Bioscience Reports, Vol. 16, No. 6, 1996

Chemotaxis of the Unicellular Green Alga *Dunaliella salina* and the Ciliated *Tetrahymena pyriformis*—Effects of Glycine, Lysine, and Alanine, and Their Oligopeptides

L. Kőhidai,[1] P. Kovács,[1] and G. Csaba[1,2]

Received June 12, 1996

Chemotactic properties of amino acids (L-alanine, glycine and L-lysine) and their oligopeptides (10^{-6} M) and binding sites to these ligands were investigated in two unicellular models, the heterotrophic *Tetrahymena pyriformis* and the autotrophic *Dunaliella salina*. Chemotaxis of *Dunaliella* induced by simple amino acids and their derivatives demonstrated that binding sites (receptors) for food molecules are not only present in the membrane but are also able to induce their basic physiological response. In *Tetrahymena*, substances with special molecular structure and properties (polar, hydrophilic character of the signal peptide chain)-5-L-Lys, 5-Gly- were required for chemoattraction, other peptides tested, lacking the required structure, were repellent. Divergences in chemotaxis and binding assays of both species suggest that trends of functional and binding parameters do not run parallel at this level of evolution.

KEY WORDS: Chemotaxis; evolution; oligopeptides; *Tetrahymena*; *Dunaliella*.

INTRODUCTION

Chemotaxis is one of the most basic biological responses, appearing at very early levels of phylogeny. According to the selection theory of receptor-evolution (18) primitive binding sites are responsible for food recognition, and appeared first. These membrane associated elements ensured selectivity of cells and their well oriented locomotion-chemotaxis-towards the first chemical external signal, nourishment. The capacity to differentiate among signals provided new families of receptors, e.g. hormone or immune receptors.

Branches of plants and animals were separated at an early stage of evolution. The two distinct forms of life represent two distinct metabolic pathways: plants are autotrophic (food producer), while animal cells are heterotrophic (food

[1] Department of Biology, Semmelweis University of Medicine, POB 370, H-1445 Budapest, Hungary.
[2] To whom correspondence should be addressed.

0144-8463/96/1200-0467$09.50/0

consumer) organisms. This difference suggests that food-particle induced chemotaxis should be more obvious in animals than in plants. However, the biological possibility of chemotactic response is given also in plants.

In the present work our goal was to study two unicellular representatives of the above mentioned evolution of metabolism, in respect to signaling. The two model cells chosen—however, both of them classified as protists—represent very distinct forms of metabolism. They were compared with respect to their capacity to responding to simple chemical signals.

The questions to be answered were:

1. Is there any detectable chemotactic response of algal cells to simple signals?
2. Is there any signal-dependent difference in the response detected in algal and ciliated cells?
3. Is there any homology of chemotactic response elicited in these two types of cells?

We applied two types of eukaryotic model cells: the halotolerant flagellar green alga *Dunaliella* salina and the holotrichous ciliate, *Tetrahymena pyriformis.* Both of the model cells chosen are well described and the signaling systems were presumably sufficient for detection and response to the "chemoattractants". *Dunaliella* possesses an inositol phosphate—diacyl glycerol based second messenger pathway (6) and its membrane (21, 26) and intracellular process (20) have also been investigated in respect to signal transmission. Until the present work there were no data about any directed motile response of *Dunaliella.* Nevertheless it has been shown to be responsive to external stimuli like gossypol (5), which induces a decrease of respiratory processes in mitochondria and also affects cell motility. Hormone-like sesquiterpenoids, e.g. germacon, germacol can also modulate *Dunaliella,* their auxin and cytokinin-like effects stimulate growth (10). In the *Tetrahymena*-model, signaling and its essential components were also described: in the membrane, receptors (2), membrane associated and cytoplasmic enzymes, second messengers (3, 12) and protein kinases (7) and at the nuclear level, hormone-dependent gene activation (11). This is why it is one of the favourite models of chemotaxis (8, 14, 22).

Three different amino acids (glycine, L-alanine and L-lysine) and their oligopeptides were chosen as chemical signals. In the peptides the increasing number of composing amino acids provide us a model of the early stage of evolution when such simple food-signals were available.

MATERIALS AND METHODS

Cells and Culturing

Dunaliella salina cells were cultured in artificial "sea water" (hereafter SW) at room temperature. Ten day old cultures were in logarithmic phase of growth, the density of culture was 4×10^7/ml. *Tetrahymena pyriformis* GL cells were originally cultured in axenic conditions in 0.1% yeast extract containing 1%

Tryptone medium (Difco, Michigan, USA) at 28°C. Then cells of logarithmic cultures were washed thrice with Losina-Losinsky physiological salt solution (19) (hereafter LL) containing inorganic ions, then they were transferred to LL containing flasks to promote the removal of exogenous organic substances which were taken up from the axenic medium. Our purpose was to avoid background effects of auto- and paracrine peptides of *Tetrahymena* secreted into extracellular space. Therefore cells were transferred to fresh LL solution every second day. The cells were tested after 120 hours of the first transfer to LL. The density of cultures was 10^4 cell/ml at that time.

