95% of the cells were lysed, as judged by light microscopy. A 2-ml portion of lysed cells was loaded on a continuous sucrose gradient prepared in a Beckman SW 41 tube (10.5 ml of 20% to 55% sucrose (w/v) over 0.5 ml of a 60% cushion). Sucrose solutions were freshly prepared in lysis solution from which benzamidine was omitted. Gradients were run at 25,000 rpm for 1 hr and 1-ml fractions were collected. Cells and lysates were kept on ice except for the duration of lysis. Before assaying for adenylate cyclase, an aliquot of the fractions was brought to 7 mM CHAPS with a stock solution of 100 mM and incubated for 5 min on ice.

Adenylate Cyclase Assay

Reactions were performed in a total volume of 100 μl containing 50 mM Tris-HCl, pH 8.0; 5 mM cAMP; 20 mM phosphocreatine; 20 μg of creatine kinase; 10 mM DTT; 0.2 mM [$\alpha-^{32}$P]ATP (50 mCi/mmol); and, unless indicated otherwise, 2 mM $MnCl_2$. A 30-μl portion of the sucrose gradient fraction was added; in the cases where CHAPS was present during the assays, the final concentration was 2.1 mM. Reactions were started by the addition of the substrate, and [^{32}P]cAMP was isolated after 10 min at 24°C, as described by Salomon et al. [9].

Solubilization of Adenylate Cyclase

The appropriate gradient fractions were diluted 5-fold with lysis solution and centrifuged (100,000g_{max}, 1 hr). The pellets were solubilized in 10 mM Tris, 1 mM EDTA, 5 mM benzamidine, 1 mM dithiothreitol (DTT), 20% (v/v) glycerol, and 15 mM CHAPS for 30 min on ice, as described in [2]. Insoluble pellets obtained after centrifugation at 100,000g for 1 hr were resuspended in the same volume of solubilization buffer.

In Vitro Sulfation

In vitro sulfation of endogenous substrates was performed with aliqots of the sucrose gradient fractions as described [10]. Briefly, 80 μl of each fraction were incubated in a total volume of 100 μl containing 50 mM Hepes-NaOH, pH 7.4, 1 mM DTT, 5 mM $MgCl_2$, 2 mM $MnCl_2$, and 10 μM [^{35}S] 3′-phosphoadenosine-5′ phosphosulfate (PAPS) for 15 min at 22°C. The reaction was stopped by the addition of 50 μl of 3× concentrated sample buffer [11], and after boiling for 3 min, the proteins were separated on a 10% SDS-polyacrylamide gel and transferred to nitrocellulose [12]. The filters were dried, dipped in 20% PPO in toluene, and exposed to Kodak XAR-5 film at −70°C.

Electron Microscopy

Sucrose gradient fractions containing peak activities of adenylate cyclase were fixed with 1% glutaraldehyde and pelleted at 100,000g for 1 hr. The pellets were washed once with lysis solution, embedded in 2% agarose, and postfixed in 1% osmium tetraoxide. After dehydration in a graded series of ethanol and embedding in Epon, thin sections were stained with uranyl acetate and lead citrate and viewed in a Jeol JEM-100 CX electron microscope at 80 kV.

Immunoblots

A 50-μl portion of the sucrose fractions were solubilized by the addition of 25 μl of 3 × concentrated sampler buffer [11], boiled for 3 min and run on a 10% SDS-polyacrylamide gel. Contact sites A (csA) were detected after transferring the proteins

to nitrocellulose filters and labelling with the radioiodinated monoclonal antibody 33-294-17 directed against the protein part of csA as described [13].

RESULTS

When cells harvested 4.5 hr after the onset of starvation were lysed and fractionated on a sucrose gradient as described in Materials and Methods, adenylate cyclase activity was concentrated in a single band in an area of high sucrose concentration (average ± standard deviation of 17 experiments 43.6% ± 2%), corresponding to a band of visible turbidity (designated "heavy peak"). However, when the same fractions were assayed in the presence of the detergent CHAPS, a second peak at 33% ± 1.2% sucrose was revealed ("light peak"), whereas the activity of the first peak was reduced (Fig. 1).

