J. Cell Sci. **22**, 49–58 (1976)
Printed in Great Britain

A THEORETICAL STUDY OF THE EFFECTS OF CYCLIC AMP PHOSPHODIESTERASES DURING AGGREGATION IN *DICTYOSTELIUM*

VIDYANAND NANJUNDIAH AND DIETER MALCHOW*
Biozentrum der Universität, CH-4056 Basel, Switzerland

SUMMARY

During aggregation the larger *Dictyostelium* species use cAMP as a chemoattractant and possibly also as a transmitter. In passage from cell to cell, cAMP levels are modulated by diffusion and by enzyme hydrolysis. It appears that the important cAMP-hydrolysing enzyme is a phosphodiesterase bound to the cell membrane, the main roles of which are (1) very fast hydrolysis of cAMP and (2) steepening of spatial cAMP gradients. An extracellular phosphodiesterase has no function, so far as can be conjectured from present data.

INTRODUCTION

Triggered by starvation, unicellular amoebae of the cellular slime mould species aggregate in characteristic patterns via coordinated movements which are often pulsatile (Bonner, 1967). These movements are chemotactic, and a naturally occurring chemoattractant in the larger *Dictyostelium* species is cyclic AMP (cAMP; Konijn, 1972). After release by source cells, the amount of cAMP detected by another cell is modulated by (*a*) diffusion on and into the substratum, and (*b*) hydrolysis. Hydrolysis occurs on account of 2 cAMP-phosphodiesterases: (1) an extracellular enzyme (ePD; Chang, 1968) released by the cells during aggregation together with a macromolecular inhibitor (Riedel, Malchow, Gerisch & Naegele, 1972; G. Gerisch, in preparation); and (2) a cell membrane-bound enzyme (mPD; Pannbacker & Bravard, 1972; Malchow, Naegele, Schwarz & Gerisch, 1972) accessible to external cAMP, increasing in activity from the end of growth on, and reaching maximal activity at the time of aggregation ($t = 8$ h). These 2 enzymes differ in that *in vivo*, the mPD is not inhibited by the inhibitor of the ePD; and whereas the ePD exhibits Michaelian kinetics, the mPD is markedly non-Michaelian (Malchow, Fuchila & Nanjundiah, 1975).

Here we report an analytical and numerical study of some of the effects that these enzymes can be expected to have, when considered together with diffusion, in modulating cAMP levels that occur during aggregation at the site of a responding cell. In parts this work is an extension of that of Cohen & Robertson (1971), with the new information that there are 2 phosphodiesterases, and with more accurate enzyme data. All numerical values are from observations on *Dictyostelium discoideum* at the aggregation-competent stage.

* To whom reprint requests should be addressed.

PARAMETER VALUES

We indicate briefly below how these were estimated. A list of the numerical values used by us is given in Table 1.

Table 1. *Values for the different parameters used in this study, assumed to hold for aggregation-competent Dictyostelium discoideum amoebae*

	Parameter	Value	Source
Amoebae	Minimal density for aggregation by wave propagation	$5 \times 10^4/cm^2$	Konijn & Raper (1961)
	Close-packing on a plane	$1 \times 10^6/cm^2$	Own estimate
	'Standard' density	$1 \times 10^5/cm^2$	
	Cell volume	5×10^{-10} ml	
	Cell diameter on a plane	10 μm	
cAMP	Diffusion coefficient in agar	1×10^{-5} cm^2/s	Cohen *et al.* (1975)
	Pulsatile release	1×10^7 mol/amoeba	Own estimate, from Gerisch & Wick (1975)
ePD	Activity on agar surface during aggregation at close-packed cell density	1×10^{-6} M/s	Own estimate, based on Gerisch (in preparation)
	K (0·5)	4×10^{-6} M	Riedel *et al.* (1972)
mPD	K (0·5)	1×10^{-6} M	Malchow *et al.* (1975)
	Effective Hill coefficient H	0·7	
	Negative-cooperativity parameters	Chosen to yield H (min.) = 0·7 K (0·5) = 1×10^{-6} M	Own estimate; model of Russel *et al.* (1972)
	Maximum activity on a per-cell basis	4×10^8 mol cAMP/amoeba-min	Malchow *et al.* (1975)
cAMP receptor	Number of sites	5×10^5/amoeba	Malchow & Gerisch (1974)
	K (0·5)	1×10^{-7} M	

As mentioned in the text, we have also studied the effects of varying these values.

