Chemical signal-response in diatoms of the genus *Amphora*

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Summary

The marine diatom *Amphora coffeaeformis* is positively chemotactic to glucose and sugars of similar structure. A free hydroxyl group is required at the 2-position of the ring; however, D-mannose (a toxic sugar) and L-glucose promote negative chemotaxis. Sugar metabolism is not necessary for a chemosensory response. When the direction of higher concentration in a D-glucose gradient is reversed, cells already orientated reverse their direction of movement. Pretreatment of diatoms with a uniform concentration of D-glucose before exposure to a concentration gradient eliminates the chemotactic response. When diatom cells attached to surfaces in the presence of 5 mM-Ca^{2+} are exposed to 0·25–0·625 mM Ca^{2+}, their motility drops rapidly. However, when this experiment is carried out in the presence of a uniform concentration of D-glucose or 3-*O*-methyl-D-glucose, motility is sustained for 25–30 min before falling. Speed of motility is also increased during this period. These results can be interpreted in terms of an agonist binding–secretion coupling model for diatom chemotaxis.

Key words: chemical signal response, diatom, chemotaxis, secretion model, calcium, sugars, *Amphora*.

Introduction

The phenomena of recognition of and subsequent response to the external environment are important mechanisms of survival in all cells. Directed movement of simple cells as a result of an external chemical signal is an example of this and one that has its place in the evolution of higher organisms (Adler, 1985). Taxes of various kinds provide visual evidence of a response to an external stimulus. Chemosensory responses have been studied extensively in such organisms as bacteria (for a review see Ordal, 1985), slime moulds (Janssens & Van Haastert, 1987), and leucocytes (Zigmond, 1978), but there have been few investigations utilizing microalgae. This is somewhat surprising considering the ubiquitous nature of the diatom niche, i.e. an illuminated aquatic environment.

We have shown previously that both gliding motility and adhesion to substratum in *Amphora coffeaeformis* are calcium-dependent (Cooksey & Cooksey, 1980; Cooksey, 1981) and that the processes saturate with respect to calcium at approximately 2 mM. At this calcium concentration or higher, *Amphora* migrates towards higher concentration levels in glucose gradients prepared in agar (Cooksey & Cooksey, 1986). Tracks observed on the surface of the agar in the absence of glucose are a series of connected ellipses. Those seen in the presence of a glucose gradient, however, are zigzags, with the cell moving towards the higher concentration. Measurements of diatom motility on agar-containing glucose gradients showed correlation between successive changes in direction and cell polarization and thus support our proposal that *Amphora* demonstrates true chemotaxis. There are no previous studies of diatom chemotaxis so far as we are aware.

In this paper we extend our previous observations, describe some stereochemical and concentration requirements for the chemosensory response in *Amphora coffeaeformis* and propose a simple sensory model to explain our results. Some of the more pertinent experiments with *A. coffeaeformis* were repeated with another and larger species of *Amphora*.

Materials and methods

Materials

All sugars and Tris buffer were obtained from Sigma Chemical Co. (St Louis, MO 63178, USA). Inorganic chemicals used in growth media and minimal media were analytical reagent grade. Radiochemicals D-glucose [6-^{3}H], 25 Ci mmol^{-1}; D-mannose [2-^{3}H], 20 Ci mmol^{-1}; 3-*O*-methyl-D-glucose [*methyl*-^{3}H], 20 Ci mmol^{-1}; and 2-deoxy-D-glucose [1,2-^{3}H], 30 Ci mmol^{-1} were obtained from ICN, Irvine, CA 92713, USA.

Organisms and their growth

The isolation, growth physiology and Ca^{2+} requirements for adhesion and motility of *Amphora coffeaeformis* (Agardh) Kutz. have been described previously (Cooksey & Chansang, 1976; Cooksey & Cooksey, 1980; Cooksey, 1981). *Amphora* sp. (an unidentified species, 27 μm × 12 μm) was isolated from brackish

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water, using standard methods. Both isolates were maintained in axenic culture and grown for experimental purposes in the ASP-2 medium of Provasoli *et al.* (1957) at 28°C and $100\,\mu E\,m^{-2}\,s^{-1}$ from cool-white fluorescent lights.

