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NOTES

Purine-Containing Compounds, Including Cyclic Adenosine 3',5'-Monophosphate, Induce Fruiting of *Myxococcus xanthus* by Nutritional Imbalance

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Induction of *Myxococcus xanthus* fruiting by a number of different purine-containing compounds, including cyclic adenosine 3',5'-monophosphate, is defective in a mutant resistant to 2,6-diaminopurine. Furthermore, the purine-induced fruiting of wild-type cultures is uniquely blocked by a low concentration of added glycine. These results imply that different purine-containing compounds induce fruiting through a single mechanism involving nutritional imbalance.

There are surprising morphological similarities in the development of fruiting bodies of the procaryote *Myxococcus xanthus* and the eucaryote *Dictyostelium discoideum* (6, 11). The fact that cyclic AMP (cAMP) plays a central role in *D. discoideum* development (11) has led to the search for effects of cAMP on *M. xanthus* development. It has been observed that fruiting of *M. xanthus* on an undefined, low-nutrient medium is strongly stimulated by cAMP and ADP, and more weakly stimulated by adenosine and adenine (3). This was interpreted to imply a role for cAMP in triggering fruiting body formation by activating transcription at differentiation-specific promoter sites, or by interaction of nucleotides at the cell surface (3). Figure 1 extends the observations to show that low concentrations of cAMP, adenosine, adenine, and hypoxanthine stimulated fruiting of wild-type *M. xanthus* (strain DK501) on a defined minimal medium, A1 medium (2). To determine whether these purines and purine-containing compounds stimulate fruiting through related mechanisms, a mutant (designated DK590) that could form colonies in the presence of the adenine analog 2,6-diaminopurine was isolated. In contrast to the parent strain (Fig. 1), this mutant failed to fruit when treated with adenine, cAMP, hypoxanthine (Fig. 2A to C), or adenosine (data not shown), although it fruited normally under other conditions, such as treatment with threonine (Fig. 2D) or starvation for phenylalanine (data not shown). Thus it appears that this mutant is defective in a function specifically required for fruiting induction by purines and purine-containing compounds, such as their transport or metabolism, indicating that inductions by these compounds all have at least one step in common. An independently isolated mutant (DK591) selected for resistance to adenine (740 μM on A1 medium) failed to fruit when treated with adenine or cAMP, but did fruit in response to hypoxanthine or adenosine addition (unpublished experiments). This mutant appears to be defective in a function needed for fruiting induced by adenine and cAMP but not by hypoxanthine or adenosine.

In view of the general correlation between fruiting of *M. xanthus* and the elevation of guanosine polyphosphate levels (8, 9), nucleotide levels were measured in *M. xanthus* cultures treated with purine-containing compounds. Figure 3A and B shows a two- to threefold increase in guanosine pentaphosphate by 4 h after adenosine or cAMP addition to DK501 cells growing in A1 medium. This increase in guanosine pentaphosphate level is comparable to that observed during induction of fruiting by gradual amino acid starvation of *M. xanthus* (9) and suggests the possibility that purine and purine derivatives might induce fruiting by indirectly imposing amino acid limitation. However, unlike direct amino acid limitation (9), adenosine addition also led to increases in the levels of the guanosine polyphosphate precursors ATP and GTP (Fig. 3C and D). No corresponding changes in the CTP or UTP levels were observed (data not shown). The ATP level increase was also seen after cAMP addition, although to a lesser extent. Other chromatograms showed that these increases in the levels of nucleoside triphos-

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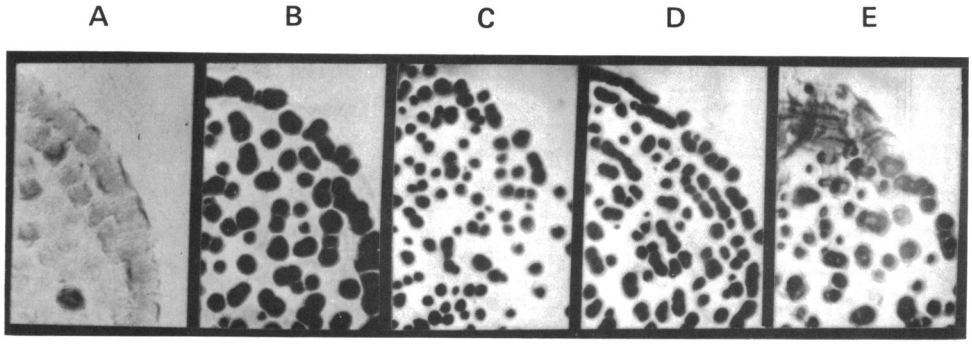
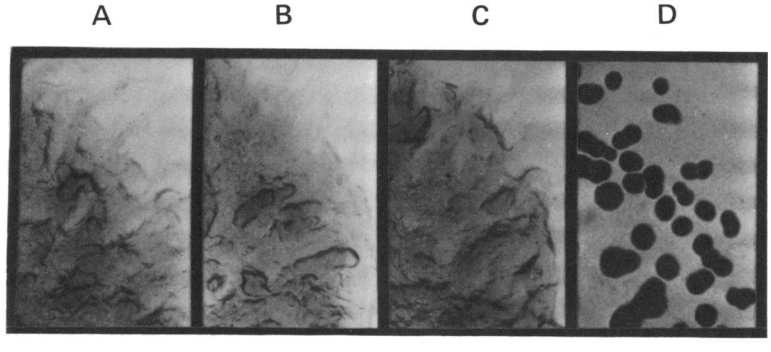


