Chemotropism of *Achlya ambisexualis* to Methionine and Methionyl Compounds

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The chemotropic response of the water mould *Achlya ambisexualis* to nutrients was investigated. Among individual amino acids only methionine was active. Other amino acids were active only in combinations containing cysteine. Methionine was unique among the amino acids tested in its ability, when incorporated uniformly into the water agar substratum, to disrupt chemotropism towards an attractant mixture of amino acids. Among non-amino acid compounds tested for chemotropic activity only *S*-adenosylmethionine was active. Carboxyl methylation of protein was promoted by amino acid mixtures.

INTRODUCTION

Hyphae of oomycetous water moulds (Fischer & Werner, 1955) and the rhizoids of the chytridiomycetous fungus *Blastocladiella emersonii* (Harold & Harold, 1980) grow chemotropically towards casein hydrolysate or amino acid mixtures. Using *Achlya bisexualis*, Musgrave *et al.* (1977) devised a method for quantifying the chemotropic response. Although they did not report a systematic survey of all L-amino acids, they agreed with the conclusion of Fischer & Werner (1955) that no single amino acid was chemotropically active. When comparing the relative chemotropic potency of single amino acids, they did the tests on agar containing dilute casein hydrolysate.

Reports that tripeptides such as *N*-formylmethionyl-leucyl-phenylalanine (fMet-Leu-Phe) are chemotactically active in leucocytes (Showell *et al.*, 1976) prompted a re-examination of the chemotropic responses of *Achlya*. Our work indicated that in *A. ambisexualis* (male strain, E87) L-methionine functioned on its own as a chemotropic stimulus, a finding that differed from those of Fischer & Werner (1955) and Musgrave *et al.* (1977). We examined the role of methionine and methionyl derivatives in chemotropism of *Achlya*.

METHODS

Spore preparation. *Achlya ambisexualis* Raper (male strain, E87) was grown in peptone/yeast-extract/glucose medium (PYG; Cantino & Lovett, 1960) at room temperature (21–23 °C) with gentle agitation on a reciprocating shaker for 24 h, when the culture reached the mid-exponential phase of growth. The mycelium was rinsed with and suspended in 5×10^{-4} M-CaCl₂ to induce sporulation (Griffin, 1966). The mycelium was removed by aseptic filtration and spores were stored for a maximum period of 2 weeks in CaCl₂ at 4 °C until they were used for chemotropic tests.

Screening for chemotropic activity. (a) *General procedure.* A sector of agar was removed from a 9 cm Petri dish containing 20 ml 1.5% water agar (Difco or Gibco). An agar donor block (S) containing the test compound (4×10^{-5} M to 1×10^{-3} M) was placed in close contact with the cut edge of the agar (Fig. 1a). The inoculum (0.1 ml of a suspension containing 1×10^5 spores ml⁻¹) was applied as a narrow band (I) parallel to and 4 cm from the donor block.

(b) *Reorientation test.* A reorientation test provides a useful criterion for distinguishing between growth stimulation and chemotropism (Musgrave *et al.*, 1977). The procedure is described in Fig. 1(d).

Comparison of chemotropism-inducing activity. Since the activity comparisons are based on the assumption of approximately similar diffusion rates in 1.5% water agar, we compared the diffusion rates of selected amino acids,

Fig. 1. Chemotropism assays (see Methods). (a) General test format using a 9 cm Petri dish: I, zone of inoculum; S, donor block with test compound(s), 4 cm from inoculum. (b) Magnified representation of spores in the zone of inoculum. (c) Magnified representation of hyphal growth after 3 d incubation, with parallel orientation of germ tubes proximal to donor block containing attractant and random orientation distal to donor. (d) Representation of reorientation test: spores were allowed to germinate and grow at room temperature for 48 h after which the initial donor block S_1 was removed, and a fresh donor placed in position S_2 . Reorientation was reported if the hyphal tips bent towards S_2 within 24 h. (e) For comparing relative chemotropic activities, test compounds in varying concentrations were supplied in donor blocks (S). Individual amino acids used in mixtures were each equimolar to compounds used alone. The inoculation zone (I) was at a 45° angle to the donor block. After 3 d at room temperature, the perpendicular distance (D) from the donor block to that point on the line of inoculum beyond which no chemotropic growth could be observed was measured. The lowest active concentration of the attractant was determined by extrapolation from a linear plot of D versus log concentration of the test compound in the donor block, as exemplified in Fig. 2. All tests were in duplicate, and each point represents the mean of two independent determinations.

