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Chemotactic Responses of *Chlamydomonas reinhardtii*

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A capillary chemotaxis assay revealed that among a wide range of inorganic and organic chemicals, only ammonium ion (NH_4^+) could serve as an attractant for *Chlamydomonas reinhardtii*. NH_4^+ (10^{-2} M) gave the maximum response, with up to a 15-fold increase in accumulated algae being measured. No repellents for the chlorophyte were detected. The response to NH_4^+ was influenced by exogenous levels of calcium, but not by L-methionine. The optimal pH for positive chemotaxis was 7.0; however, attraction was measurable from pH 4.0 to 9.0. Positive chemotaxis was stimulated by performing the assay under fluorescent illumination rather than in the dark.

Although there has been extensive work done on evaluating chemotactic responses induced by specific algal-produced sexual pheromones (3), relatively few quantitative studies have been applied to assess the abilities of motile algae to respond to a wide spectrum of inorganic and organic chemicals. Levandowsky et al. (6) used the tendency of *Crypthecodinium cohnii* to imbed in agar gels containing various concentrations of test compounds as a measure of the chemoresponses of this dinoflagellate. Their results have been summarized (5), and of interest are the algal responses to a number of neurologically active chemicals such as catecholamines and quarternary amines. A capillary assay has been used in a study with *Chlamydomonas reinhardtii* labeled with $NaH^{14}CO_3$ (2). It is reported that this chlorophyte is attracted to $CoCl_2$ and $MnSO_4$ and repelled by L-arginine.

A rapid, quantitative capillary assay in which algal numbers are determined with an electronic particle counter has shown that the motile, neritic, and littoral chlorophyte *Dunaliella tertiolecta* is attracted strongly by ammonium ion (NH_4^+) (11), L-tyrosine, L-tryptophan, and L-phenylalanine (12). The alga apparently possesses one chemoreceptor that binds only NH_4^+, and another that binds NH_4^+ and the three amino acids. In the present study, the same assay procedure was used to evaluate the chemotactic responses of *C. reinhardtii* to a wide range of chemicals. If future studies on the biochemical mechanisms or on the ecological significance of algal chemoreception are to be facilitated, it is essential that attention be given to factors that influence chemosensory behavior in these eucaryotes. Thus, we have evaluated some of these parameters with *C. reinhardtii*.

MATERIALS AND METHODS

C. reinhardtii Dangeard strain 90 was obtained from the Culture Collection of Algae (Austin, Tex.) and was cultivated in a modified Bold basal medium (1) containing urea (2 mM) as the sole nitrogen source and supplemented with thiamine (1.0 μg/liter), pyridoxine (10 μg/l), and cyanocobalamine (1.0 μg/l). Cultures in 75 ml of growth medium were incubated at 21 ± 1°C in 250-ml Erlenmeyer flasks over continuous fluorescent illumination ("Gro'n Show," General Electric Co., Schenectady, N.Y.) at 4 klx. This lighting was also used in all subsequent studies. After a population density of 5×10^6 to 1×10^7 organisms per ml was attained, the culture was centrifuged at $270 \times g$ for 10 min, and the algae were carefully resuspended in a medium composed of K_2HPO_4 (0.47 mM), KH_2PO_4 (1.29 mM), NaCl (0.43 mM), $MgSO_4 \cdot 7H_2O$ (0.3 mM), and $CaCl_2 \cdot 2H_2O$ (1 mM), in distilled water. This wash medium (pH 7.0) also served as the chemotaxis medium. Assays were done within 2 h after the suspension of the algae in the chemotaxis medium. Algal numbers were determined with an electronic particle counter (model Zb; Coulter Electronics, Inc., Hialeah, Fla.). Cultures of *C. reinhardtii* were maintained on agar slants composed of sodium acetate (2 g), yeast extract (4 g), and agar (15 g), per liter of modified Bold basal medium.

