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ROLE OF A GUANINE NUCLEOTIDE BINDING PROTEIN, G_α 2, IN REGULATION OF ADENYLATE CYCLASE IN DICTYOSTELIUM DISCOIDEUM

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Two substances, cAMP and 2,3-dimercapto-1-propanol (BAL) are known to induce transient activation of adenylate cyclase in Dictyostelium discoideum. A frigid mutant (HC85) has a deletion in a gene for G_α 2, a guanine nucleotide binding protein and cannot activate the cyclase in response to cAMP. We found that BAL induced activation in the frigid mutant. This result suggests that the BAL-induced activation is independent of G_α 2 and that BAL mimics a role of activated G_α 2. We also found that cAMP promoted the BAL-induced activation. This result suggests that cAMP plays a role in activation through a mechanism in which G_α 2 is not involved. We lastly showed that continuous cAMP stimulation could not inhibit the BAL-induced activation in the frigid mutant. Since the cAMP-induced inhibition observed in the wild type strain (NC4) proceeds with the time course identical to the cAMP-induced adaptation (Oyama, submitted), this result suggests that G_α 2 is involved in adaptation of adenylate cyclase.

Starvation triggers a shift from the growth phase to the developmental phase in the cellular slime mold, Dictyostelium discoideum. In the developmental phase, cAMP acts as a pheromone to induce morphogenesis and cell differentiation [for review, 1]. Extracellular cAMP binds to receptors on the cell surface [2,3,4] and activates various types of intracellular signal transduction systems. Adenylate cyclase is one example regulated by such systems [5,6,7]. The cAMP receptors on the cell surface consist of at least two types, A and B [8,9] and they co-operatively work

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Abbreviations: BAL, 2,3-dimercapto-1-propanol; cAMP, adenosine-3':5'-cyclic monophosphate; dcAMP, 2'-deoxyadenosine-3':5'-cyclic monophosphate.

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for activation of adenylate cyclase. G_α 2, a guanine nucleotide binding protein, is thought to be associated with B-receptors [9,10]. A frigid mutant (HC85) [11] which is a deletion mutant of G_α 2 gene [10] cannot activate adenylate cyclase in response to cAMP stimulation [12] although this mutant has cAMP receptors and adenylate cyclase [11, 12].

Oyama [13], Oyama and Kubota [14] and Oyama et al. [15] proposed that reducing reagents, such as BAL or dithiothreitol, act on the intracellular signal transduction pathway(s) independently from the receptor and induce and/or modify the receptor-mediated events. The most interesting aspect of the effect of BAL is that BAL induces or enhances activation of adenylate cyclase [13,15].

Since BAL directly acts on the signal transduction pathway(s) in activation of adenylate cyclase, this reagent may mimic a role of G_α 2. We found this is the case. Taking advantage of the BAL-induced activation of adenylate cyclase in the frigid mutant, we investigated the role of G_α 2 in the receptor-mediated activation and adaptation of adenylate cyclase.

MATERIALS and METHODS

Frigid mutant, HC85 and its parental strain, HC6 (which is an axenic mutant) were shake-cultured with Escherichia coli B/r in phosphate buffer. Vegetatively growing amoebae were collected, washed and resuspended in 20 mM phosphate buffer pH 6.4 containing 10 mM KCl and 1.2 mM $MgSO_4$ at $6x10^6$ cells/ml. The cell suspension was shaken at 120 rpm at 21 °C for 10 h. The starved cells were washed and resuspended in 20 mM phosphate buffer pH 6.4 containing 10 mM KCl (PBK buffer) at $1x10^7$ cells/ml. One ml of the cell suspension was shaken in a 20 ml vial for at least 30 min and then stimulated with 3 mM BAL (Sigma), 10 μM dcAMP (Sigma) or the mixture of them. One hundred μl of the suspension was sampled into a micro-test tube containing $HClO_4$. The cell lysate was neutralized with $KHCO_3$. cAMP in the supernatant was assayed with isotope dilution assay (Amersham) [16].

RESULTS

We investigated the effect of BAL on activation of adenylate cyclase in a frigid mutant (HC85) and its parental strain (HC6). Since in a preliminary expeiment we found that activation of adenylate cyclase in the frigid cells starved for 8-10 h were more pronouncedly induced by BAL than in those starved for 4.5-6 h (data not shown), we used 10 h starved cells for further investigation.

We first confirmed that dcAMP did not cause any accumulation of cAMP in the frigid cells while it induced a transient accumulation in its parental cells (Fig. 1). dcAMP was used for stimulation since it binds to cAMP-receptors and induces receptor-mediated events without interfering with cAMP quantification [16].

We found that BAL induced the activation of adenylate cyclase in both the frigid and parental strains (Fig. 1). The accumulation of cAMP began with a lag after the addition of BAL in both strains. The lag times in the parent and the frigid cells were 1 min and 3-5 min respectively.

When dcAMP and BAL were used together for stimulation, immediate accumulation of cAMP occurred in both the frigid and the parental cells (Fig. 1). In the parenal strain cAMP accumulation was terminated within 2 min, similarly to the case of stimulation by dcAMP alone. By marked contrast, in the frigid strain the accumulation continued beyond 6-8 min after the stimulation (Fig. 1).

