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Phorbol 12-myristate 13-acetate modulates the cAMP-induced light-scattering response of a *Dictyostelium discoïdeum* cell population

R. Thiery, R. Klein and I. Tatischeff

Institut Curie, Section de Physique et Chimie LPCB, URA 198 CNRS, 11, rue Pierre et Marie Curie, 75231 Paris, Cedex 05, France

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The effect of phorbol 12-myristate 13-acetate upon the light-scattering response to cAMP of a *D. discoïdeum* cell suspension was investigated. It was found that the first spike of the cAMP-mediated light-scattering change (peaking at about 15–20 s after stimulation) was inhibited by the phorbol ester. This effect was concentration dependent with an half-maximum value for the inhibition of 4 nM. The inhibition was found to be maximal after a 10–20 min incubation time. The phorbol ester was shown to affect the dose-response relationship between the cAMP concentration and the relative amplitude of the light-scattering change, more by decreasing the number of cAMP receptors than by decreasing their apparent affinity for cAMP.

cyclic AMP; Aggregation; Chemotaxis; Phorbol ester; (*Dictyostelium discoïdeum*)

1. INTRODUCTION

The eucaryotic cellular slime mold *Dictyostelium discoideum* displays an original life cycle. Unicellular amoebae grow as long as nutrients are available and a developmental process is induced by starvation of the cells, beginning with the aggregation of 10^3–10^5 cells and leading to an organized multicellular structure containing only two major differentiated species [1,2].

Since the discovery of the chemotactic role of cAMP during the aggregation [3], a lot of studies have been focused on the molecular components of the chemosensory system. A framework of the events occurring at the plasma membrane level during the chemotactic reaction has emerged (reviews [4,5]). It became progressively clear that this simple organism is particularly interesting with respect to gaining insight into the mechanisms of signal transduction from the membrane receptors to intracellular targets, occurring in higher eucaryotic systems. In *D. discoideum*, stimulation of the cells by cAMP induces transient formation of intracellular cAMP and cGMP. The newly synthesized cAMP is mainly excreted thus relaying the signal, while cGMP is most probably involved in the chemotactic reaction [6].

Recently, experimental evidence has appeared for the existence of a phosphatidylinositol pathway beside the well known cAMP-related components (i.e., cAMP receptors, adenylate cyclase, cAMP phosphodiesterase and a phosphodiesterase inhibitor). It was shown that the inositol 1,4,5-triphosphate elicited an increase of intracellular cGMP and calcium in saponin-treated cells [7,8], and an increase of inositol trisphosphate was evidenced in cells stimulated by cAMP [9]. Although no direct formation of diacylglycerol has as yet been demonstrated these

Correspondence address: R. Thiery, Institut Curie, Section de Physique et Chimie, LPCB, URA 198 CNRS, 11, rue P. et M. Curie, 75231 Paris, Cedex 05, France

Abbreviations: PMA, phorbol 12-myristate 13-acetate; 4-*O*-methyl-PMA, 4-*O*-methyl phorbol 12-myristate 13-acetate; PKC, protein kinase C

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results strongly support the idea that a functional phospholipase C, coupled to the cAMP receptor, exists in *Dictyostelium* cells. In vertebrates, a specific protein kinase, PKC, is further synergistically activated by calcium and diacylglycerol produced by this phosphatidylinositol breakdown (for review see [10]).

In this paper, we report the effects of PMA, a potent tumor promoter which is supposed to specifically activate the protein kinase C [11], on the cAMP-induced changes in the light scattered by a *D. discoideum* cell suspension.

2. MATERIALS AND METHODS

2.1. *Cell growth and developmental conditions*

Cloned *D. discoideum* cells, strain AX2, were grown in HL5 medium [12], in rotatory agitated suspensions (175 rev./min) at 22°C. To initiate the development, cells were harvested during the exponential phase of growth, centrifuged at 700 × *g* for 2 min, washed twice in potassium phosphate buffer (pH 6.8), and resuspended in the same buffer at a final density of 10^8 cells/ml. The cellular suspension was kept at 22°C and shaken at 175 rev./min until the light-scattering experiment.