Lucite chemotaxis plates (9) and 3-μl capillaries (Drummond Scientific Co., Broomall, Pa.) were used in the assays. Capillaries were cleaned by being thoroughly rinsed in distilled water and in reagent grade acetone. For the assay, capillaries were filled with chemotaxis medium containing test chemicals at known concentrations and then submerged in the algal suspension. The accumulation of algae in these capillaries after 30 min of incubation at 21 ± 1°C was compared to the accumulation in capillaries containing chemotaxis medium only. During the assay the lucite plates were covered in glass petri dishes to minimize evaporation and were incubated between fluorescent lamps. For dark incubation studies, the petri dishes were wrapped with aluminum foil.

After incubation, the capillaries were removed from the algal suspension, and the exterior surfaces were carefully wiped dry. Each capillary with accumulated algae was then dispensed into a counting vial containing 20 ml of balanced electrolyte solution (Isoton II; Coulter Electronics, Inc.). To insure the elution of all algae, each capillary was rinsed in this solution five times. Triplicate counts of algae in each vial were made with the Coulter electronic particle counter, and the results were averaged. Each assay was repeated at least twice with different algal cultures. When appropriate, the effect of test chemicals on population motility was determined by including the compound at the same concentration both in the capillary and in the algal suspension. All chemicals used were of reagent grade and were tested in 10-fold dilutions from 10^{-1} to 10^{-6} M unless prohibited by limitations of solubility. Solutions containing fatty acids were adjusted to pH 7.0 with NaOH before testing.

RESULTS

Under the conditions of our assay, *C. reinhardtii* showed positive chemotaxis only to NH_4Cl and other inorganic ammonium salts, including $(NH_4)_2SO_4$ and NH_4NO_3. A typical concentration-response curve with NH_4Cl is shown in Fig. 1. The minimum concentration needed to elicit a detectable response was 10^{-5} M, and maximum attraction occurred with 10^{-2} M NH_4Cl in the capillary. The alga did not respond to KCl (Fig. 1), NaCl, KNO_3, $NaNO_3$, or Na_2SO_4, showing that attraction was due to NH_4^+. At 10^{-1} M NH_4Cl, a portion of the attracted algae was observed to be accumulated outside the capillary tip, accounting for the apparent decrease in positive chemotaxis. Presumably, chemoreceptor saturation by high concentrations of the attractant occurs at an area outside the capillary mouth, and thus the decrease may be considered as an assay artifact. At 10^{-2} M NH_4Cl, the algae formed a thick green band inside the capillary.

Preliminary time course experiments with 10^{-2} M NH_4Cl showed that 30 min was a sufficient assay incubation period. The response to the attractant also depended on the algal population density: the number of attracted algae sharply decreased if fewer than 5×10^6 organisms per ml were used (data not shown). Similar results have been observed in a chemotaxis assay with *D. tertiolecta* (11).

C. reinhardtii did not respond to any of the following compounds: amino acids (L-form) (the 20 amino acids commonly found in proteins were tested); the carbohydrates D-glucose, D-fructose, and D-lactose; the fatty acids formic acid, acetic acid, propionic acid, *n*-butyric acid, and valeric acid; and the inorganic compounds $CoCl_2$, $MnCl_2$, $FeCl_3$, KNO_3, $NaNO_3$, KCl, NaCl, and Na_2SO_4. All compounds were tested from 10^{-1} to 10^{-6} M except for the aromatic amino acids (10^{-3} to 10^{-6} M). Urea and adenosine also elicited no chemotactic responses.

The concentration of calcium (as $CaCl_2 \cdot 2H_2O$) included in the chemotaxis medium influenced the attraction of *C. reinhardtii* to 10^{-2} M NH_4Cl (Fig. 2). No response was detectable if calcium (Ca^{2+}) concentrations were 10^{-6} M or 10^{-1} M. The maximum response was obtained when 10^{-3} M Ca^{2+} was supplied exogenously. Population motility was not affected if the algae were incubated in Ca^{2+} from 10^{-2} to 10^{-6} M, and these results are consistent with the lack of any

