Chemotaxis of *Physarum polycephalum* towards Carbohydrates, Amino Acids and Nucleotides

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A simple method for studying the chemotactic responses of the plasmodium of *Physarum polycephalum* was designed. A positive chemotactic response was detected towards the sugars D-glucose, lactose, D-maltose and D-galactose, the amino acids L-leucine, L-serine and DL-phenylalanine, and the nucleotide uracil. The significance of chemotaxis in *Physarum* is discussed.

INTRODUCTION

The fascinating phenomena of chemotaxis, movement and plasma shuttle streaming in plasmodia of myxomycetes have attracted the attention of many scientists for almost a century (Pfeffer, 1883; Kamiya, 1959; Carlile, 1970; Chet & Mitchell, 1976).

In recent years, analysis of receptors and a better understanding of their mode of action in bacteria has been achieved (Adler, 1975). The role of chemotaxis in cell aggregation of the cellular slime-mould was comprehensively reviewed by Bonner (1971) and his collaborators. The plasmodium of acellular slime-moulds shows a similar response towards chemical attractants (Konijn, 1973). There has been much interest in the biochemical and physiological analogies between the plasmodium of acellular slime-moulds and the muscular systems in vertebrates (Kessler, 1972; Komnick, Stockem & Wohlfarth-Botterman, 1973).

In this paper we describe a system developed to measure chemotactic responses towards carbohydrates, amino acids and nucleotides.

METHODS

Microplasmodia of *Physarum polycephalum* (Madison strain M₃C) were grown in 250 ml flasks containing 20 ml semi-defined nutrient solution prepared according to Daniel & Baldwin (1964), and incubated for 48 to 72 h at 24 °C in darkness on a reciprocal shaker (70 cycles min⁻¹). The microplasmodia were centrifuged at 150 g for 5 min, and washed twice in salt solution (Daniel & Baldwin, 1964). Samples (0.1 ml) of microplasmodia were placed on sterile medium containing 0.02 M-phosphate buffer (pH 6.4) and 2 % (w/v) agar (Difco). Within 12 h fusion of the microplasmodia took place, and after 24 h 1.0 × 0.5 cm pieces of plasmodium were cut and transferred to the test system.

The 'star' configuration of the test system was cut in the agar (12 ml 0.02 M-phosphate buffer pH 6.4 and 1 % agar) as shown in Fig. 1. Test substance (0.1 ml, 0.05 M) was applied to one of the four holes (8 mm diam.) cut in the agar. The other three holes contained the control buffer solution.

The glucose gradients formed in the agar were measured by covering its surface with 5 ml of solution containing 1 mg 2,3,5-triphenyltetrazolium chloride ml⁻¹ (Sigma) for 10 min in darkness (Stahal & Kaltenbach, 1965). The reagent was washed off with water. Strips of agar (1 × 5 cm) were cut and placed on microscope slides and the colour intensity was recorded by a Varian spectrophotometer model 635 (Varian Techtron, Mulgrave, Australia) at 600 nm. This test showed that a gradient of 1 to 2 µg ml⁻¹ mm⁻¹ between 40 to 60 µg glucose ml⁻¹ was sufficient for a positive chemotactic response. Such a gradient was reached 30 mm from the hole containing the attractant after 24 h of diffusion. A positive chemotactic response was

Fig. 1. Positive chemotaxis of a plasmodium of *Physarum polycephalum* towards 0.1 ml of 0.05 M-glucose in the 'star' chemotaxis test, carried out in a 9 cm diam. Petri dish. The other three holes contained 0.02 M-phosphate buffer as controls.

recorded when the plasmodium moved towards the hole containing the test substance and touched it within 24 h.

Data of all tests were analysed for distribution of frequency of events at a significance level of 0.05. The most probable outcome of random migration gave the result of one positive response in four successful tests. A successful test was recorded when the plasmodium touched any of the four holes within 24 h. All tests were carried out at 24 °C in darkness.