The amoebae

These are considered to be distributed on a plane surface in a density range of $5 \times 10^4/cm^2$ (minimal density for aggregation) (Konijn & Raper, 1961) to $1 \times 10^6/cm^2$ (confluency); a 'standard' density used for making various comparisons is $1 \times 10^5/cm^2$. The amoebae were assumed to be in an aqueous film of depth 1 μm. We have looked at the effect on signal profiles both when the cell distribution is approximated by a continuum and when it is taken to be discrete (a ring of amoebae centred about the source, with no intervening cells).

The cAMP signal

We assume cAMP release by the cells to be pulsatile, with 1×10^7 molecules released by a cell in one pulse. This figure follows from measurements of extracellular cAMP levels in cell suspensions (Gerisch & Wick, 1975), and it is consistent with the fact that with such a pulse size the peak cAMP concentration at nearest-neighbour

distance at minimal aggregation density (50 μM) is about 2×10^{-8} M, sufficient for a chemotactic response by an amoeba (Konijn, 1972). After release, cAMP is assumed to diffuse in the aqueous film with a diffusion constant (Cohen, Drage & Robertson, 1975)

$$D_{cAMP} = 10^{-5} \text{ cm}^2/\text{s}. \quad (1)$$

Diffusion was taken to be two-dimensional, and concentrations were obtained by dividing by the film thickness of 1 μm. We have verified that the errors caused by this approximation to three-dimensional diffusion were small (Appendix A, p. 57).

ePD activity under agar-plate conditions

As mentioned earlier, under these conditions the amoebae release enzyme as well as its inhibitor during interphase; however, the actual amounts vary very much with the (wild-type) strain used, within the same species. From the experimental data, one can take into account inhibitor action and estimate the ePD activity on an agar surface during aggregation; for *D. discoideum* NC-4, this is

$$A_{ePD} = 1 \times 10^{-6} \text{ M/s} \quad (2)$$

with a close-packed cell layer. With strain M1, the ePD activity is lower than this by a factor of ten or more. We shall use the NC-4 figure in what follows as a useful upper limit.

The kinetics of cAMP hydrolysis by ePD was taken to be Michaelian, with a Michaelis constant (Riedel *et al.* 1972)

$$K_{ePD} = 4 \times 10^{-6} \text{ M}. \quad (3)$$

mPD activity

The cAMP-hydrolysing effect of the mPD is sharply localized in space: it is non-zero only inside a distance d from the cell surface. d is determined by the relative rates of free cAMP diffusion and of equilibration with the cell surface enzyme. If we make the reasonable assumption that diffusion of cAMP to the cell is rate-limiting for formation of the enzyme-substrate complex, and take a figure of 1 ms as an upper limit for the cAMP equilibration-time with the enzyme, we get $d = 1$ μm. On this basis, taking a maximal rate of cAMP hydrolysis by mPD (Malchow *et al.* 1975) of

$$V_{mPD} = 4 \times 10^8 \text{ mol of cAMP/cell-min} \quad (4)$$

the activity of the mPD near the cell surface works out to

$$A_{mPD} = 2 \times 10^{-5} \text{ M/s}. \quad (5)$$

To take into account the non-linear kinetics of cAMP hydrolysis by mPD (Malchow *et al.* 1975) we have considered 2 alternatives: (*a*) an enzyme with a constant Hill coefficient

$$H = 0{\cdot}7 \quad (6)$$

and substrate level at half-maximal activity

$$K_{mPD} = 1 \times 10^{-6} \text{ M}, \quad (7)$$

and (*b*) an enzyme with the same K_{mPD} but with a varying Hill coefficient whose *minimal* value is 0·7 (from the model of Russel, Thompson, Schneider & Appleman (1972) for a 2-sited phosphodiesterase).