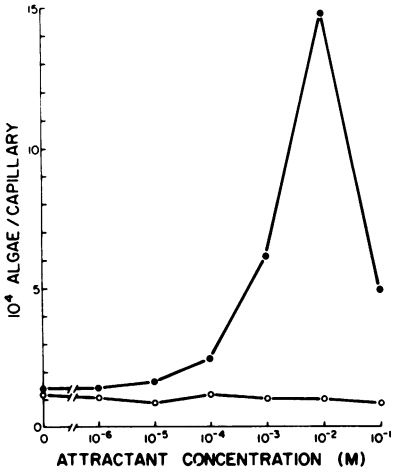

FIG. 1. *Attraction of C. reinhardtii to NH_4Cl (●) and KCl (○).*

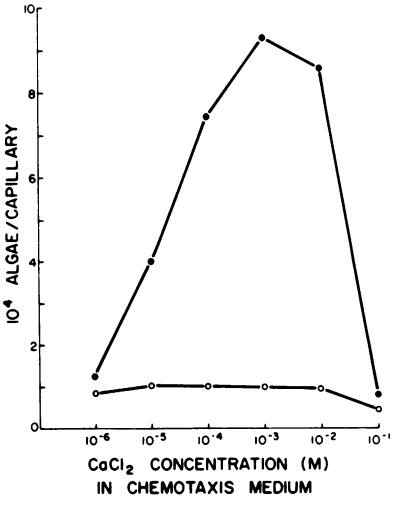

FIG. 2. *Effect of $CaCl_2$ in chemotaxis medium on the attraction of C. reinhardtii to 10^{-2} M NH_4Cl (●) and on population motility (○; NH_4Cl omitted from capillaries).*

Ca^{2+} effect on the translational velocity of individual *C. reinhardtii* organisms (10). However, as indicated in Fig. 2, population motility, and thus positive chemotaxis, was greatly reduced when the chlorophyte was suspended in chemotaxis medium containing 10^{-1} M Ca^{2+}.

The components of the chemotaxis medium (see Materials and Methods) were sufficient to allow for positive chemotaxis in *C. reinhardtii*. Inclusion of other components of the Bold basal medium (ethylenediaminetetraacetic acid, $FeSO_4$, H_3BO_3, trace salts, or B vitamins) in the chemotaxis medium did not affect algal attraction to 10^{-2} M NH_4Cl. Further, an exogenous supply of L-methionine (10^{-5} M) was not essential for, nor did it stimulate, positive chemotaxis (data not shown).

The pH of the chemotaxis medium affected positive chemotaxis in *C. reinhardtii* (Fig. 3). Maximum attraction to 10^{-2} M NH_4Cl occurred at pH 7.0; however, responses were detectable from pH 4.0 to 9.0. Stimulation of population motility was observed from pH 6.5 to 7.5. At pH 3.0, algae in the suspension were not motile, and aggregate formation was observed.

The response of *C. reinhardtii* to 10^{-2} M NH_4Cl was inhibited if the chemotaxis assay was performed in the dark rather than under fluorescent illumination (Table 1). Repeated experiments consistently showed that light stimulated positive algal chemotaxis. The number of accumulated algae in capillaries containing only

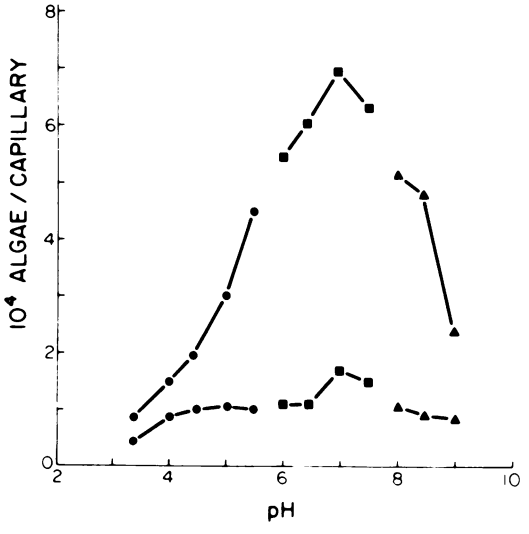

FIG. 3. *Effect of chemotaxis medium pH on the attraction of C. reinhardtii to 10^{-2} M NH_4Cl. Capillaries in the upper curves contained NH_4Cl; capillaries in the lower curves contained no NH_4Cl. Buffer systems (10^{-3} M) used: citrate (●), phosphate (■), and borate (▲).*