Chemicals

The amino acids tested (L-alanine, glycine and L-lysine = Ala, Gly and Lys thereafter), their di-, tri-, tetra- and penta-peptides were obtained from Sigma Chemicals (St. Louis, MO, USA). The materials were applied in concentration 10^{-6} M.

LL solution was an aqueous mixture of 1% NaCl, 0.1% $MgCl_2$, 0.1% $CaCl_2$, 0.1% KCl and 0.2% $NaHCO_3$. SW was also a mixture of anions (Cl^-, SO_{42-}, NO_{3-}, HNO_{3-}, PO_{4-}) and cations (Na^+, Mg^{2+}, K^+, Ca^{2+}, Fe^{3+}). Composition of PBS was 0.05 M phosphate buffer pH 7.2, containing 0.9% NaCl.

Assay of Chemotaxis

The chemotaxis assay applied was a modification of Leick's two-chamber capillary assay (17) described by us (16). Here we used a multichannel micropipette and a microtitration plate. The wells of the plate were filled with the cultures while the tips of the pipette served as inner chambers filled with compounds to be tested. The incubation time was 15 minutes. (The relatively short time of incubation allowed us to monitor the oriented movement—chemotaxis, and not the biased random movement—chemokinesis.) Following the incubation the samples were fixed in 4% formaldehyde in LL or phosphate buffer (PBS) respectively.

The trials were done in five parallels. The samples were counted in a Neubauer hemocytometer.

Binding-assay of FITC Labelled Oligopeptides

All the oligopeptides applied were labelled with fluorescein iso-thiocyanate (FITC, BDH, England) in our laboratory according to (25). Cultures of *Dunaliella* were fixed in 4% formaldehyde in SW. Then the samples were washed in SW three times. The washed cells and the FITC-labeled oligopeptides were mixed (v:v = 1:1) and the samples were incubated at room temperature for 60 minutes. Following this the samples were washed thrice with SW. The cells were dropped onto slides. In case of *Tetrahymena* the main steps of the procedure were identical to the steps described above, however the buffer used was LL.

The binding of FITC-labelled oligopeptides was assessed by cytofluorimetry using a Zeiss Fluoval cytofluorimeter which was connected with an analogue-digital converter and an HP-41CX microcomputer. The system provided us the statistical analysis of data (inter-group variation by Student t-test, standard deviation, variance).

In control groups the FITC-labelled oligopeptides were omitted and aliquots of washed cells and SW or LL were mixed and incubated for 60 minutes. The background fluorescence of cells was measured as it was mentioned above and the averages of data obtained served as the control.

Statistical Analysis of Data

In those cases where the built-in statistical analysis was not a property of the system, Sigma Plot 4.0 and Origin 2.8 were applied to evaluate data. Calculation of Student t-test, standard deviation, variance were the standard statistical probes.

RESULTS

Chemotaxis

In the case of *Dunaliella* the chemotactic responses elicited by glycine and its oligopeptides are shown in Fig. 1A. The maximal response was elicited by 1-Gly. The increasing length of the chain resulted in a slight decrease of chemotaxis, however the longest peptide tested, 5-Gly was also significantly chemoattractant. Responses of *Tetrahymena* were different. 1-Gly—4-Gly forms were repellent and there was no difference in their effectiveness. Nevertheless 5-Gly had strong chemoattractant character and this potency of 5-Gly was more expressed in *Tetrahymena* than in *Dunaliella.*

In the case of lysine we could observe a similar profile of curves (Fig. 1B), however in the case of Dunaliella the chemotactic capacity of 1-Lys and their peptides was significantly higher. 1-Lys, 2-, 3- and 4- were repellent to *Tetrahymena,* and the oligopeptide composed of five residues—5-Lys—exhibited an "uncommon" chemoattractant response, like 5-Gly.

In the case of alanine we observed a special curve. Each substance tested was chemoattractant to *Dunaliella,* but the criss-cross feature of the curve was very interesting (Fig. 1C). There is a significantly decreased chemotactic potency of molecules containing odd numbers of amino acid residues (1-Ala, 3-Ala, 5-Ala) compared to molecules containing even numbers of residues (2-Ala, 4-Ala, 6-Ala).

The described chemotactic character of alanine was observed also on *Tetrahymena,* though 2-Ala does not fit to the scheme. In the case of *Tetrahymena* the general effect of alanine and its oligopeptides was either neutral (1-Ala and 6-Ala) or repellent. Only Ala-4 had chemoattractant effect but this effect was significantly below the response elicited in *Dunaliella.*

