Effects of Chemical Substances on Rate of Locomotion in the Amoeba *Mayorella penardi*¹

NORIKO OSHIMA,* FUMIKAZU TAKEDA,** and KEICHI ISHII**

*Department of Biology, Faculty of Science, Toho University, Miyama, Funabashi, Chiba 274, Japan and **Laboratory of Biology, Hosei University, Fujimi, Chiyoda-Ku, Tokyo 102, Japan

ABSTRACT. The relationship between the locomotive velocity of amoeba which had not been fed for 24 h and the concentration of the test solutions was examined. With solutions of L-amino acids, protein substances, and alcian blue 8GS, an increase in velocity began at very low concentrations, reaching a maximum at a higher concentration and as the concentration increased further, the velocity fell to zero. In contrast, no increase was observed with D-glutamic acid and 8-alanine. Moreover, the velocity of well fed amoebae did not increase significantly even in L-amino acid solution. These results may suggest a correlation between orthokinesis and amoeboid phagocytosis.

Many previous researchers have reported that a large variety of proteins and peptones act as inducers of a feeding response in amoebae (2, 7, 8–10). On the other hand, Nomhi & Tawada (5) showed that a certain negatively charged protein (MW 24,000) extracted from *Tetrahymena pyriformis* functions as a chemotactic substance for *Amoeba proteus*. They also stated that most other proteins were not chemical attractants for the amoeba and could not induce pseudopodium formation. It was discovered, however, that an extract from hydra, containing a positively charged protein, was effective as chemotactic agent (4). Brewer & Bell (1) found that in an amoeba the pseudopodium was induced by an electrostatic imbalance at the surface of the amoeba, which was brought about by the direct attachment of cations such as quaternary ammonium ions. Thus, the possible inducer of food cup formation in phagocytosis seems to remain enigmatic.

As an approach to this subject, therefore, we have examined the locomotive velocity of amoebae in solutions of various chemicals and discussed the relation between kinetic behavior and formation of food cups.

MATERIALS AND METHODS

*Mayorella penardi*, clone 2, isolated in our laboratory from wild strains and fed with *Chilomonas paramecium* in KCM solution (KC, 35 mM, CaCl₂, 135 mM, MgSO₄, 16 mM, pH 6.8–7.2) at 22°C, was used. This clone is appropriate for measuring the rate of locomotion because the data obtained are more consistent than with other strains. For example, when 30 organisms having body lengths of 70–80 μm were selected, the mean velocity was 120.9 ± 2.9 μm/min.

Amoebae were taken from cultures that had not been fed for 24 h. The amoebae were washed 3 × with fresh KCM and then rinsed 7 × with a test solution in order to replace completely for improved microscopic observation was put on the assembly. A group (lo−1 M) was 100 × higher than that of the first one. In the concentration range from control velocity to total stoppage of locomotion, the velocity decreased slowly and the concentration of solution were mounted on a glass slide on which a pair of parallel objective lens), illuminated at less than 50 lux through an Interference Cold Cut Filter (Nihonshinku, Tokyo). Thirty cells were measured in each experiment. In some experiments, amoebae well fed with bacteria were employed.

Chemical agents were dissolved in KCM solution, and the pH of the test solutions was adjusted to 6.5–7.0 with 0.1 N NaOH or 0.1 N HCl. The test solutions were as follows: proteose-peptone (Difco, Detroit), Bacto peptone (Difco), Bacto tryptone (Difco), meat juice (Valentine), bovine serum albumin (Wako Pure Chemical, Osaka), alcian blue 8GS (Chroma, Stuttgart). 15 sorts of amino acids (Ajinomoto, Tokyo), and sodium glutamate (Wako Pure Chemical). Chicken liver extract was prepared as follows: 10 g of sliced chicken liver was boiled in distilled water for 3–4 min, then homogenized and diluted by distilled water to make 100 ml. This solution was filtered through a paper filter with pores 10–15 μm in diameter. All experiments were done at a room temperature between 23 and 25°C.