Contact site A is a developmentally regulated cell surface 80-kd glycoprotein of *D. discoideum*, implicated in the formation of EDTA-stable cell – cell contacts [14]. Antibodies to csA were used to determine the position of plasma membranes within the sucrose gradient. Figure 2 shows that after incubation with the iodinated monoclonal antibody mAb 33-294-17, the label was mainly associated with the fractions containing the heavy peak of adenylate cyclase activity.

Sulfation, a post-translational modification of lysosomal, plasma membrane and secreted proteins, has been shown to occur in the Golgi apparatus of *D. discoideum* [10]. Transfer of the sulfate group from the donor (PAPS) to carbohydrate residues of endogeneous acceptor glycoproteins was performed in vitro in order to determine the position of Golgi membranes in the sucrose gradient. Sulfotransferase activity was detected in the fractions forming the top of the gradient and was found to overlap only slightly with the light peak of adenylate cyclase activity (Fig. 2, compare with Fig. 1).

In order to further characterize the 2 peaks of adenylate cyclase activity, they were examined by electron microscopy. These studies showed that vesicular structures predominate in the light peak, whereas large membrane fragments and mitochondria make up the heavy peak (Fig. 3). The origin of the vesicular structures found in the light peak cannot be inferred from these pictures. Most are small, with diameters of 0.2 μm or less. It should be mentioned that the method of fractionation was devised in order to

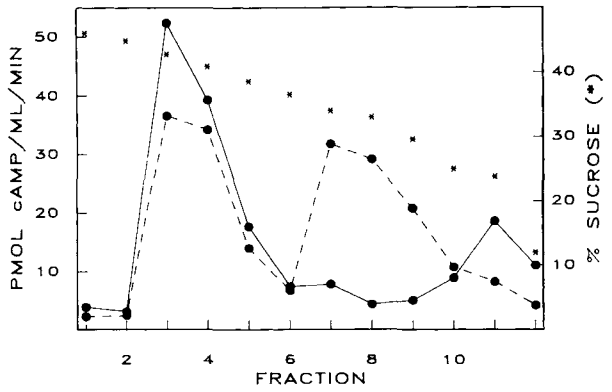

Fig. 1. Sucrose gradient fractionation of *D. discoideum* cells starved for 4.5 hr. Adenylate cyclase was assayed without (**solid line**) or with (**broken lines**) 2.1 mM CHAPS, as described in Materials and Methods.

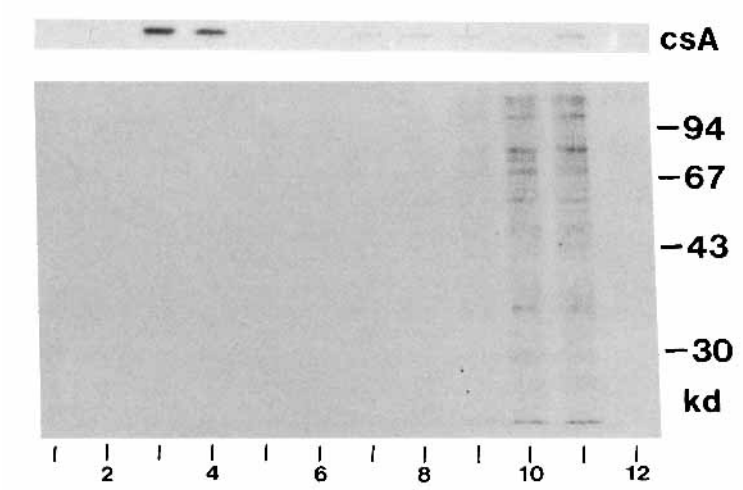

Fig. 2. **Top**, contact site A immunoblot of the fractions of the gradient shown in Figure 1. **Bottom**, sulfotransferase activity.