2.2. *Light-scattering experiments*

At t_x (after x h of starvation-induced development), cells were diluted tenfold in phosphate buffer and incubated either with a given concentration of PMA or with ethanol, 0.1% (v/v), as a control. After a given incubation time, cells were transferred onto a light-scattering chamber made up with a 20 ml plastic syringe adapted to a quartz circulation cell (Hellma). A peristaltic pump prevented the cell deposit induced by agglutination and the cells were oxygenated with a 20 ml/min oxygen bubbling. A pulse of cAMP was automatically given after 5 min using a 250 μl Hamilton syringe controlled with a 9835A Hewlett-Packard microcomputer. The maximal recorded change in the transmitted light was between 4% and 10% depending on the time of starvation and also on daily variations. Therefore, the control experiments were systematically performed every day just before and/or after measuring the response of PMA-treated cells. The microcomputer controlled both acquisition and treatment of the data.

3. RESULTS

Fig.1 shows a typical recording of the transmitted light through a *Dictyostelium* t_6 cell suspension. About 10^7 cells/ml in a total volume of 5 ml were stimulated by a 5 μl pulse of 2×10^{-4} M cAMP, giving a final cAMP concentration of 2×10^{-7} M. In these conditions, the diluting effect was negligible. In some experiments, the base line increased slowly with time; this effect was taken into

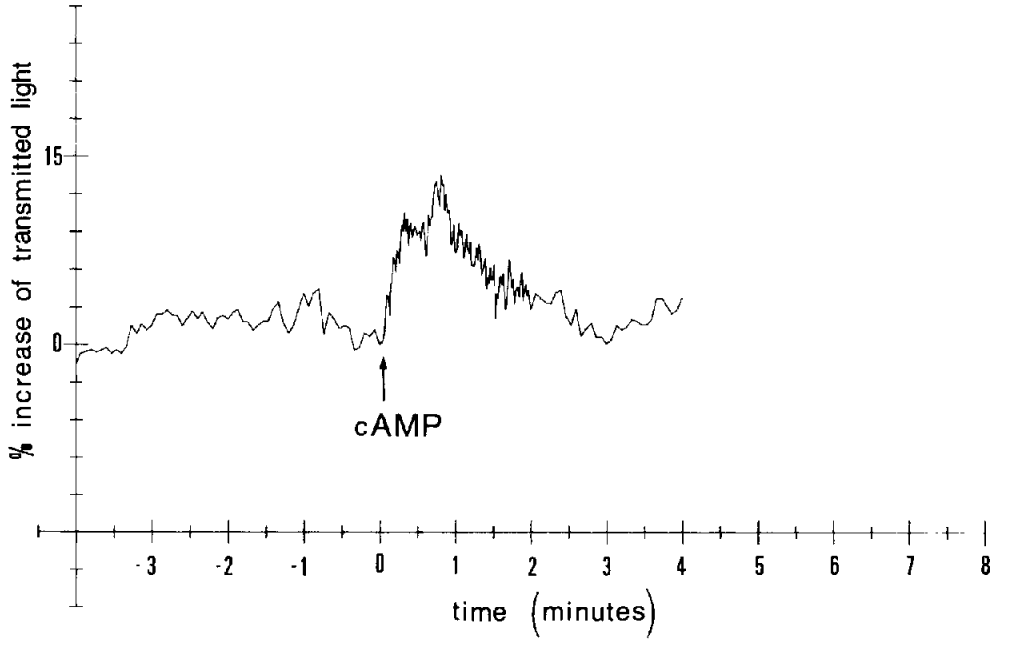

Fig.1. cAMP-induced changes in the transmitted light by a *Dictyostelium discoideum* cell suspension. After 6 h of starvation-induced development, 10^7 cells/ml were stimulated with a 2×10^{-7} M cAMP pulse (final concentration). The magnitude of the optical change is expressed as a percentage of the increase in the transmitted light after stimulation.

account to determine the percentage change in the transmitted light after stimulation. As previously shown [13], the response to cAMP was biphasic with a fast and a delayed component.