Uptake of radioactive sugars

The ability of *A. coffeaeformis* to take up radioactive sugars was measured as described previously (Chansang & Cooksey, 1977). Triplicate samples of incubation mixtures containing 1 mM-sugar ($10\,\mu Ci\,\mu mol^{-1}$) and cells ($0{\cdot}5\times10^{6}\,ml^{-1}$) were removed every 10–30 min for 3 h and the radioactivity in the filtered (Nuclepore, Pleasanton, CA 94566, USA, $1\,\mu m$ pore size), washed cells determined after their solubilization in 0·5 ml Protosol (New England Nuclear, Wilmington, DE 19898, USA) and neutralization with 0·05 ml glacial acetic acid. Aquasol II (New England Nuclear Corp.) liquid scintillation cocktail was used in a Packard counter (Model Tricarb 460CD). Counts were corrected for quenching using an external standard. Radioactivity accumulated by cells was plotted *versus* time and the uptake rate calculated from the linear slope of the graph.

Chemotaxis experiments

A chemosensing assay based on that of Zigmond (1977) was used essentially as originally described, but substituting diatoms for polymorphonuclear leucocytes as the test organisms. The bridge of the chamber was, however, 2 mm in width rather than 1 mm, as described by Zigmond (1977). Diatoms were attached to microscope slide cover-slips using a procedure described for an adhesion assay (Cooksey, 1981). To avoid crowding of cells on the cover-slips and thus the potential interference of contact–inhibition response (Abercrombie & Dunn, 1975), the initial cell densities used to prepare the cover-slips for experimentation were kept within the range of $4\text{–}6\times10^{4}$ cells ml^{-1}. This produced a surface density of approximately 60–90 cells $field^{-1}$. Before assembly of the chamber, diatoms were wiped from all but a 2-mm section of the cover slide. This section was positioned over the bridge of the chamber. To enable monitoring of a time-course lasting 90 min, assembled chambers were incubated at room temperature (25°C) and $100\,\mu E\,m^{-2}\,s^{-1}$ in a humid chamber made from a Petri dish. By staggering the starting time for several chambers, measurements could be made over an extended period of time. In each experiment, minimal medium (Cooksey, 1981) containing 5 mM-Ca^{2+} was used as a control, and test substances, except where stated, were dissolved in the same medium. In these experiments, gradients were always from zero concentration to the concentration stated in the experiment. To prepare glucose-pretreated organisms, cells were exposed to 0·5 mM- or 1·0 mM-glucose during their adhesion to the cover-slips. To remove free glucose, cover-slips were allowed to stand in minimal medium for 20 min before use in chemotaxis experiments.

All experiments were recorded in β III mode on a Toshiba recorder (Model V8500T). Images were obtained from a Reichert phase-contrast microscope fitted with an RCA video camera (Model TC2011). The video image was modified to contain a time–date signal (RCA Model TC1440B). The overall magnification was 330× (microscope 16×; electronic, approximately 20×).