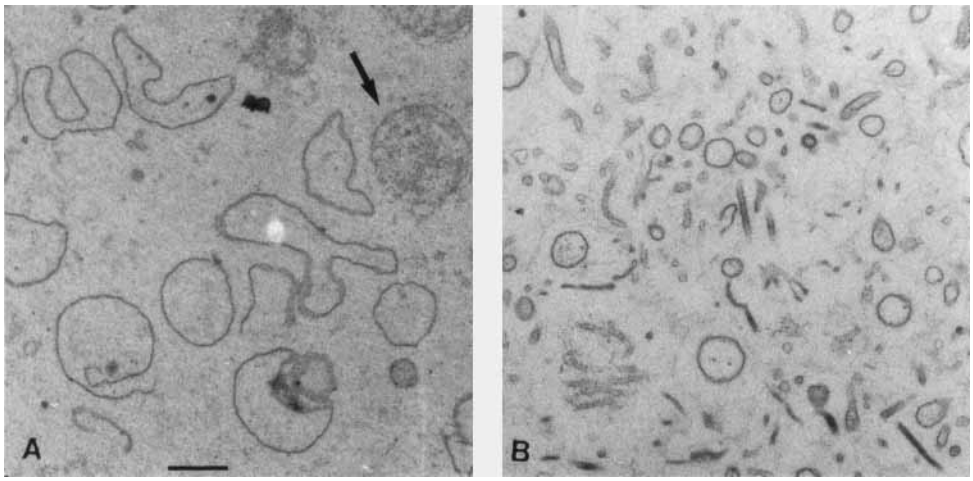

Fig. 3. Electron microscopic analysis of the heavy peak (**A**) and light peak (**B**). The arrow points to a mitochondrion. The length of the bar is 0.5 μm.

quickly separate the 2 adenylate cyclase compartments described. Complete separation of organelles can not be expected, hence the heterogenous appearance of the 2 pictures shown in Fig. 3.

I then examined whether the characteristics of the enzyme found in the 2 peaks differed from each other. It has been reported previously that up to 60% of the adenylate cyclase activity of *D. discoideum* can be solubilized by CHAPS [2]. The solubility in CHAPS of the two adenylate cyclase peaks was therefore investigated. The fractions closest to 44% and 33% sucrose were selected; and after dilution and centrifugation at 100,000*g*, the pellets were extracted with 15 mM CHAPS, as described in Materials

and Methods. Eighty-four percent (21.1 pmol cAMP/ml/min) of the enzyme activity recovered from the light peak was CHAPS soluble. However, only 19% (15.7 pmol/ml/min) of the activity in the heavy peak could be solubilized.

The kinetic behaviour of *D. discoideum* adenylate cyclase assayed in cell homogenates is nonMichaelian in the presence of Mn^{2+} [15, and personal observations], but Michaelian when Mg^{2+} is used as the divalent cation [Hagmann, unpublished observation]. Michaelian kinetics were also obtained when CHAPS-solubilized enzyme was assayed in the presence of Mn^{2+} (Hagmann, unpublished). Kinetic experiments were performed with the 2 peaks of adenylate cyclase activity. Michaelian kinetics were observed in the heavy peak with both cations. The K_m for the Mn^{2+}-dependent activity was 15 μM; with Mg^{2+}, it was 0.1 mM. The inhibitory effect of CHAPS on the Mn^{2+}-dependent activity of the heavy peak was noncompetitive (CHAPS completely inhibits Mg^{2+}-dependent activity [2]). In the light peak, however, Mn^{2+}-dependent activity was Michaelian only when detergent was included in the assay (Fig. 4). In this case, the K_m was the same as in the heavy peak (15 μM). In the absence of CHAPS, the kinetics were nonMichaelian with Mn^{2+}, and Michaelian with Mg^{2+}. Moreover, the curves for Mn^{2+}-dependent activity with and without CHAPS intersect the ordinate close to each other, indicating that the V_{max}, and hence probably the amount of enzyme, are similar. The ratio between the V_{max} of the Mn^{2+}-dependent and the V_{max} of the Mg^{2+}-dependent activity was 3 for the heavy peak and 5.5 in the light peak. Most likely, the Mg^{2+}-dependent activity and the activity measured at low ATP concentration in the light peak represent contaminating heavy peak enzyme.