RESULTS AND DISCUSSION

Effects of ePD

cAMP hydrolysis by ePD can be described by a time-constant

$$\tau_e \equiv K_{ePD}/A_{ePD}. \tag{8}$$

This is under the assumption that peak cAMP concentrations reaching one amoeba on account of release from another are much smaller than $K_{ePD} = 4 \times 10^{-6}$ M. Higher peak levels make the time-constant concept invalid, but do not affect the conclusions to follow. From (2) and (3) at close-packing densities one has

$$\tau_e = 4 \text{ s}. \tag{9}$$

Now, given a cell density σ, one has a mean cell-cell separation $\Delta r \equiv (4/\pi\sigma)^{\frac{1}{2}}$. From this a mean cell-cell diffusion time for cAMP can be estimated as

$$\tau_{Diff} \equiv (\Delta r)^2/6D_{cAMP}. \tag{10}$$

At close-packing densities this yields

$$\tau_{Diff} = 2 \times 10^{-2} \text{ s}. \tag{11}$$

The ratio

$$\tau_e/\tau_{Diff} = 200 \tag{12}$$

is cell-density independent. Thus cAMP diffusion is 'too fast' for its level to be affected by ePD within cell-cell diffusion times. As mentioned after (2), the ePD activity is much lower for another wild-type strain of *D. discoideum*, thus strengthening this conclusion.

What we have said so far relates to cAMP hydrolysis by ePD at one point in space; one can also ask how ePD might affect spatial concentration gradients of cAMP. It can be shown that if (*a*) the ePD is uniformly distributed in space, and (*b*) the source releases cAMP *either in a pulse or at a uniform rate in time*, then diffusion-limited concentration gradients of cAMP will be made less steep by ePD action, given that no CAMP enters the cell from outside. Crudely speaking, the reason for this is that because the rate of hydrolysis by an enzyme is a non-decreasing function of substrate concentration, within a given amount of time higher substrate levels will be 'pulled down' more than lower levels, thus flattening the concentration profile. However, with the level of ePD activity estimated by us, even this effect is very small. It can be shown that the steepness of the cAMP profile in the presence of ePD will be $\leqslant e^{-1}$ of its value in the absence of ePD at a distance x from the source, where

$$x[A_{ePD}/(K_{ePD} \cdot D_{cAMP})]^{\frac{1}{2}} \geqslant 2{\cdot}2. \tag{13}$$

From (1), (2) and (3), this gives

$$x \geqslant 140\ \mu\text{m}. \tag{14}$$

Therefore one ought to expect no significant effects at typical cell-cell separations, which are of the order of 50 μm and smaller (Konijn & Raper, 1961) during aggregation.

The conclusion follows from (12) and (14), that ePD should be expected to have no significant effects on cAMP levels during aggregation. Clearly, the line of reasoning used in these arguments is no longer valid if there exist other regulatory loops involving the ePD – for instance, a secretion of ePD by the cells in response to extracellular cAMP (Klein & Darmon, 1975). However, any such dependence would have to be fairly drastic in order for an ePD effect to be noticeable, since as mentioned above the values of τ_e/τ_{Diff} and x obtained above are extremely large in relation to our scales of interest.

In the numerical simulation, the estimated activity of ePD had no significant influence on any of the signal profiles; typically at any given position and time a relative difference of less than 5 % was obtained in the value of a quantity estimated with and without ePD taken into account (results not shown).

Effects of mPD

General considerations. A comparison of (2) and (5) shows that the mPD should be 20 times as effective as the ePD in limiting cAMP signals received on the surface of an amoeba. Apart from this difference in the enzymes arising from their differing spatial distributions, there is the matter of the mPD's non-linearity, mentioned earlier. On account of this property, given 2 enzymes with the same K (0·5), at substrate concentrations less than K (0·5) the one with a Hill coefficient less than 1 hydrolyses the substrate much faster than the Michaelian enzyme (Malchow *et al.* 1975).