RESULTS

Proteose-peptone, Bacto peptone, Bacto tryptone, meat juice, chicken liver extract. The relationship between concentration and velocity was similar for all five media (Fig. 1). In the solution of proteose-peptone, velocity increased at concentrations between 10⁻¹¹ and 10⁻⁶ M/liter. The greatest increase was about 25% (10⁻⁷ M/liter) compared with control. At higher concentrations, above 10⁻³ M/liter, the velocity decreased slowly and the amoebae stopped completely and floated at 10⁻² M/liter. In Bacto tryptone solution of 10⁻⁶ M/liter, accelerated velocity rose to 140%, which was the maximal rate of increase in this experiment. Results obtained with beef meat juice and chicken liver extract were similar so the data are not presented.

Bovine serum albumin. The velocity curve was like that described above. If the molecular weight is assumed to be 60,000, the threshold concentration for acceleration of velocity was about 10⁻¹⁰ M (pH 6.8), which was the lowest one among all the solutions tested in the present experiments (Fig. 2).

Amino acids. As patterns of velocity curves for 12 kinds of L-amino acids and glycin were almost similar to one another, only four typical graphs are shown in Fig. 3. A schematic diagram of the curve and more detailed data obtained are exhibited in Fig. 4 and Table I. Amino acids tested can tentatively be classified into two groups in reference to the concentrations at which organisms became immobilized (point A). In the first group, which contains negatively or positively charged amino acids (e.g., glutamic acid, arginine, etc.), the concentration of point A ("stop concentration") is 10⁻¹⁰ M. Point A of the second group (10⁻¹ M) was 100 × higher than that of the first one.

The concentration range from control velocity to total stoppage was expressed as X = 2 on a logarithmic scale (Fig. 4). For example, X = 2 signifies a hundredfold range of concentration. For acidic amino acids X = 0.5; for basic amino acids and trypt-
Concentration (g/L)

Fig. 1. Relationship between the locomotive velocity of amoebae and the concentration of test solutions. Each point is the mean of 30 measurements on different cells. Abcissa: logarithmic scale. pH: 6.8–7.0. A: Bacto tryptone, B: Bacto peptone, C: proteose-peptone.

tophan X = 1.5–2.0; for the other amino acids X lies between 2 and 4. Minimal concentration for velocity-increase (threshold, point B), concentration for maximal increase (point C), and the rate of increase of velocity (Y) seem not to be related to the structure of the amino acids; however, it is very remarkable that an increase in velocity always appeared in L-amino acids while in the D-amino acid tested and in the non-protein amino acid no acceleration occurred (Table I). In L-amino acid solutions of higher concentration at which the movement of organisms was stopped, amoebae showed cytolysis within several minutes while in the D-amino acid they were not lysed.

Alcian blue 8GS. In general, biological dyes contain large amounts of contaminants. The concentration of alcian blue 8GS used in this experiment was estimated as being only 60% pure. Increase in velocity commenced at $M$, the velocity reached its maximum (C), i.e. 110% of control. The range of increased velocity was very narrow; velocity decreased above $5 \times 10^{-3}$ M, and at $8 \times 10^{-3}$ M organisms were stopped (A, Table I). Point A in alcian blue 8GS as well as in albumin was lower than that in the amino acids tested in this experiment. Even after the cessation of movement, the organisms were not
cytolysed, and pinocytosis was not induced until the concentration of alcian blue was raised 10 to 100 times higher than that needed to arrest movement.

Response of satiated amoebae to sodium glutamate and alcian blue 8GS. We examined whether the locomotive velocity of well fed amoebae also increased at very low concentrations of amino acid solution. As Mayorella penardi is known to feed by choice on bacteria as well as protozoa, the amoebae for this experiment were maintained as follows. To the plain agar plate (0.6Yo), which had been spread with a little fresh KCM solution on the previous day, more solution with a proper quantity of amoebae were added. The next day these amoebae were used for experiments. As controls, starved amoebae were employed. That is, after a large number of amoebae were mounted on the plain agar plate (0.6 Yo) so as to gather at one edge of the petri dish, the liquid medium was sucked up completely. About 5 h after this, amoebae which had moved a long distance were picked up.