The adenylate cyclase of *D. discoideum* is developmentally regulated, and under the conditions used here the steepest increase occurs between 2 and 5 hr after the onset of starvation. In order to establish whether the distribution of adenylate cyclase changes between the 2 peaks during development, cells were lysed after 3 and 6 hr of starvation and run on a sucrose gradient, and adenylate cyclase was assayed in the absence or presence of CHAPS. Figure 5 demonstrates that after 6 hr of starvation, the distribution

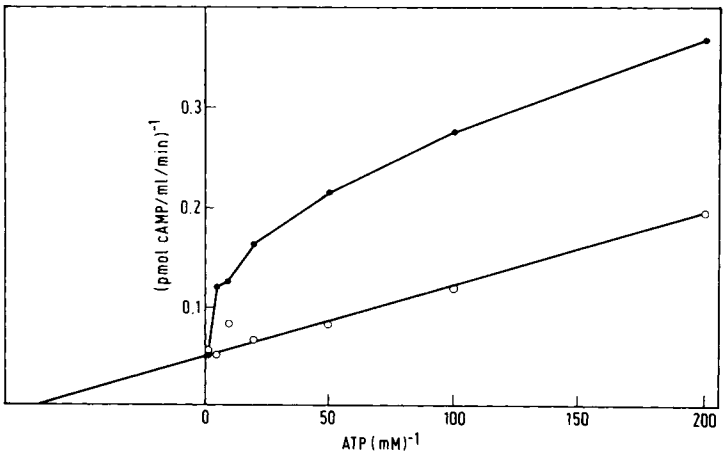

Fig. 4. Lineweaver-Burk plot of light peak adenylate cyclase assayed with 2mM $MnCl_2$ in the absence (●) or presence (○) of 2.1 mM CHAPS. Cells were starved for 4.5 hr.

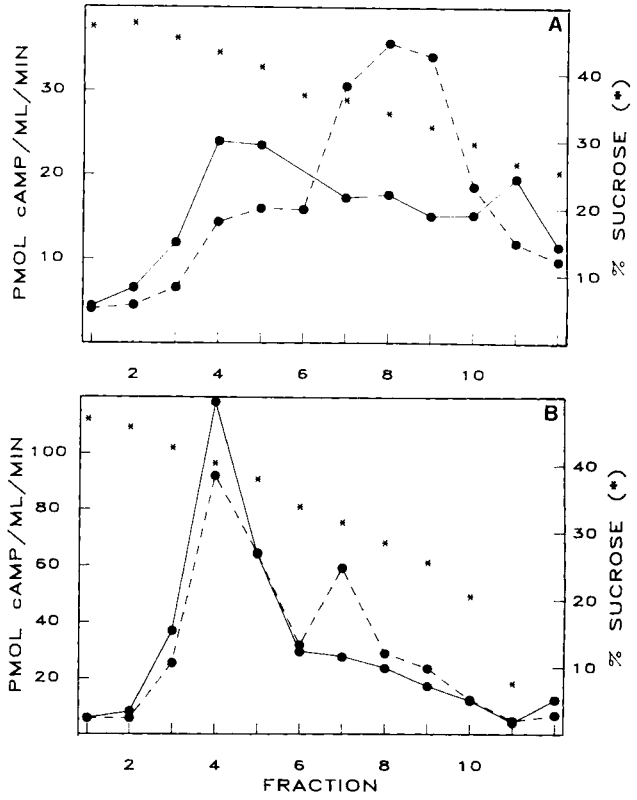

Fig. 5. Sucrose gradient analysis of adenylate cyclase of cells starved for 3 hr (**A**) or 6 hr (**B**). Symbols as in Figure 1.

of adenylate cyclase activity is shifted towards the heavy peak. In addition, the amount of heavy peak adenylate cyclase activity which is inhibited by CHAPS decreases during development. The shift in adenylate cyclase distribution and CHAPS sensitivity was also present in lysates of cells starved for 4 hr in the presence of 10^{-5} M cAMPS, a non-hydrolysable analog of cAMP, and in cell lysates prepared after pulsatile addition for 20 nM cAMP every 6 min (not shown).

