Induction by Ethylene of Macrocyst Formation in the Cellular Slime Mould

*Dictyostelium mucoroides*

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A hitherto unidentified volatile substance(s) is known to induce macrocyst formation in a strain (Dm 7) of *Dictyostelium mucoroides* and in a mutant (MF 1) derived from it. The properties of this substance suggested that it might be ethylene, and here it is shown that this is indeed the case. The addition of ethylene to MF 1 cells, in conditions otherwise favouring sorocarp formation, induced the formation of macrocysts. Conversely, the addition of mercury perchlorate, an absorbent of ethylene, inhibited macrocyst formation and induced sorocarp formation under conditions otherwise favouring macrocyst formation. Two inhibitors of ethylene synthesis, aminooxyacetic acid and aminoethoxyvinyl glycine, also inhibited macrocyst formation. Production and release of ethylene by *D. mucoroides* cells was confirmed by gas chromatography.

**INTRODUCTION**

After ceasing to grow, slime mould cells usually construct a sorocarp consisting of an apical mass of spores and a supporting cellular stalk. Under certain environmental conditions, however, some strains of *Dictyostelium mucoroides* form a macrocyst as a final developmental structure via a morphogenetic pathway dissimilar to sorocarp formation (Blaskovics & Raper, 1957). The macrocyst of the cellular slime mould has been considered to represent a true sexual phase with zygote formation and transient diploidy (MacInnes & Francis, 1974). The initial sign of macrocyst formation by Dm 7 cells is the formation of large aggregates that are subdivided into smaller masses (precyst), each of which is surrounded by a fibrillar sheath. At the centre of the precyst there arises a cytophagic cell (giant cell) which proceeds to engulf all the other cells in the precyst. The enlarged cytophagic cell finally becomes surrounded by a thick wall to form the mature macrocyst (Filosa & Dengler, 1972).

Development toward sorocarp or macrocyst formation can be controlled by environmental conditions. Dm 7 cells form sorocarps in the light and macrocysts in the dark (Weinkauf & Filosa, 1965). However, in the presence of charcoal the cells form sorocarps even in the dark, suggesting that volatile substance(s), absorbed by charcoal, are necessary for macrocyst formation (Weinkauf & Filosa, 1965). The volatile substance must also be lipid soluble, because the cells form macrocysts in water (Filosa & Dengler, 1972), but sorocarps in mineral oil (M. F. Filosa, personal communication). Furthermore, the removal of CO₂, an antagonist of ethylene (Abeles, 1973), seems to enhance the effect of the volatile substance (Filosa, 1979). From these observations, ethylene was considered as a candidate for the volatile substance, and several kinds of experiment were done to determine whether or not ethylene is a naturally occurring inducer of macrocyst formation.

A mutant, MF 1, isolated by M. F. Filosa from Dm 7, forms macrocysts in the light as well as in the dark, and also produces the volatile substance absorbed by charcoal (Filosa, 1979). Since

**Abbreviations:** AOA, aminooxyacetic acid; AVG, aminoethoxyvinyl glycine.
the developmental pathway of MF 1 can be controlled by changing the amount of the volatile substance, even in the light (Amagai & Filosa, 1984), the mutant cells were used mainly in the present work.

METHODS

Organisms and cultivation. A spontaneously occurring mutant, MF 1, isolated from the wild-type Dictyostelium mucoroides-7 (Dm 7) was used in addition to Dm 7. Escherichia coli B/rw was used as a source of food. Spores of MF 1 were obtained from sorocarps formed over activated charcoal (Filosa et al., 1979). A 0.1 ml sample of a dense suspension of Dm 7 or MF 1 spores together with E. coli was spread with a glass rod over the surface of nutrient agar (Bonner, 1967). The agar plates were incubated at 22 °C for a General Electric cool white fluorescent lamp. After 1 d, vegetative amoebae were collected and shaken in 20 ml-phosphate buffer, pH 6.2, with E. coli for 21 h (Gerisch, 1960). Amoebae were harvested during the growth phase and washed three times in Bonner’s saline (Bonner, 1947). A sample of cell suspension was put on 2 ml of 2% plain agar in a glass Petri dish (3 cm diameter). After the amoebae had settled onto the substrate for 30 min, excess water was removed and the surface allowed to dry. The plates were then incubated for 48 h at 22 °C in the light. The final developmental forms were observed under a dissecting microscope.

Ethylene application. The 3 cm diameter plates on which MF 1 cells had been deposited were transferred into glass dishes of 9 cm diameter and covered with the same size of glass dishes (air space: 250 cm²). A 3 ml volume of 1% ethylene gas was injected into the air space through a narrow space between the two facing dishes, using a 5 ml glass syringe, and after the dishes were sealed with vinyl tape and Parafilm they were dipped in water to minimize gas leakage, and incubated at 22 °C.