Individual fields of diatoms at the centre of the chamber bridge were monitored and recorded for time intervals between 3 and 6 min. So that all the cells examined were subject to the same gradient (i.e. the midpoint in concentration), only the y-axis knob of the movable stage was used to change microscopic fields. The videotape was replayed for statistical analysis of movement. Individual cells moving at less than 45° to an imaginary line parallel to the gradient were scored as either positively or negatively oriented, depending on their movement up or down the gradient, i.e. cells moving in a 90° sector were counted. To aid in the decision concerning the angle of movement, lines were drawn on the TV monitor screen parallel to the gradient and at 45° to it. The selection of the size of the quadrant for counting reflects the degree of stringency of the test for chemotaxis, with an included angle of 2° being the most stringent, i.e. only cells moving parallel to the gradient (±1°) would be counted. In our case, the angle of 45° (included angle 90°) was not chosen arbitrarily. When *Amphora* responds to a chemical gradient, cells move up the gradient by making a series of changes in direction of about 40°. This can be followed microscopically in the Zigmond chamber or by the tracks left on the surface of agar when the chemotaxis experiments are performed in agar-containing chemical gradients (Cooksey & Cooksey, 1986). The adoption of the 45° angle therefore includes only those cells whose net movement is in effect almost parallel to the gradient, i.e. it is a conservative measure of cellular chemotaxis. In judging direction of movement, each cell was observed once for a distance of at least one cell length, i.e. $20\,\mu m$, or about 10 s. Actual translational distances for individual cells were measured by marking the position of a cell on the screen with a felt tip pen and re-marking its position at a later time. Marking the screen in this way also allowed an accurate assessment of the angle of movement with respect to the gradient to be made. The percentage of cells showing the desired characteristic, i.e. movement at 45° or less to a line parallel to the gradient, were compared with a hypothetical standard of 50% using a normal approximation of a binomial distribution and $P<0{\cdot}05$ (Documenta Geigy, 1970; Bailey, 1981). In these circumstances, 50% oriented cells means that the population is moving randomly. Only populations showing movement statistically different from 50% ($P<0{\cdot}05$) were considered to be showing directed movement and were included in the Tables 1–3 under the heading positive or negative taxes. Where these analyses indicated that the distribution of cells showing positive or negative taxis is not different from 50% ($P<0{\cdot}05$), the cells were considered to be random in their movement (see Tables 1–3). The numbers of observations (n) were pooled from at least three experiments and reported as the mean ± one standard deviation. Where only two experiments are reported, the range of the determinations is quoted. Experiments were terminated if the number of cells in several fields along the centreline of the diffusion chamber bridge became too low because of migration of the cells to regions of higher concentration of attractant.

Speed of movement

This was measured directly from the television screen. Diatom movement was traced directly on the screen and measured using dividers. Times were obtained from the time–date generator signal. Cells were followed for at least two cell lengths but usually for about $90\,\mu m$. These distances translate to between 15 and 30 mm on the TV monitor screen and to times of 15–30 s.

Agonist sustained motility

These experiments were carried out in a similar manner to the chemotaxis experiments, but the putative agonist was present in each well of the Zigmond chamber so, after the first few minutes, no concentration gradients were present. Putative agonist sugars were prepared in 0·25 mM- or 0·625 mM-Ca^{2+} in minimal media. The percentage motility and speed of movement of the cells were measured as a function of the incubation time with the sugar. To make statistical comparisons between treatments and controls after specific incubation times, Stu-

Table 1. *Chemotactic response of* A. coffeaeformis *to sugars*

Sugar concentration* (mM)	Cells responding† (%)	Time for response‡ (min)	Taxis§
None	44 ± 8 (15)¶	0–50	Random
D-glucose			
0·1	59·5+5 (8)	34–62	+
0·5	72 ± 2 (3)	34–50	+
1·0	71 ± 8 (11)	32–45	+
10·0	79 ± 6 (2)	20–26	+
D-mannose			
0·1	45 ± 2 (2)¶	0–49	Random
0·5	100 (2)	0–20	No motility
0·5	72 ± 10 (6)	20–25	–
1·0	100 (2)	0–20	No motility
3-*O*-methyl-D-glucose			
1·0	65 ± 3 (6)	35–53	+
10·0	67 ± 2 (3)	38–50	+
2-deoxy-D-glucose			
10·0	52 ± 12 (2)¶	30–43	Random
D-glucoheptose			
1·0	51 (2)¶	6–35	Random
10·0	66 ± 3 (5)	6–33	+
L-glucose			
10·0	65 ± 1 (3)	21–27	–
D-maltose			
1·0	69 ± 4 (3)	6–28	+
10 0	70 ± 5 (5)	16–33	+

* Concentration gradient of zero to the concentration mentioned.

† Mean percentage of orientated cells ± one standard deviation; (*n*) is pooled number of observations spread between at least two experiments; 30–60 cells were counted for each observation. Where the percentage of cells responding to the gradient was not significantly different from 50 % (¶), the motility is regarded as random.