Caffeine added at a 5 mM concentration to cells of *D. discoideum* cultured in nutrient medium induces the expression of adenylate cyclase [16]. When cells were maintained under these conditions for 5 hr, and lysed and fractionated on a sucrose gradient, a picture identical to the one shown in Figure 5A was obtained. Figure 6 shows the results from an experiment, in which 5 mM caffeine was present during 6 hr of starvation. Caffeine did not prevent the shift of activity from the light peak to the heavy peak during development. The CHAPS sensitivity of the heavy peak, however, persisted under these conditions. In developing cells, caffeine has the additional effect of blocking the activation of adenylate cyclase by extracellular cAMP and, consequently, endogenous oscillations of cAMP production. Other responses to extracellular cAMP tested, e.g., increased cGMP levels, chemotaxis, and light scattering, were not affected [17]. I therefore examined whether pulsatile addition of 20 nM cAMP every 6 min overcame the effect of caffeine. Cells were starved for 4.5 hr with caffeine, and Figure 6C shows

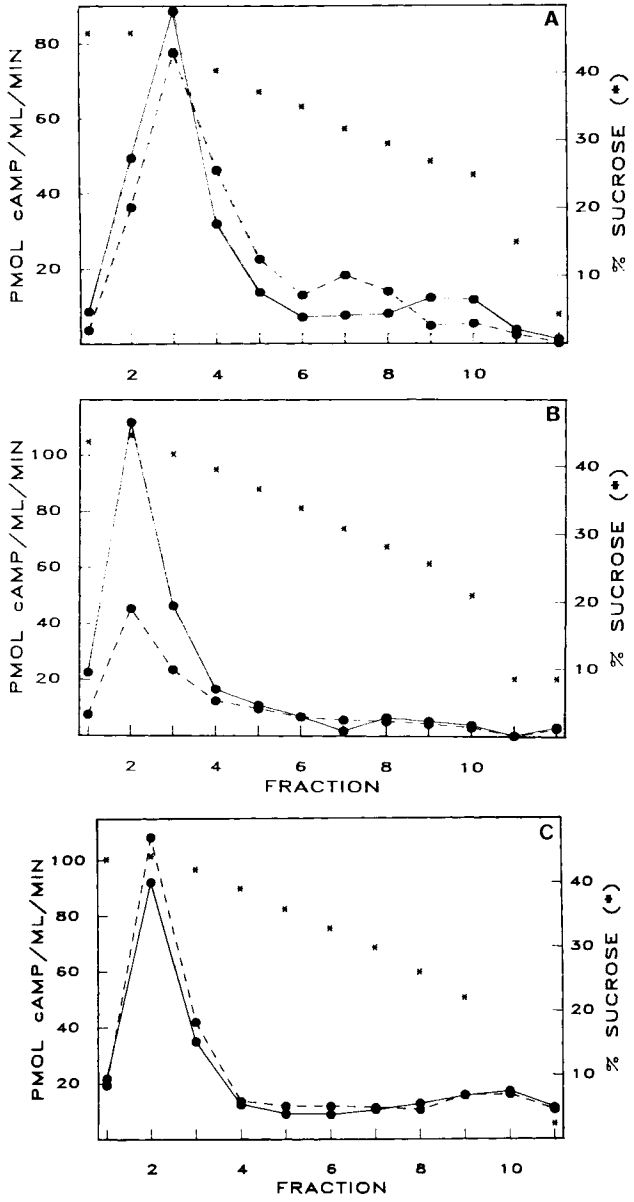

Fig. 6. Effect of caffeine on the distribution of adenylate cyclase. **A**, control; **B**, 5 mM caffeine; **C**, 5 mM caffeine and pulsed with 20 nM cAMP every 6 min. Symbols as in Figure 1. Cells in A and B were starved for 6 hr, cells in C for 4.5 hr.

that simultaneous pulsing with cAMP enabled the cells to acquire CHAPS-resistant heavy peak adenylate cyclase.