RESULTS AND DISCUSSION

Our method of measuring chemotactic responses improves that previously used (Madelin, Audus & Knowles, 1975; Knowles & Madelin, 1975) in that the 'star' cuttings prevent diffusion and mixing of substances in the different holes (Fig. 1). Using this test we have shown that the plasmodium of *Physarum polycephalum* responds positively to the monosaccharides D-glucose and D-galactose, and to the disaccharides lactose and D-maltose. With cellobiose and D-mannitol, a response was suspected but was not proved (Table 1). These results are in accord with those reported by Carlile (1970), except for the absence of chemotaxis to mannose which may be due to the different strain tested.

In contrast to the effect of glucose and galactose analogues on bacterial chemotaxis (Adler, 1969), no effect of α -methylglucoside, 2-deoxyglucose or raffinose on plasmodial chemotactic movement was observed.

Out of 10 amino acids tested, only L-leucine, DL-phenylalanine and L-serine were attractants (Table 1). Similarly, zoospores of *Allomyces* sp. showed a positive chemotactic response to L-serine (Carlile & Machlis, 1965; Machlis, 1969). The effective response of bacterial membrane receptors to the amino acid L-serine was demonstrated by Adler (1975) and Mesibov & Adler (1972).

In view of the active nucleic acid metabolism which presumably occurs during growth of the plasmodia of *P. polycephalum* (Rusch, 1970), the failure of most of the tested nucleotides and related compounds to attract the plasmodium was unexpected. Positive chemotaxis was confirmed only with uracil (Table 1).

Recently, Carlile (1976) concluded that specific receptors for attractants are present in *P. polycephalum*. The ability to distinguish between compounds affecting motility and chemotaxis in the plasmodium of *P. polycephalum* may be helpful in the biochemical and physiological research on the principles of chemotactic movement of eukaryotic organisms.

Table 1. *Chemotactic response of a plasmodium of Physarum polycephalum to some carbohydrates, amino acids and nucleotides*

The 'star' test was used with phosphate buffer as the control solution.

Substrate (0.05 M)	No. of successful tests	No. of positive responses	Probability of results* (%)	Response†
Carbohydrates				
Glucose	10	9	2.7×10^{-4}	+
Lactose	10	7	3.08×10^{-1}	+
Maltose	10	6	1.6	+
Galactose	10	6	1.6	+
Cellobiose	10	5	5.8	0
Mannitol	15	6	9.1	0
Sucrose	15	4	85	0
Fructose	10	4	14.6	0
Rhamnose	15	3	100	0
Ribose	15	5	16.5	0
D-Arabinose	15	5	16.5	0
Xylose	5	1	100	0
α -Methylglucoside	15	5	16.5	0
2-Deoxyglucose	15	5	16.5	0
Mannose	5	2	23.6	0
Raffinose	5	2	23.6	0
Amino acids				
DL-Phenylalanine	15	10	6×10^{-3}	+
L-Leucine	5	4	1.4	+
L-Serine	13	9	3×10^{-2}	+
L-Histidine	5	1	100	0
L-Cysteine	5	1	100	0
L-Alanine	5	1	100	0
L-Threonine	5	2	23.6	0
L-Proline	5	2	23.6	0
L-Arginine	10	4	14.6	0
L-Isoleucine	10	1	100	0
Peptone	10	6	1.62	+
Nucleotides				
Uracil	5	4	1.4	+
Uridylic acid	5	0	100	0
Adenylic acid	5	1	100	0
Thymine	5	1	100	0
Adenine	5	2	23.6	0
Uridine	5	1	100	0
Cytosine	5	2	23.6	0
Cytidine	5	2	23.6	0
Thymidine	5	1	100	0
Adenosine	5	0	100	0

* Probability (%) of the results being due to random migration.

† +, Positive chemotaxis at the 5% significance level ($P = 0.05$); 0, no significant response.

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