Its high local activity makes the mPD able to limit sharply in time an incoming cAMP signal. Further, precisely because this enzyme is cell-bound and because the diffusion-limited 'falling phase' of an incoming pulse of cAMP is comparatively slow, the mPD will steepen concentration gradients in the direction of the source, for a short time after the signal has already peaked.

Numerical simulation

(*a*) *Fast hydrolysis.* Fig. 1 shows that mPD has a drastic effect in cutting down cAMP levels. An mPD with negative-cooperative kinetics (Russel *et al.* 1972) is faster in hydrolysing cAMP than a purely Michaelian enzyme but slower than a non-linear enzyme (constant Hill coefficient less than 1 at all substrate concentrations). In general, the enzyme effect becomes more noticeable the further away one goes from the source (compare Fig. 1 C with A or B). Fig. 2 shows the effect of varying cell density on the signal profile in time, assuming a negative-cooperative mPD on all cells. The source strength has been taken as proportional to cell density σ; the cAMP profile takes on more of a pulse character with increasing σ.

(*b*) *Steepening of spatial gradients.* If we consider a situation where there are no other cells between source and responder, the local-sink action of the mPD steepens signal gradients ahead of an amoeba – that is, in the direction towards the source

(Table 2), while at the same time making gradients less steep in the opposite direction. This phenomenon is observed for a short time after the signal has peaked. It is likely that the macroscopic signal input for chemotaxis in *D. discoideum* is a spatial gradient of cAMP (Mato, Losada, Nanjundiah & Konijn, 1975). In case a cell measures the

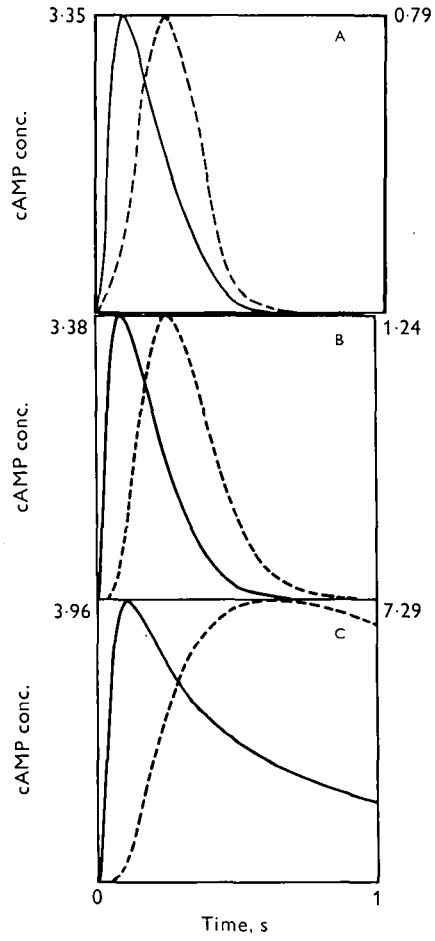

Fig. 1. cAMP concentrations as a function of time (abscissa, 0–1 s) at distance of 22·5 μm ($\times 10^{-6}$ M: left ordinate, full line) and 52·5 μm ($\times 10^{-7}$ M: right ordinate, broken line) from the source (10^7 mol at time 0): Cell density $\times 10^5$/cm^2. A, with a non-linear mPD (Hill coefficient = 0·7 at all substrate concentrations); B, with a negative-cooperative mPD; C, in the absence of mPD. ePD was present in all cases.

local gradient around its surface (Gerisch, Huelser, Malchow & Wick, 1975), such a steepening in the direction of the source and flattening in the opposite direction should enhance chemotactic response by an amoeba.