DISCUSSION

Whole cell lysates of *D. discoideum* starved for 4.5 hr demonstrate 2 peaks of adenylate cyclase activity at sucrose concentrations of 44% and 33% ("heavy" and "light" peaks). The light peak was detected in the presence of the detergency CHAPS, the addition of which inhibited heavy peak activity to some extent. Lysis of the amoebae was achieved by high voltage electric impulses, a quick and gentle method. Breakdown of the cell membrane occurs at a transmembrane potential of about 1 V, resulting in cell permeabilization, cell–cell fusion and, at higher voltages, cell lysis [18]. Since the transmembrane potential is proportional to the diameter of the vesicular structures, small intracellular organelles are left intact even after repeated impulses. Estimated transmembrane potentials at the conditions used here are 7 V for the cell membrane and 140 mV for vesicles with a diameter of 0.2 μm [19]. The observations described in this paper are not an artifact of the lysis method: the nonMichaelian kinetics which characterize Mn^{2+}-dependent adenylate cyclase activity of the light peak are also found in homogenates of whole cells prepared by freezing and thawing and in particulate fractions obtained after nitrogen cavitation [Hagmann, unpublished] and have first been reported by de Gunzburg et al., who used cells homogenized in a Thomas potter [15]. In addition, several batches of particulate fractions showed an increase in activity when CHAPS was included in the assay (data not shown).

Detection of the light peak only in the presence of the detergent CHAPS could be due to the formation of sealed outside-out plasma membrane vesicles containing adenylate cyclase inaccessible to the substrate. While this possibility cannot be excluded entirely, such outside-out vesicles would have to fulfill the following requirements: (1) they would have to form from specialized domains of the celll membrane since the quantity of csA, a cell membrane glycoprotein [14], does not correlate with the light peak activity; (2) adenylate cyclase localized in these vesicles would have to be functionally and/or structurally different from the enzyme found in the heavy peak membrane fragments, since the latter resists solubilization by CHAPS, whereas the former does not. More likely, light peak adenylate cyclase is localized in an intracellular vesicular compartment. An intracellular localization of *D. discoideum* adenylate cyclase has been suggested by several authors: Hintermann and Parish [5] have reported results of subcellular fractionation studies which showed that cyclase activity was not enriched in fractions containing cell membrane markers. However, cell membranes were prepared in the presence of Triton X-100 which might have interfered with their assays. More recently, Padh and Brenner [6] have observed increased cyclase activity when radioactive substrate was present during cell lysis. They concluded that the enzyme is localized in the membranes of intracellular vesicles, the catalytic site inside, and that with stimulation or cell lysis the vesicles become transiently permeable to ATP. This view is compatible with the results reported here. CHAPS may permeabilize such vesicles, rendering the catalytic site accessible to ATP. Furthermore, the nonMichaelian kinetics could be due to limited transport of ATP. In vivo, after stimulation by extracellular cAMP, the vesicles may fuse with the cell membrane and discharge the cAMP which has been synthesized. However, the catalytic site would then be on the cell surface. Yet, I was unable to demonstrate adenylate cyclase activity when the substrate was added to intact cells.

Moreover, resuspension of washed light peak material in buffer containing 0.25 M sucrose or in the absence of sucrose did not affect adenylate cyclase activity measured at 0.1 mM ATP and 2 mM $MnCl_2$, indicating either that sequestering is not involved or that the vesicles are resistant to strong osmotic changes. And finally, trypsin treatment of the light peak decreased cyclase activity to the same extent when it was subsequently measured in the presence or absence of CHAPS (data not shown). An alternative explanation is that adenylate cyclase is inserted in intracellular vesicles with the catalytic site facing the cytoplasm. In the absence of CHAPS, the enzyme might be masked or blocked by an inhibitor and show anticooperative kinetics towards ATP.