‡ Time during which directed movement was obtained. The first number is the time after addition of sugar to the chamber when orientation of the cells became significantly different from 50 % ($P < 0{\cdot}05$), i.e. non-random. The second is the time when cells reverted to random movement. In some cases no directed movement was obtained and the times then represent the period over which the cells were observed.

§ + and – indicate positive or negative taxis. The population is considered to be exhibiting random movement when the cells responding to the gradient was not significantly different ($P < 0{\cdot}05$) from 50 %.

dent's *t*-test for paired observations was used with $P < 0{\cdot}05$ (Bailey, 1981).

Results

Chemotaxis to sugars and analogues

The responses to sugar gradients of two diatoms were examined and the results are shown in Tables 1, 2 and 3. *A. coffeaeformis* demonstrated positive taxis to D-glucose and the analogue 3-*O*-methyl-D-glucose. If the reducing part only of the molecule is considered, D-heptose also behaved as a glucose analogue in that it supported directed movement. However, a much steeper concentration gradient (0–10 mM) was necessary to demonstrate significant directed movement with this compound. Gradients of D-maltose also attracted this diatom. On the other hand, D-mannose (0·5 mM), an epimer of D-glucose, caused negative taxis. This sugar also produced a toxic effect when the cells were exposed to the steeper 0–1·0 mM-mannose gradient, in that motility was inhibited completely. L-Glucose produced a negatively chemotactic response in *A. coffeaeformis* only when cells were exposed to a steeper concentration gradient, i.e. 0–10 mM. 2-Deoxy-D-glucose did not cause directed movement in any of the concentration gradients tested. Where several concentrations of the same sugar were examined, it was evident that cellular response to the chemical gradient was concentration-dependent. Using the formula described by Lauffenburger & Zigmond (1981) and a distance of 20 μm for the distance from bridge to cover glass, we calculated that the gradient in our chamber reached 90 % of linearity in 20 min and did not fall to a value lower than this in less than 6 h. In our experiments cells at the centre of the bridge in the chamber experienced an approximate concentration of 0·05 mM-glucose at the shallowest concentration gradient tested. This represents a change in glucose concentration of approximately 1 μM or 1 %, over a distance of one cell length. When diatom cells were pre-exposed to a uniform concentration of a sugar, they showed random movement when placed subsequently in a gradient of the same sugar (Table 3), in contrast to the directed movement demonstrated when not pre-exposed. Where the sugar in the gradient was not the one to which the cells were pre-exposed, chemotactic responses were still detected.

Table 2. *Chemotactic response of diatoms after pretreatment with glucose*

Pretreatment	Gradient*	Cells responding† (%)	Time for response‡ (min)	Taxis§
A. coffeaeformis				
None	0·5 mM-glucose	62 ± 10 (3)	18–36	+
0·5 mM-glucose	0·5 mM-glucose	50 ± 5 (11)¶	4–44	Random
1·0 mM-glucose	1·0 mM-glucose	51 ± 5 (6)¶	5–32	Random
1·0 mM-glucose	1·0 mM-mannose	74 ± 7 (4)	5–25	–
1·0 mM-glucose	0·5 mM-mannose	70 ± 4 (2)	17–27	–
Amphora sp.				
1·0 mM-glucose	1·0 mM-glucose	57 ± 11 (12)¶	9–50	Random
1·0 mM-glucose	1·0 mM-mannose	81 ± 9 (5)	12–33	–
1·0 mM-glucose	0·5 mM-mannose	54 ± 14 (5)¶	4–28	Random

*·†·‡·§ See footnotes to Table 1.