The effect of PMA on the amplitude of the 2×10^{-7} M cAMP-induced optical change was investigated in the concentration range 10^{-10}–10^{-5} M and for an incubation of 10 min. The results (fig.2) are plotted on a relative scale compared to the control obtained with 0.1% ethanol-treated cells. In these conditions, the magnitude of the fast light-scattering change was strongly reduced giving a maximal inhibition of about 50% at 10^{-6}–10^{-5} M. The half-maximum effect was reached at a concentration of about 4 nM PMA. The phorbol ester 4-*O*-methyl-PMA, which does not activate PKC, did not induce any decrease of the response in this concentration range.

The kinetics of the inhibition was investigated for 10^{-7} M PMA-treated cells (fig.3). It was observed that the maximal inhibition was reached after 10–20 min of incubation with PMA and that the effect was practically suppressed after a 60 min treatment.

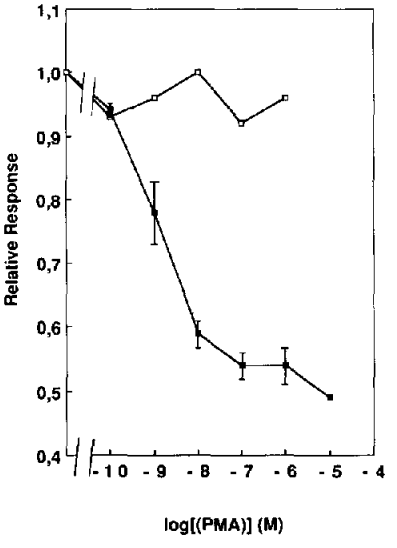

Fig.2. Influence of phorbol ester on the magnitude of the fast light-scattering response. Cells were incubated for 10 min with PMA (■), 4-*O*-methyl-PMA (□) or with ethanol 0.1% (control). The results are plotted as a relative response: height of the cAMP-induced fast response obtained with cells incubated with phorbol ester as compared to the control. The concentration of the cAMP pulse was 2×10^{-7} M. The shown results are the mean ± SD of three independent experiments.

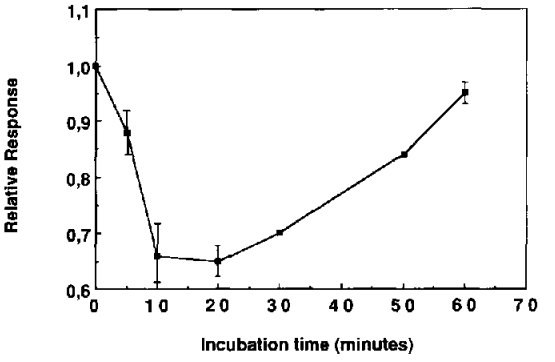

Fig.3. Kinetics of the phorbol ester effect. The time of incubation of the cells with 10^{-7} M PMA, before stimulation by 2×10^{-7} M cAMP was varied up to 60 min. The results are expressed as in fig.2. The results shown are the mean ± SD of three independent experiments.

Fig.4 shows the cAMP dose-response curve of the optical change obtained for cells treated with 10^{-7} M PMA for 10 min at 22°C as compared to the control. Again an inhibition was observed for PMA-treated cells. The two curves displayed a plateau for higher cAMP concentrations ($> 10^{-6}$ M) but it was higher for control cells than for PMA-treated cells. Both curves gave a half-maximum cAMP concentration around 10 nM.

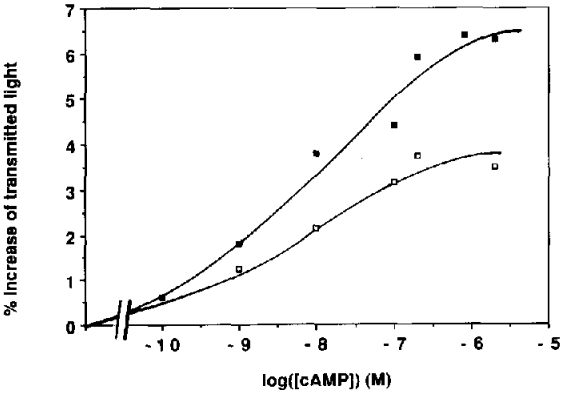

Fig.4. Dose response curves of the cAMP-induced fast response. The magnitude of the first peak, obtained with cells incubated for 10 min with 10^{-7} M PMA (□) or with ethanol 0.1% (■), is plotted as percentage of the increase in the transmitted light after stimulation.