	NO ADDITIONS	+cAMP	+ADENOSINE	+ADENINE	+HYPO–XANTHINE
AGG	±	+	+	+	+
SPO	+	+	+	+	+

FIG. 1. *Fruiting induction by purines and purine derivatives. Cells were spotted onto A1 minimal medium (2) containing (A) no additions, (B) 15 μM cAMP, (C) 19 μM adenosine, (D) 3.7 μM adenine, or (E) 37 μM hypoxanthine. DK501 cells (8) were grown in CTT broth supplemented with 7.4 μM adenine, 9.0 μM cytosine, and 8.9 μM uracil, then spotted and incubated as previously described (9). The spots were photographed and scored for aggregation (AGG) and spores (SPO) after 4 days (9). Many fewer spores were observed in the spot on the plate with no additions (A) than in any of the other spots shown. Each frame shows an area of 3.6 by 2.4 mm.*



	+ADENINE	+HYPO–XANTHINE	+cAMP	+THREONINE
AGG	–	–	–	+
SPO	–	–	–	+

FIG. 2. *Fruiting induction of a mutant resistant to 2,6-diaminopurine. DK590 was selected for the ability to grow on agarose plates containing A1 minimal medium and 270 μM 2,6-diaminopurine. Mutant cells were grown, spotted, and scored for fruiting as described in Fig. 1 and reference 9. Plates contained A1 medium supplemented with (A) 3.7 μM adenine, (B) 37 μM hypoxanthine, (C) 15 μM cAMP, and (D) 17 mM threonine. In the same experiment, spots of DK501, the wild-type parent of DK590, fruited in response to the treatments shown in (A) to (D).*

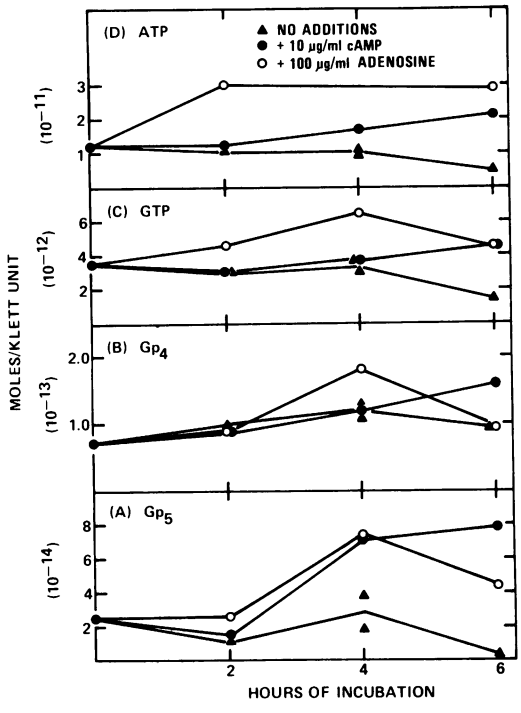


FIG. 3. *Nucleotide levels after adenosine and cAMP treatments. DK501 cells grown in low-phosphate A1 medium (9) were treated with cAMP (30 μM or 10 μg/ml, final concentration)* (●), *adenosine (375 μM or 100 μg/ml)* (○), *or an equivalent volume of water* (▲). *Nucleotides were analyzed chromatographically using solvent system 2 as previously described (8). Gp_4, Guanosine tetraphosphate; Gp_5, guanosine pentaphosphate.*

phates in adenosine-treated cells occur predominantly as rATP and rGTP rather than dATP and dGTP (C. Manoil, Ph.D. thesis, Stanford University, Stanford, Calif., 1978).

If purine addition causes an amino acid starvation, then addition of the limiting amino acid would block purine-induced fruiting. Indeed, the fruiting activity induced by adenine, cAMP, hypoxanthine, or adenosine was reversed by a low concentration of glycine (Fig. 4 and data not shown). None of 14 other amino acids at low concentrations reversed fruiting induction by adenine, and glycine did not inhibit fruiting under other conditions, e.g., phenylalanine limitation. It is possible that purine-containing compounds induce fruiting by specifically preventing intracellular glycine biosynthesis, although the mechanism by which this would occur is unknown. Alternatively, glycine may reverse fruiting induction indirectly by acting as a source of glyoxylate and of α amino groups for transamination reactions (7). This latter alternative would also account for the strong growth rate enhancement observed after glycine supplementation of defined media (5), including A1 minimal medium (unpublished data). The fact that glycine reverses adenine-, adenosine-, and hypoxanthine-induced fruiting as well as cAMP-induced fruiting argues that all these compounds induce fruiting by similar mechanisms.