De Gunzburt et al. assayed *D. discoideum* adenylate cyclase in cell homogenates with $MnCl_2$ and obtained nonMichaelian kinetics [15]. I confirmed these results but found that the kinetics were Michaelian when $MgCl_2$ was the divalent cation or when CHAPS-solubilized enzyme was used with $MnCl_2$ [unpublished]. The results presented in this paper show that nonMichaelian kinetics are contributed by the Mn^{2+}-dependent light peak activity and that addition of CHAPS to the light peak changes the kinetics to Michaelian. Two points of interest are: first, the K_m of the Mn^{2+}-dependent light peak activity with CHAPS (15 μM) is the same as the K_m of the Mn^{2+}-dependent heavy peak activity (with or without CHAPS). This suggests that the enzyme is the same in both peaks. Secondly, Mg^{2+}-dependent activity is Michaelian in both peaks, and the ratio between the V_{max} of the Mn^{2+}-dependent and Mg^{2+}-dependent activities is higher in the light peak. This argues for the possibility that light peak activity is inactive with $MgCl_2$ and that the Mg^{2+}-dependent activity observed in these fractions is due to contaminating heavy peak enzyme (the extent of the contamination by cell membranes of fractions in the low sucrose range was variable as judged by csA profiles). In the preparation used for the kinetic experiments, the ratio between the Mn^{2+}-dependent adenylate cyclase activity in the presence and absence of CHAPS was 1.88 at 5 μM ATP in the light peak. This is similar to the ratio of 1.83 obtained when the ratios V_{max} with Mn^{2+}/V_{max} with Mg^{2+} between the light peak, (5.5), and the heavy peak (3), are compared. This agrees with the hypothesis that heavy peak adenylate cyclase is Mg^{2+}- and Mn^{2+}-dependent and that light peak adenylate cyclase is only Mn^{2+}-dependent and not detected in the absence of CHAPS when low concentrations of ATP are used. It also agrees with the suggestion made above that adenylate cyclase activity detected in fractions of the light peak with Mg^{2+} or in the absence of CHAPS at low ATP concentrations with Mn^{2+} is due to contaminating heavy peak activity. Whether light peak adenylate cyclase represents a form uncoupled from a GTP-binding protein remains to be clarified.

Two changes were observed during development: a relative shift of activity from the light to the heavy peak and increased CHAPS resistance in the heavy peak. Pulsatile additions of cAMP and, to a lesser extent, the continuous presence of cAMPS, a nonhydrolysable cAMP analog [20], accelerate early development in *D. discoideum*. Both also stimulate the changes mentioned. Caffeine has 2 effects on the adenylate cyclase system of *D. discoideum*. In nutrient medium, it induces *expression* of the enzyme without development [16]. In starved cells it inhibits *stimulation* of adenylate cyclase activity by extracellular cAMP and the subsequent release of cAMP [17]. Both effects may be due to an increase in free cytosolic Ca^{2+} concentrations [17]. These effects were used to study the cAMP dependence of the 2 developmental changes mentioned. The shift of activity from the light peak to the heavy peak appears to be independent of extracellular cAMP. In fact, a large fraction of the activity was found

in the heavy peak when adenylate cyclase was induced in nutrient medium by caffeine. When 5 mM caffeine was included in the starvation buffer, thereby blocking endogenous stimulation of adenylate cyclase and release of cAMP, the shift still occurred (Fig. 6B). Acquisition of CHAPS-resistance, on the other hand, seems to depend on extracellular cAMP: it is blocked by caffeine, but the inhibition can be overcome by the pulsatile addition of cAMP (Fig. 6C). Since caffeine blocks the stimulation of adenylate cyclase by extracellular cAMP, but not of guanylate cyclase [17], intracellular cGMP might be responsible for the change in detergent sensitivity observed. Interestingly, the mutant HSB1, which expresses the markers characterizing the aggregation-competent stage including adenylate cyclase but lacks activatability of adenylate cyclase [17a], shows the same behaviour as wild-type cells starved in the presence of caffeine when adenylate cyclase is analyzed on sucrose gradients (data not shown).