4. DISCUSSION

First, it can be stressed that monitoring the cAMP-induced light-scattering changes is very useful in studying the effect of an exogenic agent on the chemosensory system. The method is simple and does not involve disruption of the cells, which can be further studied for their aggregation and development ability after the light-scattering experiment. The biphasic cellular response is thought to reflect the morphological changes associated with cell motion toward the cAMP source. According to this scheme, the fast response is directly linked to the chemotactism, whereas the delayed response represents the changes in the cell shape associated with the relay of the cAMP signal. It has already been put forward that the fast and delayed components of the response are graded, their magnitude being respectively proportional to the fractional occupancy of the high and low affinity cAMP-binding sites [14]. Furthermore, the time analysis of the cAMP-dependent processes clearly shows that the fast and delayed light-scattering responses are respectively correlated with the transient increase of intracellular cGMP and cAMP [15,16]. Thus, monitoring the relative magnitude of the fast and delayed processes gives an indirect but rapid way to determine which particular step – chemotactism or relay – is affected.

By using this technique, the main results of the present report are as follows: (i) the magnitude of the fast light-scattering response to cAMP is inhibited up to 50% when the cells are preincubated with PMA; (ii) this decrease depends on the PMA concentration, with a half-maximal effect of about 4 nM, which is in the range of the protein kinase C affinity for PMA, and is not observed when an inactive phorbol ester is used; (iii) the effect is optimal when the cells are preincubated for 10–20 min with PMA; (iv) the effect of PMA seems to originate mainly from a decreased number of active cAMP receptors, rather than from a shift in their apparent affinity for cAMP.

Both the concentration dependency and the specificity of the phorbol ester effect indicate that PMA acts via PKC activation. However, it is noteworthy that even the higher concentration of added PMA only inhibited ≈50% of the response. This may indicate that two subpopulations of cAMP receptors exist with regard to their link with the PKC system. Another interesting point deals with the kinetics of the inhibition. It was found that the maximum inhibitory effect is reached within 15–20 min after the drug addition and that a full reversibility is obtained after 60 min. The origin of this reversibility is not yet understood, it could possibly arise from a plasma membrane renewal.

As the results, reported in this paper, showed a significant inhibition of the fast light-scattering response to cAMP, but not of the delayed one (not shown), we suggest that the PMA-induced activation of PKC regulates one of the components of the transduction pathway leading to guanylate cyclase activation.

Considering that the PMA-mediated activation of PKC leads to an inhibition of intracellular cGMP formation, the nature of the affected component remains unclear. A negative feedback control of this protein over membrane receptors was evidenced in higher eucaryotic systems (review [17]). For *Dictyostelium*, the number of high affinity, slowly dissociating sites of the cAMP receptors is increased twofold by ATP [18] and this effect depends on the presence of calcium and PMA, suggesting that PKC is involved. On the other hand, a regulatory G-protein could also be the substrate of PKC. Such components have been evidenced in *D. discoideum*, since the affinity of the fast and slow-dissociating binding sites [19], respectively coupled to adenylate cyclase and to guanylate cyclase [20,21], can be modulated by guanine nucleotides [22]. This last hypothesis is supported by recent findings derived from transformation studies using wild type and mutated *ras*-gene [23]. Cellular extracts obtained from these transformed cells showed a calcium and ATP-dependent decrease of the number of cAMP receptors. PMA and GTP shifted the calcium requirement of the process to the submicromolar range for wild type transformants, whereas it remained unchanged (10^{-7} M) in cells expressing the mutated *ras*-gene.

Finally we conclude that, although the phorbol ester effect is not yet understood at the molecular level, our results, together with other recent reports, strongly support the idea that the signal transduction during *Dictyostelium* chemotaxis involves complex biochemical interactions between the cAMP receptors and the components of the

phosphatidylinositol pathway including the activation of protein kinase C.

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