As shown in Fig. 5A, no increase in locomotive velocity of satiated amoebae was observable with the solution of sodium
glutamate whereas starved organisms responded to it at the concentration of $10^{-8}-5 \times 10^{-5}$ M by an increase in velocity. With the solution of alcian blue 8GS, the situation was almost the same (Fig. 5B).

**DISCUSSION**

In relation to the feeding behavior of amoebae, it is significant that increase in velocity occurred in solutions containing a trace of L-amino acids or protein substances such as might exude readily from prey. In addition, the observations that there is no increase in velocity with a D-amino acid and a non-protein amino acid and that the velocity of satiated amoebae does not increase even in the solution of L-amino acid, suggest a probable correlation between phagocytosis and orthokinesis.

Minimal molar concentration for the stoppage of advancing amoebae was not the same for different amino acids. That is, the concentration was $10^{-3}$ M in one group, and $10^{-1}$ M in the other. This classification corresponds approximately with the existence of electric charge on each amino acid. We supposed that in amino acids of $10^{-3}$ M group, bovine serum albumin

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Table I. Results of the relationship between the locomotive velocity of amoebae and the concentration of amino acids and pinocytotic inducers.

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<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>X</th>
<th>Y</th>
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<td>$8 \times 10^{-4}$</td>
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* Refer to Fig. 4.
A concentration gradient of chemicals such as amino acids is effective in acceleration of pseudopod formation, possibly acting as attractants in chemotaxis (on which a further study will be done). Alcian blue 8GS, which is well known as an inducer of pinocytosis, showed the expected velocity curve. Pinocytosis began at a concentration 10–100 times higher than that for maximal velocity. In both serum albumin and alcian blue 8GS, most organisms were not cytolysed at the stop concentration or even higher. In contrast, L-amino acids and polypeptide of the same concentration caused amoebae to be cytolysed within several seconds or minutes. So it is supposed that substances having the capacity to increase velocity may be able to induce endocytosis in amoebae, but among these substances only a few such as alcian blue 8GS, in which amoebae are not cytolysed at high concentrations, can act as inducers of pinocytosis. If this speculation is valid, one and the same substance acts as an inducer of phagocytosis at lower concentrations and also functions to induce pinocytosis at higher concentrations though the mechanisms of these responses might not be the same.

**LITERATURE CITED**


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**Polyphenol Oxidase Produced During Encystment of *Acanthamoeba castellanii***

**DONALD E. SYKES** and **R. NEAL BAND**

Department of Zoology, Michigan State University, East Lansing, Michigan 48824

**ABSTRACT.** *Acanthamoeba castellanii* has a phenol oxidase activity that is believed to be a laccase. Enzyme activity was found in the outer cyst wall, in the cytoplasm of encysting amoebae and in the encystment medium. Encystment procedures were modified to promote an increase in the amount of soluble enzyme secreted during encystation. *Acanthamoeba* polyphenol oxidase has a pH optimum of 6.0 and a $K_m$ value of 0.21 mM with dihydroxyphenylalanine. The enzyme does not oxidize tyrosine, and it is inhibited by chloride but not by inhibitors of peroxidase. Its synthesis coincides with encystation, and known inhibitors of polyphenol oxidase prevent encystation. Polyphenol oxidase may have a role in making the cyst resistant to mechanical and chemical breakdown.

*Acanthamoeba castellanii* is a free-living organism, found in soil and fresh water, which forms a dormant, non-reproductive cyst. The cyst has a thick, wrinkled outer wall and a cellulose-containing inner wall (8, 24). The outer wall is resistant to chemical and physical analysis (7, 24) so that little is known about its composition and molecular organization. The cyst-vegetative cycle of amoebae is an ideal model for studying cytodifferentiation (10, 12, 28), but it is limited by the lack of a well defined, cyst-specific, biochemical marker. Polyphenol oxidase activity found in the cyst of *A. castellanii* provides a cyst-specific, biochemical marker. The objective of this research was to characterize this phenol oxidase and to study its function in encystation.

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