A method similar to the one described here, sucrose gradient centrifugation of whole cell lysates, was used by Harden et al. and Toews et al., who studied the desensitization of β-adrenergic receptors in astrocytoma cells [21,22]. They observed a biphasic distribution of ligand binding and a shift towards a low sucrose peak upon desensitization with β-adrenergic agonists. Their conclusion was that β-adrenergic receptors shuttle between an intracellular vesicular compartment and the cell membrane. I suggest that in *D. discoideum*, adenylate cyclase is transported from a vesicular, intracellular compartment which is not identical with the Golgi (Fig. 2) to the cell membrane. However, this transit seems to be unidirectional, since activity accumulates in the heavy peak during development. In addition, functional and/or structural changes occur, from a Mn^{2+}-dependent form which can be solubilized by CHAPS to a Mg^{2+}- and Mn^{2+}-dependent (GTP-binding protein coupled?), CHAPS-sensitive form, which acquires CHAPS- resistance in the presence of extracellular cAMP. Possibly, these changes are related to the mechanisms of enzyme activation and cAMP secretion. An intriguing prediction based on an unidirectional model is that activation of adenylate cyclase would depend on newly synthesized enzyme. Small intracellular vesicles which fuse with the cell membrane have been observed in *D. discoideum*. Their number oscillates concomitantly with endogenous or exogenous cAMP pulses; it was suggested that these vesicles contain adenylate cyclase [23]. Further research should clarify the nature of the vesicles, the orientation of adenylate cyclase, and the molecular basis for the functional changes described in this paper.

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REFERENCES

1. Devreotes PN: In Loomis WF (ed): "The Development of Dictyostelium discoideum." New York: Academic Press, 1982, pp 117–168.
2. Hagmann, J: Cell Biol Int Rep 9:491–494, 1985.
3. Van Haastert PJM: Biochem Biophys Res Commun 124:597–604, 1984.
4. Theibert A, Devreotes PN: J Biol Chem 261:15121–15125, 1986.
5. Hintermann R, Parish RW: Exp Cell Res 123:429–434, 1979.
6. Padh H, Brenner M: J Biol Chem 260:3613–3616, 1985.

7. Malchow D, Nagele B, Schwarz H, Gerisch G: Eur J Biochem 28:136–142, 1972.
8. Neumann E, Rosenheck K: J Membr Biol 10:279–290, 1972.
9. Salomon Y, Londos L, Rodbell M: Anal Biochem 58:541–548, 1974.
10. Hohmann H-P, Gerisch G, Lee RWH, and Huttner WB: J Biol Chem 260:13869–13878, 1985.
11. Laemmli UK: Nature 227:680–685, 1970.
12. Towbin H, Staehelin T, Gordon J: Proc Natl Acad Sci USA 76:4350–4354, 1979.
13. Bertholdt G, Stadler J, Bozarro S, Fichtner B, Gerisch G: Cell Diff 16:187–202, 1985.
14. Mueller K, Gerisch G: Nature 274:445–449, 1978.
15. De Gunzburg J, Veron M, Brachet P: Cell Biol Int Rep 4:533–540, 1980.
16. Hagmann J: EMBO J 5:3437–3440, 1986.
17. Brenner M, Thoms SD: Dev Biol 101:136–146, 1984.
17a. Bozarro S, Hagmann J, Noegel A, Calautti E: Dev Biol 123:540–548, 1987.
18. Zimmerman U: BBA 694:227–277, 1982.
19. Knight DE, Baker PF: J Membr Biol 68:107–140, 1982.
20. Rossier C, Gerisch G, Malchow D, Eckstein F: J Cell Sci 35:321–338, 1978.
21. Harden TK, Cotton CU, Waldo GL, Lutton JK, Perkins JP: Science 210:441–443, 1980.
22. Toews ML, Waldo GL, Harden TK, Perkins JP: J Biol Chem 259:11844–11850, 1984.
23. Maeda Y, Gerisch G: Exp Cell Res 110:119–126, 1977.