Detection of ethylene by gas chromatography. A 1 ml suspension of washed cells in Bonner’s saline in a 20 ml Erlenmeyer flask, tightly sealed with a silicone stopper, was shaken gently in the dark for 48 h at 100 r.p.m. Ethylene released from the cells was determined by a slight modification of Imaseki’s method (Imaseki et al., 1968). An air/gas mixture (2 ml) was assayed by gas chromatography (Shimazu, GC7A type) on an activated alumina (60–80 mesh, Nishio Product) column (3.1 m × 3.2 mm) operated at 75 °C with helium (50 ml min⁻¹) as the carrier gas. Quantification of the ethylene peak was carried out with a printing integrator attached to the apparatus. All ethylene concentrations were adjusted for ethylene contamination in the air.

Effects of inhibitors. Agar (1 ml) containing 20 mM-MES buffer (pH 7.0) on which the MF 1 cells had been deposited at 2 × 10⁶ cells cm⁻² was transferred onto 1 ml of agar containing various concentrations of amino-oxycetic acid (AOA; Sigma) or aminoethoxyvinyl glycine (AVG) in 20 mM-MES buffer (pH 7.0). This was followed by incubation at 22 °C. AVG was kindly supplied by Dr. M. Stempel, Research Division, Hoffman LaRoche, Nutley, NJ, USA.

CO₂ application. Harvested MF 1 cells were plated on 2 ml of plain agar in the lid of a glass dish (4 cm diameter) at 1 × 10⁶ cells cm⁻². A similar lid with a small notch in it was placed over it and the two sealed with vinyl tape (air space 30 cm³). Pure CO₂ (3 ml) was injected into the air space through the notch, using a 10 ml plastic syringe. The hole caused by the needle was sealed quickly with vinyl tape. As controls, plates were incubated without added CO₂.

RESULTS

To examine whether ethylene is involved in macrocyst formation, the effects of ethylene removal and application were first determined. An agar plate with MF 1 cells deposited at 2 × 10⁶ cells cm⁻² was sealed over a glass plate containing 1 ml mercury perchlorate (0.25 M-HgO in 20 mM-HClO₄) as an absorbent for olefines such as ethylene (Young et al., 1952). Under these conditions, the cells formed sorocarps instead of macrocysts. Controls had perchlorate alone and normally these cells formed macrocysts, though they sometimes stopped their development at the aggregation stage. This incomplete development may have been due to the absorption by the perchlorate of basic gases necessary for macrocyst formation.

Table 1 shows that exogenously added ethylene (100 p.p.m.) induces macrocyst formation. Although MF 1 cells which developed in a large glass dish (air space 250 cm³) formed sorocarps due to the reduced concentration of the volatile substance (Amagai & Filosa, 1984), the addition of ethylene clearly induced macrocysts provided the cell density was high enough. At low cell density, however, cells usually stopped developing at the aggregation stage, perhaps due to subthreshold amounts of another substance(s) that might be required for complete induction of macrocysts. While a relatively high concentration of ethylene was applied in these experiments, a lower concentration of ethylene (1 p.p.m.) was also effective.
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Table 1. Effects of ethylene on MF 1 cells

The 3 cm diameter glass dishes on which MF 1 cells had been deposited were transferred into 9 cm diameter glass dishes which were faced together with the same size glass dishes (air space 250 cm²). A 3 ml volume of 1% ethylene gas was injected into the air space, and the glass dishes sealed with vinyl tape, wrapped with Parafilm, dipped in water and incubated for 48 h at 22 °C in the light. % means the proportions of the experimental cases which showed the developmental forms indicated in the Table.

<table>
<thead>
<tr>
<th>Cell density (cells cm⁻²)</th>
<th>No. of experiments</th>
<th>Ethylene (100 p.p.m.)</th>
<th>Macrocysts (%)</th>
<th>Aggregates (%)</th>
<th>Sorocarps (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 x 10⁵</td>
<td>3</td>
<td>+</td>
<td>33</td>
<td>66</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1 x 10⁶</td>
<td>4</td>
<td>+</td>
<td>25</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2 x 10⁶</td>
<td>7</td>
<td>+</td>
<td>86</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>−</td>
<td>0</td>
<td>29</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 2. Release of ethylene from Dm 7 and MF 1 cells

A 1 ml suspension of Dm 7 or MF 1 cells was put into a 20 ml Erlenmeyer flask. The flasks were sealed tightly with a silicone stopper and shaken gently in the dark for 48 h at 100 r.p.m.; 2 ml of the air/gas mixture was then taken from a flask and the ethylene released from the cells was assayed by gas chromatography.

<table>
<thead>
<tr>
<th>Cell density (cells ml⁻¹)</th>
<th>Amount of ethylene released (p.p.m.)</th>
<th>Dm 7</th>
<th>MF 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 x 10⁶</td>
<td></td>
<td>2.4 x 10⁻¹</td>
<td>1.7 x 10⁻¹</td>
</tr>
<tr>
<td>2 x 10⁶</td>
<td></td>
<td>3.3 x 10⁻²</td>
<td>3.5 x 10⁻²</td>
</tr>
</tbody>
</table>

Table 3. Effects of inhibitors of ethylene synthesis on macrocyst formation

Agar (1 ml) containing 20 mM-MES buffer (pH 7.0) on which the MF 1 cells had been deposited at 2 x 10⁶ cells cm⁻² was transferred onto 1 ml of agar containing various concentrations of AOA or AVG in 20 mM-MES buffer (pH 7.0). This was followed by incubation for 48 h at 22 °C in the light.