(*c*) *Binding to receptors.* Apart from the mPD, the surface of *D. discoideum* cells has specific cAMP-binding sites, which are developmentally controlled, and stimulation of which is presumed to elicit the chemotactic response from the cell and induce cAMP release (Malchow & Gerisch, 1974). Clearly the number of cAMP-receptor

complexes at any given time will be modulated by the cAMP-hydrolysing action of the mPD. We have tried to get a measure of this effect, assuming that equilibration of cAMP with receptors is much faster than diffusion; in other words, we have taken diffusion to be rate-limiting. With this assumption, we have calculated the time-rate of change of the number of cAMP-receptor complexes on the surface of a cell, a quantity that may have a role in directing chemotactic signal response in bacteria (Brown & Berg, 1974; however, also see Spudich & Koshland, 1975). The mPD

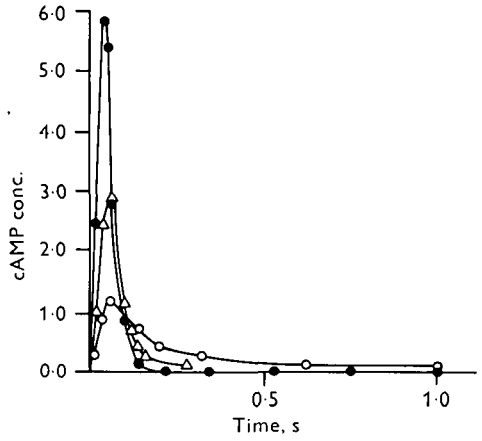

Fig. 2. cAMP concentrations at a distance of 22·5 μm from the source, at varying cell densities, in the presence of negative-cooperative mPD. Abscissa, time from 0 to 1 s. Symbols: ○, $5{\cdot}0 \times 10^4$ cells/cm^2; △, $2{\cdot}2 \times 10^5$ cells/cm^2; ●, $1{\cdot}0 \times 10^6$ cells/cm^2. A pulsatile source of 10^7 mol/cell at time 0; ordinate, concentrations in 10^{-7} M.

Table 2. *Forward diffusion gradients of cAMP at 0·5 s after a source has released 10^7 mol at the origin at time 0*

Position	cAMP gradient in 10^{-3} M/cm	
	(*a*)	(*b*)
1	0·0	0·0
2	7·4	8·2
3	17·4	19·3
4	26·0	29·2
5	34·0	37·5
6	39·4	17·8
7	42·6	25·7
8	43·4	30·3
9	42·4	32·1
10	39·6	31·8

Position 1 is at a distance of $(1-0{\cdot}5) \times 5$ μm from the source. (*a*) No amoebae in the rest of the field. (*b*) Amoebae at position 5 only, at a density of 10^5/cm^2, with non-linear mPD on their surface. A gradient of 10^{-3} M/cm implies a concentration difference of about 10^{-6} M across an amoeba.

exerts a strong effect during the falling phase of the binding; the rising phase is mainly diffusion controlled – time constants at maximum binding vary very little with cell density. The rate of change of bound receptor concentration has a sharply biphasic profile in time (Fig. 3), the sharpness increasing with cell density. The implication is that if this time-rate is important for the signal input, and if negative rates inhibit response, the mPD once again serves to enhance signal reception by the cell.

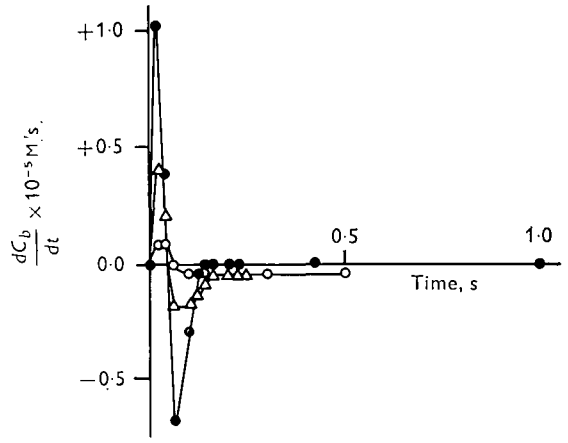

Fig. 3. Rate of change of bound cAMP receptor-concentration in time at a distance of 22·5 μm from a pulsatile source of 10^7 mol/cell at time 0. Symbols for cell density as in Fig. 2. At the peak rate of binding, the time-constants in the 3 cases are 50·0 s^{-1} (○), 47·2 s^{-1} (△), and 43·2 s^{-1} (●).