Since Oyama [13] found that continuous stimulation at a saturated level of dcAMP for 10 min completely suppresses the BAL-induced activation of adenylate cyclase in a wild type strain

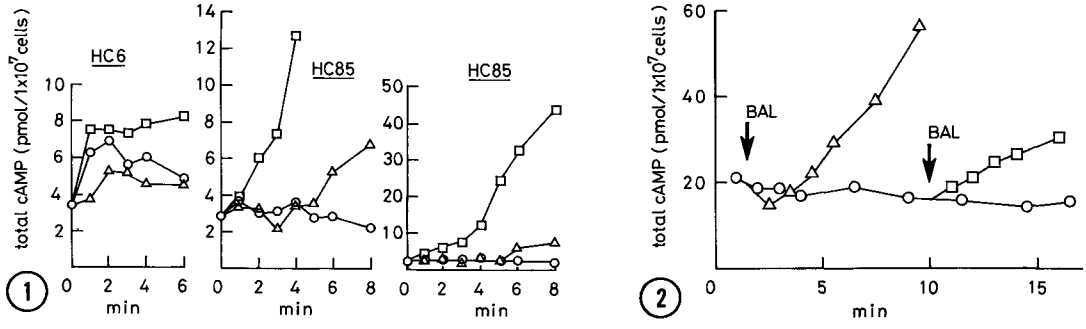

Figure 1 Activation of adenylate cyclase in the frigid mutant (HC85) and in its parental strain (HC6). Starved HC85 (center and right) or HC6 (left) cells were shake-cultured in PBK buffer. The cells were stimulated with 10 μM dcAMP (circle), 3 mM BAL (triangle) or the mixture of them (square). The cell suspension was sampled for cAMP quantification at indicated times. Center and right graphs show the same data with different vertical axes.

Figure 2 Activation of adenylate cyclase by BAL in the frigid mutant after pretreatment of 100 μM dcAMP. Starved frigid (HC85) cells were shake-cultured in PBK buffer. The cells were stimulated with 100 μM dcAMP at 0 time (circle). Part of the cell suspension was removed at 1.5 min (triangle) or 10min (square) after the dcAMP stimulation to a new vial containing 3 mM BAL. The cell suspension was sampled for cAMP quantification at indicated times. 100 μM dcAMP was detected as a background of 16.5 pmol cAMP/1x10^7 cells.

(NC4), continuous activation of adenylate cyclase induced by dcAMP+BAL in the frigid mutant may be due to the lack of the dcAMP-induced inhibition on the BAL-induced activation in this mutant.

To test this possibility, we treated the frigid cells with 100 μM dcAMP and then stimulated them with BAL (Fig. 2). We found that BAL activated adenylate cyclase even after the pretreatment of 100 μM dcAMP for 10 min while the rate of the BAL-induced accumulation of cAMP was somewhat slower at this time point than at 1.5 min of the pretreatment (Fig. 2). We could not determine whether pretreatment of dcAMP for 10 min completely suppressed the BAL-induced activation of adenylate cyclase in the parental strain (HC6) similarly in the wild type strain (NC4) since the BAL-induced activation after the dcAMP treatment was too weak in the parents.

DISCUSSION

We showed that BAL can induce activation of adenylate cyclase in a frigid mutant (HC85) as well as in its parents (HC6) (Fig. 1). Since the deletion of G_{α} 2 gene is thought to be an only mutation in HC85 in intracellular signal transduction [10], this result suggests that the BAL-induced activation of adenylate cyclase is independent of G_{α} 2 and that BAL mimics a role of G_{α} 2 to activate adenylate cyclase (Fig. 3).

dcAMP+BAL induces immediate activation of adenylate cyclase while a short lag time is observed after the stimulation with BAL alone (Fig. 1). Thus, dcAMP helps the BAL-induced activation in the frigid mutant although dcAMP alone has no effect in this mutant. These results suggest that interaction between the receptors and cAMP plays a role in the activation of adenylate

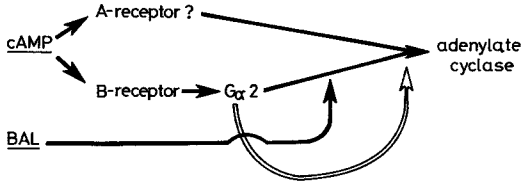

Figure 3 Hypothetical scheme for regulation of adenylate cyclase. cAMP binds to the A and B type receptors. Two types of activation signals (closed arrow) are induced by the cAMP-receptors. Cooperative actions of both signals are required for activation of adenylate cyclase. G_{α} 2 associated with the B receptor mediates induction of the activation signal (closed arrow) and the adaptation signal (open arrow).

cyclase through two cooperatively working mechanisms: one mediated by G_{α} 2 protein and the other yet unidentified (Fig. 3). Our data also suggest that adenylate cyclase is not activated through the latter unidentified mechanism alone.

It has been proposed that cAMP activates two types of receptors, A and B, and that both receptors are necessary for activation of adenylate cyclase [9]. If BAL mimics a role of G_{α} 2 but does not mimic a role of A-receptor, the synergistic effects of BAL and dcAMP in the frigid mutant are consistent with this proposal (Fig. 3).

Oyama [13] found that continuous stimulation at a saturated level of dcAMP for 10 min completely suppresses the BAL-induced activation of adenylate cyclase in the wild type strain, NC4. This dcAMP-induced inhibition proceeds with a time course identical to the cAMP-induced adaptation, which is a mechanism for termination of the cAMP-induced activation of adenylate cyclase [17]. Therefore, the result that continuous dcAMP pretreatment shows little effect on the BAL-induced activation in the frigid mutant (Fig. 2) indirectly suggests that G_{α} 2 is involved in adaptation of adenylate cyclase (Fig. 3). The prolonged period of accumulation of cAMP after dcAMP+BAL stimulation seen in this mutant might also reflect the lack of adaptation.

BAL induces weaker activation in the parental strain, HC6 than in the wild type strain, NC4. HC6 is an axenic strain and was developed in shake-culture conditions unlike the case of NC4 [13]. Some of these differences may cause less sensitive character of HC6 to BAL.

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