individually and in combination, using radiolabelled compounds. Donor blocks ($6.5 \times 0.5 \times 0.4$ cm) containing 1×10^{-3} M test substance (5.7×10^5 c.p.m. per block) were placed against the cut edge of the water agar as shown in Fig. 1(a). The test substance was allowed to diffuse into the agar at room temperature for 24 h. The position of the advancing front of the diffusant, the diffusion distance, was determined by removing 3 mm diameter agar discs along the axis perpendicular to the donor block at 0.5 cm intervals and by measuring the radioactivity associated with each sample by liquid scintillation counting.

The lowest chemotropically active concentrations of selected compounds were determined by a procedure modified from that of Musgrave *et al.* (1977) and described in Fig. 1(e).

Determination of methylation. (a) *Pretreatment of mycelium.* Mycelium grown in PYG medium for 24 h was washed thoroughly with, and resuspended in, Tris/glucose medium (0.01 M-Tris, 7.5 mM-glucose, pH 6.9). The suspension was incubated for 18 h at room temperature to attenuate endogenous nutrients before use in methylation experiments.

(b) *Protein methylation.* Quantities of approximately 0.25 g wet weight of mycelium, pretreated as described above, were incubated for various times in 10 ml-Tris/glucose medium containing *S*-adenosyl-L-[methyl- 3 H]methionine (specific activity 2.2×10^4 c.p.m. ml $^{-1}$) in the presence and in the absence of amino acid mixtures. Chemotropically active mixtures, as determined by tests on agar media, contained cysteine, aspartic acid, glutamic acid, alanine and leucine, each at 5×10^{-4} M; inactive mixtures had serine substituted for cysteine. Reactions were terminated by adding 10% (w/v) trichloroacetic acid. The mycelia were harvested and washed. Proteins were extracted using the procedure of Paik & Kim (1980) and measured using the Lowry method. Radioactivity associated with total protein was determined by liquid scintillation counting. Treatments were in triplicate and the experiment was repeated three times.

Fig. 2. Determination of the lowest active concentration of the tripeptide fMet-Leu-Phe that promotes chemotropism. The method is described in Fig. 1(e).

Chemicals. The radiochemicals *S*-adenosyl-L-[methyl- ^3H]methionine (sp. act. 72 Ci mmol $^{-1}$), L-[methyl- ^3H]methionine (sp. act. 78 Ci mmol $^{-1}$), [^{14}C]amino acid mixture (sp. act. 1 mCi mg $^{-1}$), L-[U- ^{14}C]leucine (sp. act. 55 mCi mmol $^{-1}$) were with the exception of the [^{14}C]amino acid mixture (ICN Pharmaceuticals Inc., Irvine, Calif., USA) obtained from Amersham (1 Ci = 37 GBq). All other chemicals were obtained from Sigma.