Table 3. *Chemotactic response of* Amphora *sp. to sugars*

Sugar concentration* (mM)	Cells responding† (%)	Time for response‡ (min)	Taxis§
None	53 ± 9 (17)¶	0–50	Random
D-glucose			
0·5	75 ± 4 (3)	24–33	+
D-mannose			
1·0	81 ± 3 (3)	21–25	−
3-*O*-methyl-D-glucose			
10·0	71 ± 6 (10)	16–49	+
2-deoxy-D-glucose			
1·0	56 ± 7 (10)¶	4–40	Random
10·0	54 ± 11 (10)¶	4–45	Random
D-glucoheptose			
1·0	79 ± 4 (5)	9–24	+
10·0	81 ± 7 (9)	6–26	+
L-glucose			
1·0	74 ± 5 (5)	26–44	−
10·0	77 ± 6 (10)	12–27	−
D-maltose			
1·0	70 ± 5 (3)	6–18	+
10·0	79 ± 7 (7)	6–24	+

*·†·‡·§ See footnotes to Table 1.

Table 4. *Incorporation of sugars into* A. coffeaeformis

Sugar	Incorporation rate ($nmol/10^6$ cells h^{-1})*	Incorporation pattern
D-glucose	0·44 ± 0·007 (2)	45-min lag
D-mannose	0·31 ± 0·12 (4)	No lag
3-*O*-methyl-D-glucose	0·21 ± 0·11 (2)	No lag
2-deoxy-D-glucose	0·02 ± 0·03 (2)	No incorporation

* Means of *n* experiments ± standard deviation or range where *n* = 2.

Where glucose-exposed cells were in a 0–1·0 mM-mannose gradient, they exhibited negative chemotaxis. Pre-exposure to 1·0 mM-glucose, however, appeared to reduce the toxic effect of mannose since without such pretreatment, cellular motility would have been inhibited completely (Table 1, lines 7, 9).

Of the sugars mentioned in Table 1, only D-glucose supported heterotrophic or mixotrophic growth (Cooksey & Chansang, 1976; results not shown). However, D-mannose, D-glucose and 3-*O*-methyl D-glucose, but not 2-deoxy-D-glucose, were taken up by cells of *A. coffeaeformis* (Table 4). The ability of these cells to take up D-glucoheptose, L-glucose and D-maltose was not measured.

Many of the experiments performed with *A. coffeaeformis* were repeated with another larger species of *Amphora* (Table 2, lines 6–8; Table 3). In all cases the results were qualitatively similar to those obtained with the former organism. *Amphora* sp. was less sensitive to the toxic effects of mannose. Thus, cells in a 0–1·0 mM-mannose gradient were able to move, whereas those of *A. coffeaeformis* were not. *Amphora* sp. responded chemotactically to a lower concentration of D-glucoheptose than did *A. coffeaeformis*.

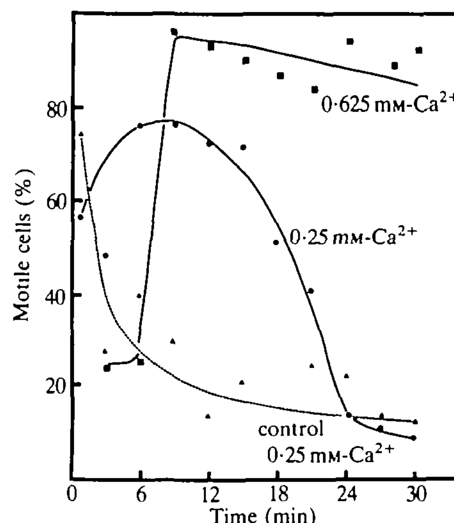

Fig. 1. Glucose-sustained motility in *A. coffeaeformis*. Cells attached to cover-slips were exposed to 0·25 mM-Ca^{2+} in minimal medium (▲); 0·25 mM-Ca^{2+} in minimal medium containing 1 mM-D-glucose (●); or 0·625 mM-Ca^{2+} in minimal medium containing 1 mM-D-glucose (■). Results obtained with 0·625 mM-Ca^{2+} in minimal medium are not shown since they were similar to 0·25 mM-Ca^{2+}. Each point represents one field containing 60–90 cells.

