BIOLOGY

Effect of Chelating Agents on Plasmodium Formation by the True Slime Mould, *Didymium nigripes*

SPORES of *Didymium nigripes*, a true slime mould, germinate to liberate myxamœbæ which multiply by binary fission. After a period of exponential growth, the uninucleate myxamœbæ fuse to form macroscopic, multinucleate veins of rhythmically flowing protoplasm called plasmodia. Following an indefinite period of growth, the plasmodia differentiate into spore-containing fruiting bodies. Kerr and Sussman¹ grew *D. nigripes* in monoxenic culture with *Aerobacter aerogenes* on a dilute glucose-peptone-yeast extract agar medium buffered with phosphate at pH 6.2 (hereafter called GPY/5). All stages of the life-cycle were regularly reproduced in less than 7 days. When 0.25 per cent (w/v) brucine was added to the GPY/5, plasmodia did not appear. Instead, the myxamœbæ remained indefinitely as such and could be sub-cultured as amœbæ. The myxamœbæ quickly formed plasmodia when harvested from the GPY/5-brucine plates and washed by differential centrifugation.

The morphogenesis of uninucleate myxamœbæ to multinucleate plasmodia is one of the most interesting, but least understood, stages in the life-cycle of the true slime moulds. According to Cadman² and Ross³, plasmodium formation involves the pair-wise fusion of myxamœbæ as gametes to form zygotes. Presumably the zygotes develop into plasmodia by growth, nuclear division, and fusion with other zygotes. Since an understanding of this morphogenesis is an essential prerequisite to studies on the genetic life-cycle, and to studies of heterokaryosis and nuclear-cytoplasmic interrelationships in the plasmodium, an elucidation of its mechanics has been undertaken. The mechanism by which brucine inhibits plasmodium formation was studied in the hope that it would yield information on the normal process of plasmodium formation.

One possible action of brucine might be to stimulate the bacterial associate to produce a metabolite which in turn would inhibit plasmodium formation. This possibility was tested by growing myxamœbæ on GPY/5-brucine with a variety of bacterial associates, including *Escherichia coli*, *Sarcina lutea*, *Pseudomonas fluorescens* and *Aerobacter aerogenes*. Plasmodia did not appear in any instance. It therefore seemed probable that brucine was acting directly on the myxamœbæ rather than via the bacterial associate.

Several lines of evidence indicated that brucine might be inhibiting plasmodium formation by chelating with ions in the medium. First, although the medium used was saturated by 0.1 per cent (w/v) brucine, the inhibitory action of brucine became more and more complete as its concentration was raised to 0.25 per cent. This might be expected if brucine were acting as a chelating agent, but not if it were acting as a metabolic inhibitor. Second, brucine inhibits the formation of plasmodia rather than plasmodia themselves, for if plasmodia are sub-cultured on GPY/5-brucine, they grow and fruit normally. Third, the density of myxamœbæ profoundly influences the number of plasmodia which they form. Even in the absence of brucine, plasmodium formation was delayed when the myxamœbæ

quickly reached a high density. Plasmodia appeared in greatest number when the myxamœbæ exhausted their bacterial food supply while still at a relatively low density. Brucine-inhibition was complete only when the myxamœbæ covered the entire plate at high density before exhausting their food supply; when myxamœbæ were plated clonally on GPY/5-brucine, occasional plasmodia appeared.

As a result of these observations, experiments were devised to test the hypothesis that multivalent cations are essential to plasmodium formation. The results are reported in Table 1. Chelating agents were added to GPY/5 media which were spread with spores of *D. nigripes* and 0.3 ml. of a broth culture of *A. aerogenes*. In each case the pH was adjusted to 6.0-6.5. The plates were incubated at 22° C. for 4 days and then scored for growth of myxamœbæ and appearance of plasmodia. Tetrasodium ethylenediamine tetraacetic acid at 10⁻³ M completely prevented the formation of plasmodia, even when the spores gave rise to clones. Either 10⁻³ M Mn⁺⁺ or 10⁻³ M Fe⁺⁺ reversed the inhibition of ethylenediamine tetraacetic acid to permit numbers of plasmodia to form. None of the other ions tested was effective. Other chelating agents, including sodium citrate, were also effective in inhibiting plasmodium formation.

Table 1. THE GROWTH OF MYXAMŒBÆ AND APPEARANCE OF PLASMODIA ON GPY/5 MEDIA TO WHICH VARIOUS AGENTS HAD BEEN ADDED

Addition	Amœbæ	Plasmodia
Control	+++	+ + +
0.25 per cent (w/v) brucine	+++	0
0.1 M Sodium citrate	++	0
10 ⁻³ M Ethylenediamine tetraacetate acid	++	0
5 × 10 ⁻⁴ M Ethylenediamine tetraacetic acid	+++	+
10 ⁻³ M Ethylenediamine tetraacetic acid		
+ 10 ⁻³ M calcium chloride	++	0
+ 10 ⁻³ M strontium chloride	++	0
+ 10 ⁻³ M magnesium chloride	++	0
+ 10 ⁻³ M manganese chloride	++	++
+ 10 ⁻³ M ferrous chloride	++	++

The experimental results thus confirm the hypothesis that multivalent cations are necessary for plasmodium formation. A likely mode of action of brucine is as a chelating agent, removing these ions from the medium.

The development of a technique which will measure plasmodium formation in a quantitative manner is in progress. These experiments will be reported elsewhere.

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¹ Kerr, N. S., and Sussman, M., *J. Gen. Microbiol.*, **10**, 173 (1958).

² Cadman, E. J., *Roy. Soc. Edin. Trans.*, **57**, 93 (1931).

³ Ross, I. K., *Amer. J. Bot.*, **44**, 843 (1957).

Effect of Extracts from Fucoids in promoting Settlement of Epiphytic Polyzoa

IN a previous communication¹ it was suggested that the simple ciliated larvæ of some species of sedentary organisms might respond to qualitative differences in bacterial films formed on surfaces immersed in the sea, as was suggested by Wilson to explain the behaviour of the larvæ of *Ophelia bicornis* Savigny².