RESULTS AND DISCUSSION

Table 1 shows the results obtained when a variety of substances were tested for chemotropic activity in *A. ambisexualis*. Among individual amino acids only L-methionine induced a tropic response (Table 1a). The reorientation of hyphal tips (Fig. 1d) in response to changed positions of methionine-containing donor blocks is consistent with the conclusion that this compound is chemotropically active. Although a methionyl derivative (*N*-formyl-L-methionine) elicited a tropic response, other formylated amino acids were inactive (Table 1b). Our disagreement with other reports (Fischer & Werner, 1955; Musgrave *et al.*, 1977) could stem from the use of different species of *Achlya*. It is also possible that Musgrave *et al.* (1977) may not have included methionine in their experiments using single amino acids. Their tests on agar substrate containing casein hydrolysate showed methionine to be the most chemotropically active of all the amino acids studied. While individual amino acids other than methionine were all chemotropically inactive, mixtures containing cysteine did elicit tropism. This thiol amino acid, in the presence of other amino acids such as glutamic acid, aspartic acid, leucine or phenylalanine, may serve as a substrate for methionine synthesis (Lehninger, 1975). The tropic responses of *Achlya* to small peptides are shown in Table 1(d). Of the small peptides tested only methionine-containing peptides were active; however, a peptide (methionine enkephalin) with carboxyl-terminal methionine was inactive. Other metabolically important compounds such as glucose, lactate and acetate failed to induce chemotropism (Table 1e). This lack of response to non-amino acid nutrients is consistent with findings in *A. bisexualis* (Musgrave *et al.*, 1977) and *Blastocladiella emersonii* (Harold & Harold, 1980). The methyl group donor *S*-adenosylmethionine, for which *Achlya* has an uptake mechanism (Manavathu & Thomas, 1982) was active chemotropically (Table 1e).

A comparison of the chemotropic activity of the tripeptide fMet-Leu-Phe with that of its component amino acids shows that the peptide is approximately 30-fold less efficient than the free amino acids (Table 2). Amino acids and mixtures showed similar diffusion rates (1.25, 1.25, 1.35 and 1.45 mm h $^{-1}$ for leucine, tryptophan, methionine, and a mixture of these amino acids), indicating that their varying chemotropic effectiveness is not based on differences in diffusion rate.

Table 1. *Screening for positive chemotropism in Achlya ambisexualis*

Agent	Positive chemotropism	Agent	Positive chemotropism
(a) Amino acids (4×10^{-5} and 1×10^{-3} M)*		(d) Peptides (4×10^{-5} and 1×10^{-3} M)	
Met	yes	Met-Met	yes
D-Met	no	Met-Leu	yes
Ala, Arg, Asn, Asp, Cys, Glu, Gly, Gln,		Met-Phe	yes
His, Ile, Leu, Lys, Phe, Pro, Ser, Thr,		N-formyl-Met-Leu-Phe	yes
Trp, Tyr, Val	no	Glu-Cys-Gly	no
(b) Formylated amino acids (4×10^{-4} M)		Gly-Gly-Phe-Leu	no
N-formyl-(DL)-Phe	no	Tyr-Gly-Gly-Phe-Leu	no
N-formyl-Leu	no	Tyr-Gly-Gly-Phe-Met	no
N-formyl-Met	yes	(e) Other metabolites (1×10^{-3} M)	
(c) Amino acid mixtures (4×10^{-4} M)†		Glucose	no
Leu + Phe	no	Lactate	no
Ala + Asp + Glu + Leu + Ser	no	Acetate	no
Cys + Leu	yes	S-Adenosylmethionine	yes
Cys + Phe	yes		
Ala + Asp + Cys + Glu + Leu	yes		
Casamino acids ($200 \mu\text{g ml}^{-1}$)	yes		

* Unless otherwise indicated L-amino acids were used. The original concentration of the agent in the donor block is specified in parentheses.

† Each amino acid was present at the concentration specified.

Table 2. *Relative chemotropic activity of certain amino acids and the tripeptide fMet-Leu-Phe*

Attractant	Lowest active concn* (nM)
Met	71
Met + Cys	50
Met + Phe	22
Met + Leu + Phe	14
fMet-Leu-Phe	410

* See Fig. 1(e) for method.