CONCLUSIONS

Role of ePD

For the aggregation phase, this remains a puzzle. On the one hand, it is quite possible – as our results suggest – that the ePD plays no role whatsoever during aggregation. It must be realized that this inference may be invalid, given other, feasible conditions, not met with under normal experimental circumstances: for instance, extremely small diffusion volumes, which may well occur in natural slime mould habitats.

Accepting that the ePD plays no role raises the question as to why the cells produce and release an ePD-inhibitor, also under standard agar-plate conditions (G. Gerisch, in preparation). A possible reason would be that, in the absence of such inhibition, ambient ePD levels would be so high that they would flatten cAMP diffusion profiles beyond the limit of recognition by an amoeba sufficiently distant from the source. Yet another reason for inhibition could be to increase aggregation territory sizes: as expected on theoretical grounds (Nanjundiah, 1973), low ePD levels are correlated with large territory sizes (Riedel, Gerisch, Mueller & Beug, 1973).

Role of mPD

This enzyme performs special functions both on account of its spatial distribution – on the cell surface – and on account of its non-linear kinetics. By always moving with the amoeba, the mPD creates a local sink for cAMP, thus steepening diffusion gradients in the direction of a source. In other words, as Pannbacker & Bravard (1972) suggested, the mPD enhances chemotactic response. Further, because of this localization, the neighbourhood of a cell is rapidly cleaned up of cAMP, and the receptors are kept prepared in a state in which they would be maximally sensitive to new signals. The non-linear kinetics mean that mPD hydrolyses external cAMP much more rapidly than a Michaelian enzyme with the same K (0·5) and V_{max} per cell: ideally, a pulse at the source is converted to a pulse at the receptor.

We thank Prof. G. Gerisch for supplying unpublished experimental results and for his criticisms. This work was supported by the Schweizerische Nationalfonds.

APPENDIX A. SOME DETAILS OF THE NUMERICAL SIMULATION AND THE APPROXIMATIONS MADE THEREIN

The simulation

The problem was to solve on a plane the diffusion equation for cAMP in one spatial dimension (distance from the source) and time, in the presence of known sinks. The boundary conditions were: zero concentration-gradient at the origin (on grounds of symmetry) and zero concentration at the outer boundary. Note that this implies that cAMP diffuses away from our region of interest. The diffusion equation was solved explicitly by the recursive method with a spatial step-length of 5×10^{-4} cm and a time-step of 5×10^{-4} s. Convergence of the numerical solution was tested by comparing with the analytic solution in an infinite region for (i) a pulse source of cAMP in the absence of hydrolysis; and (ii) a pulse source and only ePD.

Approximations

(*a*) In those cases in which we substitute a random, discrete distribution of amoebae by a uniform, spread-out distribution, we are unable to get the precise cAMP distribution at the cell surface. This has led to an underestimate of concentrations by an amount that should be zero at close-packing density. We can get a measure of the error analytically by comparing the solutions for discrete and uniform distributions, given a Michaelian enzyme, low substrate levels, and diffusion in one dimension. With a pulse source at time zero, our solution underestimates cAMP levels by a factor of 1·2 at 20 μm from the source.

(*b*) Considering diffusion to occur in only 2 dimensions with the mPD localized in a surface layer has also underestimated concentrations. To get an idea of the error this leads to, we have compared, in the case of a Michaelian enzyme and low substrate levels, our solution with that to be expected for 3-dimensional diffusion of cAMP with the enzyme again occupying a thin surface layer. This shows that whereas the shape of the cAMP profile is not much altered, absolute levels are: at 10^5 cells/cm^2 and

0·5 s after the pulse, the concentrations used in this study are too low by a factor of 2·5; since the enzyme is in fact not Michaelian, this factor should actually be somewhat lower.

REFERENCES

BONNER, J. T. (1967). *The Cellular Slime Molds*, 2nd edn. Princeton, New Jersey: Princeton University Press.