TABLE 1. *Effect of assay incubation in light versus in the dark on the attraction of C. reinhardtii to 10^{-2} M NH_4Cl*

Assay incubation condition	Algae per capillary
Light + 10^{-2} M NH_4Cl in capillary	2.5×10^5
Light − 10^{-2} M NH_4Cl in capillary	1.7×10^4
Dark + 10^{-2} M NH_4Cl in capillary	1.6×10^5
Dark − 10^{-2} M NH_4Cl in capillary	1.5×10^4

chemotaxis medium was always slightly greater in the light than in the dark. Whether due to photostimulation of population motility or to phototaxis, the difference was not sufficient to account for the increased chemotactic response.

Use of ^{14}C-organic chemicals that might interact with possible NH_4^+-binding algal chemoreceptors could facilitate chemoreceptor isolation and characterization. Thus, methylamine, formamide, and acetamide were tested as potential attractants for *C. reinhardtii*. At 10^{-2} M, these compounds acted as weak attractants and resulted in 1.7- to 2-fold increases of algae in the test capillaries. The responses were greater at 10^{-1} M, with 2.5- to 4.1-fold increases measured (data not shown). However, at these high concentrations, one has to consider the possible effects due to toxicity and contaminants (free NH_4^+) in the chemicals. It was clear that algal motility, and perhaps viability, was inhibited when *C. reinhardtii* was suspended in chemotaxis medium containing 10^{-1} M of any of the three compounds.

DISCUSSION

C. reinhardtii exhibits a limited capacity for positive chemotaxis since, of a wide range of organic and inorganic chemicals tested, it is only attracted to NH_4^+. No repellents were detected for the chlorophyte. The results do not parallel those found in an earlier report (2) in which *C. reinhardtii* was attracted to $CoCl_2$ and $MnSO_4$ and repelled by L-arginine. The differences may be due to the *C. reinhardtii* strains selected for study or to the components comprising the different chemotaxis media. In the earlier report (2), 10^{-1} M $CaCl_2$ and 10^{-1} M NH_4Cl were included in the algal suspending medium. Inclusion of 10^{-1} M $CaCl_2$ in our chemotaxis medium inhibited population motility and abolished algal attraction to NH_4Cl (Fig. 2). Further, 10^{-1} M NH_4Cl may be sufficient to saturate the presumed algal chemoreceptors for this attractant and cause a decreased chemotactic response (Fig. 1).

Calcium is essential for phototaxis in *C. reinhardtii* (7), and the cation couples flagellar reversal to photostimulation in this alga (10). Per-

haps a similar role of Ca^{2+} is involved in algal chemotaxis. The divalent cation also is essential for positive chemotaxis in *D. tertiolecta* (11) and regulates chemotactic behavior in bacteria (8). Methylation reactions have an essential role in bacterial chemotaxis (4), and L-methionine stimulates the response of *D. tertiolecta* to NH_4^+ (11). The observation that it was unnecessary to supply L-methionine exogenously for positive chemotaxis to occur in *C. reinhardtii* may reflect the ability of the alga to maintain sufficient intracellular levels of this amino acid.

It does not appear, at least in studies with intact cells of *C. reinhardtii*, that compounds like methylamine, formamide, and acetamide will provide sensitive probes for isolation and characterization of possible NH_4^+-binding chemoreceptors.

The number of algae attracted to 10^{-2} M NH_4Cl varied among assays, and the data from experiments in this report ranged from a minimum 7-fold increase in attracted algae over controls (Fig. 3) to a 15-fold increase (Fig. 1). However, the number of accumulated algae in the capillaries without attractant was fairly constant.

Clearly, we have not investigated all of the parameters that may influence chemotaxis of *C. reinhardtii*. The studies, however, do provide a foundation from which further investigations can be made into the biochemical, genetic, and ecological aspects of chemotaxis in this alga.

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