The experiments described above involved a linear gradient of test compounds diffusing from a donor block. In further experiments we incorporated uniformly into the agar substratum compounds which might possibly modify the chemotropic response to attractants diffusing from a donor block. For instance, the importance of methionine in chemotropism suggested that its presence might be required for chemotropism towards otherwise inactive compounds such as glucose. However, chemotropically inactive compounds such as individual amino acids and sugars (5×10^{-4} M) diffusing from agar donor blocks remained inactive in the presence of methionine (5×10^{-4} M) supplied in the agar substrate. The effects of substrate-supplied amino acids on chemotropism towards donor blocks containing Casamino acids (0.2 mg ml^{-1}) revealed (Table 3) that methionine was unique in its ability to disrupt chemotropism. In addition to virtual elimination of chemotropism, as evaluated by a chemotropic index, methionine elicited disoriented growth with random curvatures and 'looping' of hyphae, in contrast to chemotropic growth characterized by parallel, linear, sparingly-branched hyphae (Fig. 3). Although other amino acids and mixtures had varying effects on the chemotropic index, including a notable stimulation in the presence of phenylalanine, none of these treatments disrupted hyphal chemotropism. Additional experiments using varying concentrations of a number of individual amino acids in the agar substrate revealed no interference with chemotropism except by methionine.

Since the methyl donor S-adenosylmethionine was itself chemotropically active, the possible involvement of methylation in the chemotropic response was examined. Initial experiments included studies of the time course of methylation of total protein in the presence of various

Fig. 3. (a) Linear chemotropic growth along a gradient of Casamino acids. The direction of the donor block source is indicated by S. Parallel linear growth of hyphae occurred on water agar, on agar substrate with added amino acids other than methionine, or with amino acid mixtures (Table 3). When methionine (5×10^{-4} M) was incorporated into the agar substrate (b), hyphal looping occurred; bar, 50 μ m.

Table 3. *Effect of amino acids applied in the water agar substrate on chemotropic growth of Achlya towards a donor block*

Additive(s) in substrate*	Chemotropic index†	Growth towards donor block‡
None	2.0	L
Met	1.2	D
Ala + Asp + Cys + Glu + Leu	1.4	L
Cys + Leu	1.4	L
Cys + Phe	1.8	L
Gly	1.5	L
Leu	1.7	L
Cys	1.8	L
Tyr	2.1	L
Ser	2.2	L
Phe	3.2	L

* Each amino acid alone or in combinations was at a concentration of 5×10^{-4} M.

† Ratio of average linear growth towards the donor block to growth away from it; growth on water agar with a water agar donor block gave a chemotropic index of 1.1, approximating the expected value of 1.0.

‡ Donor block contained 0.2 mg amino acids ml⁻¹; growth patterns are shown in Fig. 3; L, linear; D, disoriented.

Table 4. *Effect of chemotropically active and inactive amino acid mixtures on protein methylation*

Treatment	Time ...	[³ H]Methyl incorporated (c.p.m. per 0.25 g)*		
		15 min	30 min	60 min
Control		81 $\pm$ 80	411 $\pm$ 20	1094 $\pm$ 17
Inactive mixture†		404 $\pm$ 14	1496 $\pm$ 21	2722 $\pm$ 66
Active mixture		568 $\pm$ 10	1253 $\pm$ 19	1959 $\pm$ 36

* Values represent mean $\pm$ SD; $n = 3$.

† See Methods.

chemotropic stimuli. Amino acids in combinations but not individually enhanced protein methylation. Glucose failed to enhance protein methylation. Chemotropically active and inactive amino acid mixtures both enhanced protein methylation to a similar extent (Table 4). During an initial 15 min period after applying the amino acids to nutrient-deprived mycelia, enhancement of methylation as compared with controls was greater (about fivefold) than over a 30 min (three- to fourfold) or 60 min (about twofold) period. Although no significant differences in carboxyl methylation of total proteins were observed when comparing chemotropically active

and inactive mixtures of amino acids, or when comparing methionine and the chemotropically inactive alanine, our results do not preclude the possible involvement of specific protein methylation in chemotropism. Interpretation of the results is complicated by the discrepancy between the kinetics of the chemotropism tests, which require 2 to 3 d and those of the protein methylation studies, which require 15 to 60 min. Methionine is a prerequisite for bacterial and lymphocyte chemotaxis (Adler & Dahl, 1967; O'Dea *et al.*, 1978). While protein methylation has been implicated in bacterial chemotaxis (Koshland, 1980) and in slime mould chemotaxis (van Waarde & van Haastert, 1984), its possible role in the chemotropic growth of *Achlya* remains to be determined.

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