Effects of Morphactins on Growth and Differentiation of *Acetabularia*

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With 7 Figures

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Summary

Morphactins bring about disturbances in the development and morphogenesis of *Acetabularia*: a delay in cap formation is observed at the concentrations of 2.0 and 20.0 μg/ml. At the higher concentration, there is, in addition, a reduction in the number of caps formed by the whole as well as by the anucleate algae. There are, however, some differences between the two types.

Morphactins do not favour the loss of polarity in anucleate *Acetabularia*.

They reduce growth in treated whole or anucleate algae as compared with the controls, but the whole treated ones reach finally a greater length than the controls.

The results are discussed.

1. Introduction

The development of the giant unicellular alga *Acetabularia* encompasses a series of morphogenetic events. When the normal sequence is disturbed, by the application of an inhibitor such as puromycin, morphogenesis is affected (Brachet 1963). Moreover, a regulation must occur at the cytoplasmic level since: 1. when the apex is repeatedly removed, the initiation of the cap is inhibited; 2. the triggering of cap initiation depends on external conditions such as light, in whole algae as well as in anucleate fragments.

Morphactins are fluorenols and substituted compounds. They have been shown to interfere reversibly with morphogenesis at a post-transcriptional level (Ziegler 1970). The mechanism of their action is mainly correlated with the inhibition of IAA transport. Not only indirect evidence has been provided, but Pilet (1970) demonstrated that the translocation of radioactive IAA is prevented by morphactins in epicotyl segments of *Lens culinaris*; Tognoni and Alpi (1969) have made similar observations in *Pisum sativum*. Morphactins might also partially interfere, in certain conditions, with gibberellic acids, although contradictory results have been reported in this respect (see Schneider 1970, for a review).
We have attempted to find out whether possible effects of morphactins on growth and morphogenesis in *Acetabularia* can throw some light on the problem of differentiation, although it has been claimed that morphactins do not act on algae and lower plant species (SCHNEIDER 1970): they do not produce visible changes in organisms such as *Chlamydomonas* (ZIELGLER et al. 1969).

2. Material and Methods

*Acetabularia mediterranea* was cultivated in standard laboratory conditions (LATEISR 1963). During the experiments, the culture medium (enriched sea-water) was replaced at least once a week; the morphactins, kindly provided by Merck, Darmstadt, contained 800/0 of 2-chloro-9-hydroxyfluorene-9-carboxylic methylester, ca. 200/0 of 9-hydroxyfluorene-9-carboxylic methylester and a small amount of 2.7-dichloro-9-hydroxyfluorene-9-carboxylic acid methylester. The action of the first compound is known to be the strongest in the biological tests. The morphactins were dissolved in the culture medium.

3. Experimental Results

3.1. Effect of Morphactins on Morphogenesis

3.1.1. Whole Algae

Morphactins bring about a delay in cap formation and, at the higher concentration, a reduction in the number of caps.

In 2 preliminary experiments, a delay of cap formation was observed within 2 weeks: if they are treated with 0.2 ~g/ml, algae 31 mm long form half as many caps as the controls. Smaller algae (about 11 mm long) do not form a cap in the presence of 0.2 ~g/ml morphactins in this time span.

At a concentration of 2 ~g/ml a delay of cap formation is observed with the exception of experiment 3 (Table 1). The percentages of caps have been calculated from control and treated samples comprising each from 32 to 125 algae depending on the experiment. However, the number of caps that are finally formed is the same as in the controls (Experiments 2 and 3) or only slightly smaller (Experiments 1 and 6) (see also Figs. 1-3).

At a concentration of 20 ~g/ml of morphactins, there is a longer delay in cap formation and a much more severe inhibition; the final number of caps is 1/4 to 1/2 of the controls (Experiments 4-6, Fig. 4).

Experiment 6 was designed in order to ascertain that a 20.0 ~g/ml concentration has a stronger effect than a 2.0 ~g/ml one on morphogenesis in *Acetabularia* (Table 1 and Fig. 3).

It is concluded that morphactins have a clear-cut inhibitory effect on morphogenesis in *Acetabularia*.

3.1.2. Anucleate *Acetabularia*

The experiments on anucleate fragments have been performed simultaneously with those on whole *Acetabularia*, using algae from the same batch. The results are summarized in Table 1.