In minimal medium containing glycine, even the addition of high concentrations of purine-containing compounds failed to induce fruiting (data not shown). However, growth inhibition, a response normally accompanying fruiting (9),

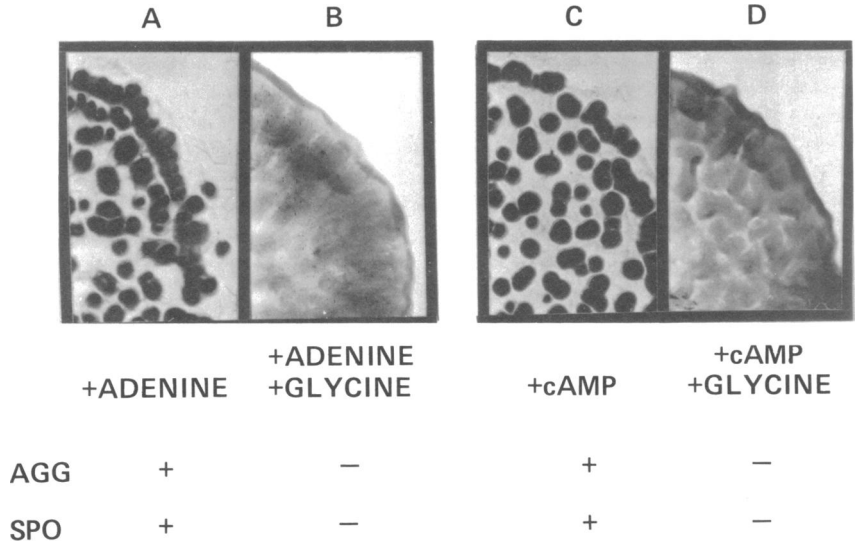


FIG. 4. *Glycine reversal of purine-induced fruiting. DK501 cells were grown, spotted, and scored as described in Fig. 1 and reference 9. Plates contained A1 medium supplemented with (A) 3.7 μM adenine, (B) 3.7 μM adenine and 1.3 mM glycine, (C) 15 μM cAMP, or (D) 15 μM cAMP and 1.3 mM glycine.*

was observed under these conditions. This is shown for adenine in Fig. 5. The growth inhibition caused by a high concentration of adenine (3.7 mM) was largely reversed by the further addition of a low concentration of thiamine (3.0 μM). Inhibition of growth on solid medium by a high concentration of cAMP (3.0 mM) was reversed by a similar treatment (unpublished data). Since adenine treatment of *Escherichia coli* cells causes a dramatic decrease in intracellular phosphoribosyl pyrophosphate levels (1), treatment of *M. xanthus* with high concentrations of adenine (and compounds related to adenine) may limit the synthesis of substances requiring phosphoribosyl pyrophosphate for their synthesis (4, 10), including thiamine (and histidine, tryptophan, and uridine).

Taken together, these data argue that cAMP and other purine-containing compounds induce

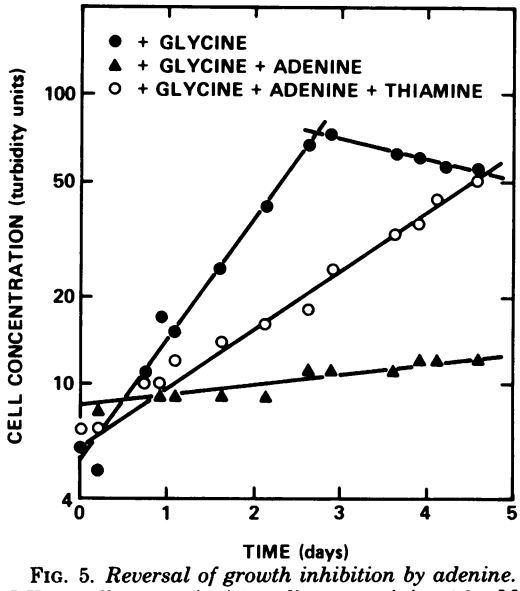


FIG. 5. *Reversal of growth inhibition by adenine. DK501 cells grown in A1 medium containing 1.3 mM glycine were diluted into fresh A1 medium containing 1.3 mM glycine (●), 1.3 mM glycine and 3.7 mM adenine (▲), or 1.3 mM glycine, 3.7 mM adenine, and 3.0 μM thiamine (○). One turbidity unit corresponds to approximately 5 × 10^6 cells per ml. A culture in A1 medium containing glycine (1.3 mM) and adenine (3.7 mM), supplemented with 645 μM histidine, 490 μM tryptophan, and 41 μM uridine in addition to 3.0 μM thiamine, grew with approximately the same kinetics as one supplemented with thiamine alone (○).*

fruiting by the same fundamental mechanism. In fact, it is possible that cAMP is converted to adenosine or adenine before it enters the cytoplasm of the cell, by degradative enzymes outside the cell or in the periplasm (6). The blockage of the effects of purine-containing compounds by low levels of common nutrients argues that purine-containing compounds induce fruiting indirectly by causing intracellular nutritional imbalances rather than by acting directly as regulatory molecules. Thus, the question of whether cAMP serves a significant regulatory function in the fruiting program of *M. xanthus* remains open.

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