<table>
<thead>
<tr>
<th>Final inhibitor concn (M)</th>
<th>Final developmental forms</th>
<th>AOA</th>
<th>AVG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Macrocysts</td>
<td>MACROS</td>
<td>MACROS</td>
</tr>
<tr>
<td>1 x 10⁻⁴</td>
<td>Macrocysts</td>
<td>MACROS</td>
<td>MACROS</td>
</tr>
<tr>
<td>5 x 10⁻⁴</td>
<td>Sorocarps</td>
<td>MACROS</td>
<td>MACROS and aggregates</td>
</tr>
<tr>
<td>1 x 10⁻³</td>
<td>No aggregation</td>
<td>MACROS</td>
<td>MACROS and aggregates</td>
</tr>
<tr>
<td>1 x 10⁻²</td>
<td>ND</td>
<td>AGGREGATES</td>
<td>AGGREGATES</td>
</tr>
</tbody>
</table>

ND, Not determined.

To measure ethylene production by cells, gases released by cells into the air space were analysed by gas chromatography, with special reference to ethylene. In general, it was necessary to use a large number of cells to produce measurable amounts of ethylene and so a suspension culture system with high cell densities was adopted. The results obtained indicate that ethylene was actually produced and released by both Dm 7 and the MF 1 derivative at almost the same levels (Table 2).

Since AOA and AVG are known to inhibit the synthesis of 1-aminocyclopropane-1-carboxylic acid (ACC), an intermediate in the conversion of methionine to ethylene (Boller et al., 1979; Amrheim & Wenker, 1979), their effects on macrocyst formation were examined. As shown in Table 3, AOA inhibited macrocyst formation and induced sorocarp formation at 5 x 10⁻⁴ M.
Fig. 1. Development of MF 1 cells with and without added CO₂. (a) Macrocysts formed without added CO₂ after 48 h incubation; (b) sorocarps formed in the presence of 10% CO₂ after 24 h incubation; (c) large aggregates formed without added CO₂ after 10 h incubation; (d) normal size aggregates formed in the presence of 10% CO₂ after 10 h incubation. Photographs were taken under a dissecting microscope. Magnification, 21 x.

Above 10⁻³ M-AOA, cells never aggregated. Although higher concentrations of AVG had inhibitory effects on macrocyst formation, sorocarps never formed under these conditions.

When cells developed under an atmosphere of 10% CO₂, an antagonist of ethylene (Abeles, 1973), sorocarps, rather than macrocysts, were formed (Fig. 1b). When CO₂ was applied to cells at the aggregation stage, it induced many tips on the surface of an aggregate within 2 h, resulting in the formation of many sorocarps. During macrocyst formation, the size of the aggregate initially formed was quite large (Fig. 1c), as compared with that formed in the presence of CO₂ (Fig. 1d).

DISCUSSION

It is generally accepted that ethylene is a potent hormone which regulates many events in plant growth and development (Abeles, 1973). Ethylene is produced naturally by higher plants and also by fungi (Ilag & Curtis, 1968) and bacteria (Freebairn & Buddenhagen, 1964), but its function in fungi and bacteria is not yet known. This is the first demonstration that ethylene regulates development in the slime mould.

Macrocysts always formed when Dm 7 and MF 1 cells were starved in submerged and shaken conditions, but in contrast, cells plated on agar were capable of either macrocyst or sorocarp for-
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The formation of macrocysts and sorocarps in slime moulds is influenced by carbon dioxide and ethylene. Carbon dioxide promotes sorocarp formation in the appropriate conditions and one of the earliest signs of this is the formation of protruding tips on the aggregates. It is therefore possible that there is an antagonistic relationship between tip and macrocyst formation. In this context, it is noteworthy that cAMP, a chemotactic substance (Konijn, 1976), greatly inhibits macrocyst formation (Amagai & Filosa, 1984). In addition, as the aggregates formed with CO₂ were smaller than those formed without CO₂ and since CO₂ and ethylene appear to be mutual antagonists, it is possible that ethylene may also be involved in regulating the size of the aggregate. As pointed out previously (Bonner & Hoffman, 1963), there may be substances which control the spacing of aggregation territories and the orientation of sorocarps, and the present results hint that these may be CO₂ and ethylene.

Although MF 1 cells mainly were used in this work, it is reasonable to suppose that ethylene also promotes macrocyst formation in Dm 7 cells, since CO₂ switched Dm 7 cells incubated in the dark from macrocyst to sorocarp formation (data not shown).

All of the results presented here strongly suggest that ethylene may function as a macrocyst-inducing hormone in slime mould development. Taken together with our previous findings (Amagai & Filosa, 1984), it is of particular importance to know more precisely the relative role of ethylene and cAMP in controlling the choice of developmental pathways.

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REFERENCES