Motility of diatom cells at low calcium concentrations

When diatom cells adhering to cover glasses were exposed to minimal medium containing 0·25 mM-calcium in place of 5 mM-calcium, they lost motility quickly (Fig. 1). Similar results were obtained when the 5 mM-calcium was replaced by 0·625 mM-calcium. However, when the medium surrounding the cells contained 1 mM-glucose in addition to 0·25 or 0·625 mM-calcium, they continued to be motile for about 20–30 min before becoming stationary. Both *Amphora* exhibited this response (Figs 1, 2), which was enhanced at the higher (0·625 mM) calcium concentration. The preparations of glucose used in these experiments contained barely detectable levels of calcium by atomic absorption spectroscopy and were not, therefore, a significant source of calcium. The same pattern of response was obtained when the speed of motility of individual cells was measured as a function of time, rather than the percentage of motile cells (Fig. 2). It was not possible to measure track velocity of cells in populations where the percentage of motile cells was low (<20 %), because although such cells could be seen to be moving, they did not move far enough in a given time to allow an accurate measurement of cellular displacement to be obtained (distance covered was less than one cell length). Experiments with glucose concerning maintenance of motility were extended to 3-*O*-methyl-D-glucose. Qualitatively, similar results were obtained (Table 5).

Reversal of a gradient

In carrying out chemotaxis experiments with the Zig-

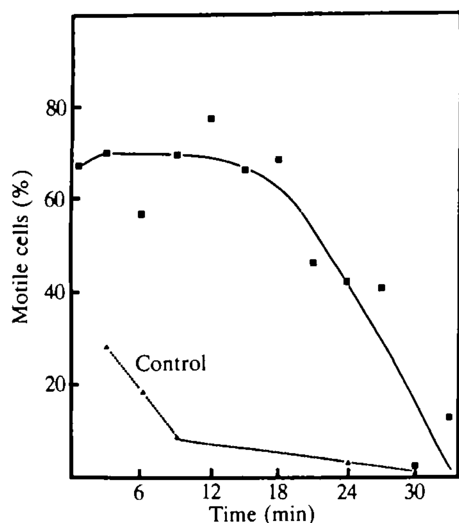

Fig. 2. Glucose-sustained motility in *Amphora* sp. (▲) cells in minimal medium containing 0·625 mM-Ca^{2+}; (■) cells in minimal medium containing 0·625 mM-Ca^{2+} and 1 mM-D-glucose. Other experimental details as Fig. 1.

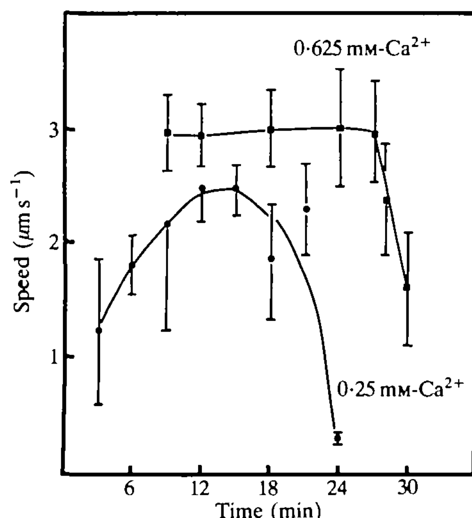

Fig. 3. Glucose-sustained speed of motility in *A. coffeaeformis*. These results were obtained from the experiment described in Fig. 1. The average speed of cells in a particular field ± one standard deviation are shown (n = 5–10 cells). (●) Cells subjected to 0·25 mM-Ca^{2+} in minimal medium containing 1 mM-D-glucose. (■) As above but minimal media contained 0·625 mM-Ca^{2+} and 1 mM-D-glucose.

mond diffusion chamber it was our practice to have the left side of the chamber filled with control minimal medium and the right side with the test substance, i.e. a gradient was established with the higher concentrations to the right of the centre-line of the bridge dividing the two chambers. Where the contents of the left-hand reservoir were replaced by a solution of higher concentration than that on the right side, the concentration profile of the gradient reversed. In a typical experiment (Table 6), after 38 min significantly more cells were

Table 5. *3-O-methyl-D-glucose-sustained motility in* A. coffeaeformis

Treatment*	Time period (min)	Motile cells† (%)
5 mM-Ca^{2+}	3–30‡	78 ± 5 (7)
0·625 mM-Ca^{2+}	27–30	7 ± 6 (3)
0·625 mM-Ca^{2+} + 1 mM-3-*O*-methyl-D-glucose	27–30	39 ± 12 (4)

* Minimal medium containing these components.