BROWN, D. A. & BERG, H. C. (1974). Temporal stimulation of chemotaxis in *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1388–1392.

CHANG, Y.-Y. (1968). Cyclic AMP phosphodiesterase produced by the slime mould *Dictyostelium discoideum*. *Science, N.Y.* **161**, 57–59.

COHEN, M. H., DRAGE, D. & ROBERTSON, A. (1975). Iontophoresis of cyclic AMP. *Biophys. J.* **15**, 753–763.

COHEN, M. H. & ROBERTSON, A. (1971). Wave propagation in the early stages of aggregation of cellular slime molds. *J. theor. Biol.* **31**, 101–118.

GERISCH, G., HUELSER, D., MALCHOW, D. & WICK, U. (1975). Cell communication by periodic cyclic AMP pulses. *Phil. Trans. R. Soc. Ser. B* **272**, 181–192.

GERISCH, G. & WICK, U. (1975). Intracellular oscillations and release of cyclic AMP from *Dictyostelium* cells. *Biochem. biophys. Res. Commun.* **65** (1), 364–370.

KLEIN, C. & DARMON, M. (1975). The relationship of phosphodiesterase to the developmental cycle of *Dictyostelium discoideum*. *Biochem. biophys. Res. Commun.* **67** (1), 440–447.

KONIJN, TH. M. (1972). Cyclic AMP as a first messenger. *Advances in Cyclic Nucleotide Research*, **1**. New York: Raven Press.

KONIJN, TH. M. & RAPER, K. B. (1961). Cell aggregation in *Dictyostelium discoideum*. *Devl Biol.* **3**, 725–756.

MALCHOW, D., FUCHILA, J. & NANJUNDIAH, V. (1975). A plausible role for a membrane-bound cyclic AMP phosphodiesterase in cellular slime mold chemotaxis. *Biochim. biophys. Acta* **385**, 421–428.

MALCHOW, D. & GERISCH, G. (1974). Short-term binding and hydrolysis of cyclic AMP to aggregating *Dictyostelium* cells. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2423–2427.

MALCHOW, D., NAEGELE, B., SCHWARZ, H. & GERISCH, G. (1972). Membrane-bound cyclic AMP phosphodiesterase in chemotactically responding cells of *Dictyostelium discoideum*. *Eur. J. Biochem.* **28**, 136–142.

MATO, J., LOSADA, A., NANJUNDIAH, V. & KONIJN, TH. M. (1975). Signal input for a chemotactic response in the cellular slime mold *Dictyostelium discoideum*. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4991–4993.

NANJUNDIAH, V. (1973). Chemotaxis, signal relaying, and aggregation morphology. *J. theor. Biol.* **42**, 63–105.

PANNBACKER, R. G. & BRAVARD, L. J. (1972). Phosphodiesterase in *Dictyostelium discoideum* and the chemotactic response to cyclic AMP. *Science, N.Y.* **175**, 1014–1015.

RIEDEL, V., GERISCH, G., MUELLER, E. & BEUG, H. (1973). Defective cyclic AMP phosphodiesterase regulation in morphogenetic mutants of *Dictyostelium discoideum*. *J. molec. Biol.* **74**, 573–585.

RIEDEL, V., MALCHOW, D., GERISCH, G. & NAEGELE, B. (1972). Cyclic AMP phosphodiesterase interactions with its inhibitor of the slime mold, *Dictyostelium discoideum*. *Biochem. biophys. Res. Commun.* **46** (1), 279–287.

RUSSEL, T. R., THOMPSON, W. J., SCHNEIDER, F. W. & APPLEMAN, M. M. (1972). 3′:5′-cyclic adenosine monophosphate phosphodiesterase: negative cooperativity. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1791–1795.

SPUDICH, J. L. & KOSHLAND, D. E. JR. (1975). Quantitation of the sensory response in bacterial chemotaxis. *Proc. natn. Acad. Sci. U.S.A.* **72**, 710–713.

(*Received* 12 *January* 1976)