† Percentage motility ± one standard deviation for n experiments, with 60–70 cells counted per experiment.

‡ Cells were motile for longer, but no data were collected.

Table 6. *Establishment and reversal of a glucose gradient*

Content of reservoirs		Time for response (min)	Cells responding (%)
Left side	Right side		
Minimal medium	Minimal medium	30–55	45 (38–51)*
Minimal medium	0·1 mM-glucose	30–54	59 (54–66)†
0·5 mM-glucose	0·1 mM-glucose	38–54	61 (51–73)‡

* Cells moving to right on video monitor counted as positively orientated. Exact confidence limits given for a binomial distribution with n = 124 cells.

† Cells moving to right counted as positively orientated. Exact confidence limits shown for a binomial distribution, n = 190 cells.

‡ Cells moving to left counted as positively orientated cells, n = 73 cells.

found to be moving to the left. In order to turn so that they were now moving up the new concentration gradient, cells initially moving parallel to the original gradient simply reversed, i.e. the front became the trailing end of the cell. In any experiment there are always some cells moving at more than 45° to the gradient. Some of these cells stopped and then, by a series of shunting and turning movements, adopted another path so that they were then travelling up the new gradient, i.e. the leading portion of the cell did not change. Others in these circumstances simply continued to turn away from the initial direction of the gradient until they had adopted the new direction; again the leading edge of the cell did not change, i.e. polarity was maintained.

Discussion

Previous work (Cooksey & Cooksey, 1986) has shown that *A. coffeaeformis*, a marine diatom, is attracted to glucose when it is presented as a gradient in agar. In this paper we extend these results to include more sugars and present some preliminary information on the specificity and concentration-dependence of the phenomenon. To obtain these results, we have used the diffusion chamber designed by Zigmond (1977) for use in her work on polymorphonuclear leucocytes (PMN). It has proved more convenient to use than agar, in that gradients of potential chemoattractants are established in about

10 min. It is evident from Tables 1, 3 that diatom cells do not show a response to sugars for approximately 10 min after being placed in the concentration gradient. Zigmond found a lag of a similar period in her work on PMNs in a gradient of *N*-formylmethionyl peptides. It is tempting to suggest that this lag in the case of our experiments was simply the delay in establishing a linear gradient which, according to Lauffenburger & Zigmond (1981), should have been complete in about 20 min. If this were so, then cells in steeper and more concentrated gradients should have responded more quickly than those in shallower gradients and of lesser concentration. This was not generally the case in our experiments (Tables 1, 3). Previously we had found that a similar lag in [^{14}C]glucose uptake could be abolished by pre-exposure of *Amphora* cells to the sugar (Chansang & Cooksey, 1977; K. E. Cooksey, unpublished results). We therefore exposed attached cells to a uniform concentration of glucose before washing briefly in glucose-free medium and using them in chemotaxis experiments. Glucose-pretreated cells showed no directed motility throughout the experiment, so it is likely that no sugar-induced molecular synthesis necessary for chemotaxis was taking place during the lag. This could be interpreted in terms of a desensitization response, which in turn could be mediated by receptor occupancy, although other explanations are possible.

Most excitable cells become adapted to an applied stimulus in time. This is borne out by experiments with PMNs (Zigmond, 1977), slime moulds (Janssens & Van Haastert, 1987) and bacteria (Taylor & Panasenko, 1984), as well as many animal tissues (e.g. Sibley & Lefkowicz, 1985). Diatoms are likely to be no different and show a return to random motility after approximately 50 min in a hexose concentration gradient. Whether this reduction in response is a desensitization or a true adaptive mechanism is unknown at this point. The gradient will have retained its steepness during this part of the experiment (Lauffenburger & Zigmond, 1981), precluding the possibility of a collapse in its steepness being the cause.

The results in Tables 1, 4 suggest that there is no apparent correlation between sugar uptake rates and the percentage response of a population of cells. Although the uptake rate for D-glucose is twice that of 3-*O*-methyl-D-glucose, there is no difference in the time taken for the cells to respond nor in the magnitude of their response.

Tables 1, 3 can be summarized in terms of a hypothetical structure embodying the required characteristics for a sensed molecule. A free hydroxyl at the 2-position of the ring is important, but substitutions at positions 3, 4 or 6 are less so. The fact that D-glucoheptose and D-maltose are also active in promoting chemotaxis implies that it is probably only the reducing end of the molecule that is important in determining whether these diatoms sense a sugar. Neither of the species used in this study grow on 2-deoxy-D-glucose or 3-*O*-methyl-D-glucose, nor are these compounds toxic (results not shown). 3-*O*-methyl-D-glucose is taken up by *A. coffeaeformis*, probably by a pathway distinct from that used by glucose since its accumulation by the cell takes place without lag (Table 4).

As complete metabolism of a compound is not necessary for it to be sensed, it is reasonable to suggest that perhaps merely binding to a membrane by a molecule external to the cell may be sufficient to initiate the sensing reaction. Implication of membrane receptors in the sensing process is supported by the experiment mentioned in Table 6. Reversal of the gradient caused cells to reorientate by various means, implying that putative receptors are located at least at the poles of the cell. In view of the amelioration of the toxic effect of mannose by glucose (Table 2), and the fact that mannose causes negative chemotaxis, there should be at least two classes of sugar receptors. One of these may be a general receptor for sugars (or hexoses) and the other for mannose and its analogues. It is difficult to envisage how a negative chemotactic effect could be produced without a unique receptor for mannose. Glucose would, however, compete with mannose at the mannose receptor. It is noteworthy that in bacteria, maltose and glucose are normally sensed by distinct receptors (Taylor & Panasenko, 1984). Only more experimentation will resolve this possibility in diatoms.

Diatoms move by the continuous production of a polymer, the secretion of which is both energy- and calcium-dependent (Cooksey & Cooksey, 1980). Chemotaxis in diatoms could be regarded therefore as a stimulus–secretion coupled system. If the system was of the type reviewed recently by Michell (1986), then binding of chemosensed molecules would lead to the release of internal Ca^{2+} and thus promote transient motility in a diatom in a medium where the Ca^{2+} concentration was too low to sustain motility.

Figs 1–3 suggest that glucose is able to sustain motility temporarily in both species of *Amphora* when they are incubated at a Ca^{2+} concentration less than 1 mM. The percentage of motile cells is increased by the presence of D-glucose or 3-*O*-methyl D-glucose. What is more, the effect of glucose is enhanced at a slightly higher calcium concentration (0·625 mM). The speed of the cells in the presence of sugars was also increased for a short time before falling precipitously after about 20 min. No differences were found between media containing 0·25 mM and 0·625 mM-calcium when speed or percentage motility were measured in the absence of a sugar. This shows that the above results can be attributed to the presence of a sensed, but not necessarily metabolized, sugar. The involvement of calcium fluxes in stimulated taxes has been observed in several other types of cells. For instance, such calcium movements have been shown to occur in *Dictyostelium*, polymorphonuclear leucocytes, *Paramecium* (reviewed by Taylor & Panasenko, 1984) and *Euglena* (Doughty & Diehn, 1982).

Any model proposed to explain chemotaxis in a diatom must also take into account the random movements exhibited by the organism. In *Amphora*, due to the placement of both raphes on the ventral surface of the cell, movement consists of a series of ellipses, with periodic back and forth shunting motions of less than one cell length. Our results for cells in a chemical gradient are consistent with a mechanism whereby binding of a spatially-sensed compound (agonist) leads to differential

raphe secretory activity. In such a gradient, the cell becomes polarized, thereby acquiring a leading edge, and moves by a series of right and left angle changes in the direction of higher concentration without any intermittent random movements. This is apparently not a biased random walk, but rather true taxis (Fraenkel & Gunn, 1961).

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