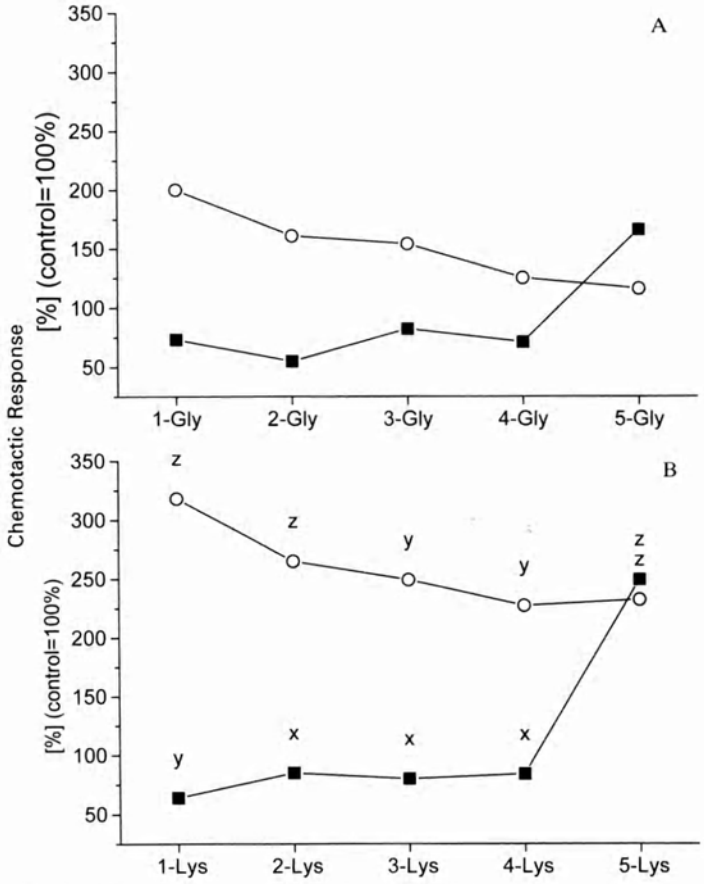

Fig. 1. Chemotactic response induced by glycine, lysine and alanine and their oligopeptides (10^{-6} M) tested on Dunaliella (open circles) and *Tetrahymena* (solid squares). Control = 100% (see Materials and Methods). x = $p < 0.05$; y = $p < 0.01$; z = $p < 0.001$ A—glycine and its oligopeptdes elicit a chemotaxis in *Dunaliella* but they were repulsive in Tetrahymena, except the longest form. B—lysine and its oligopeptides were strong chemoattractants to *Dunaliella*, while they were repellent to *Tetrahymena*, except the longest form. C—alanine and the related oligopeptides were strong chemoattractants with a special periodic profile to *Dunaliella*, but they are repellent or neutral to *Tetrahymena*, except 4-Ala.

Binding

The binding profile of the FITC-labelled oligopeptides was similar in the two taxons investigated, however there were differences in the intensity of binding.

In the case of *Dunaliella*, binding of lysine derivatives was the highest. There was a positive trend in binding with increasing number of residues. Especially the longest form (5-Lys) tested presented a strikingly increased binding compared to

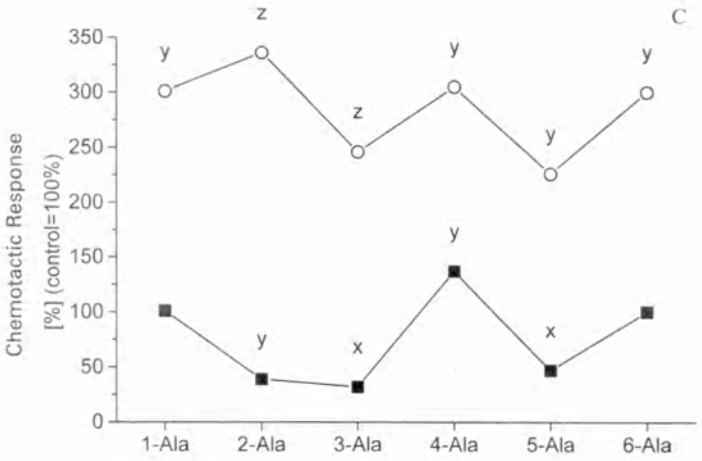

Fig. 1. (*Continued*).

the other similar peptides (Fig. 2A). In the case of the other two types of peptides there was no trend in binding. Binding of glycine related peptides was either at the control level (2-Gly and 3-Gly) or their binding was slightly increased (4-Gly and 5-Gly). In the case of alanine all the five peptides tested had an increased binding compared to the control.

Tetrahymena cells presented similar trends in binding, however, binding of all peptides was significantly above the control level. Peptides composed of lysine had a positive trend in binding, but the scale was shifted up and the lowest binding value was about 300% over the control (2-Lys), while the highest binding was 810% (5-Lys) (Fig. 2B). There was no significant difference in binding of peptides composed of glycine (range 190–240%), except for the longest form 5-Gly, the binding of which was significantly higher (300%). For the alanine peptides, there was no significant difference in binding (160–190%). Nevertheless, there was a slight tendency for the peptides to bind better with increase in length.

DISCUSSION

Chemotaxis assays are physiological tests which present data about the cells and substances investigated in different aspects. Thus, data of chemotaxis elicited in unicellular systems provides information about the i) specificity of the receptors (binding sites) of the responsive cell; ii) the environmental effects influencing the process; iii) the signal character of the attractant or repellent molecule applied. In this work our aim was to study such molecules—amino acids and their oligopeptides—which might be responsible for the selective bifurcation of simple

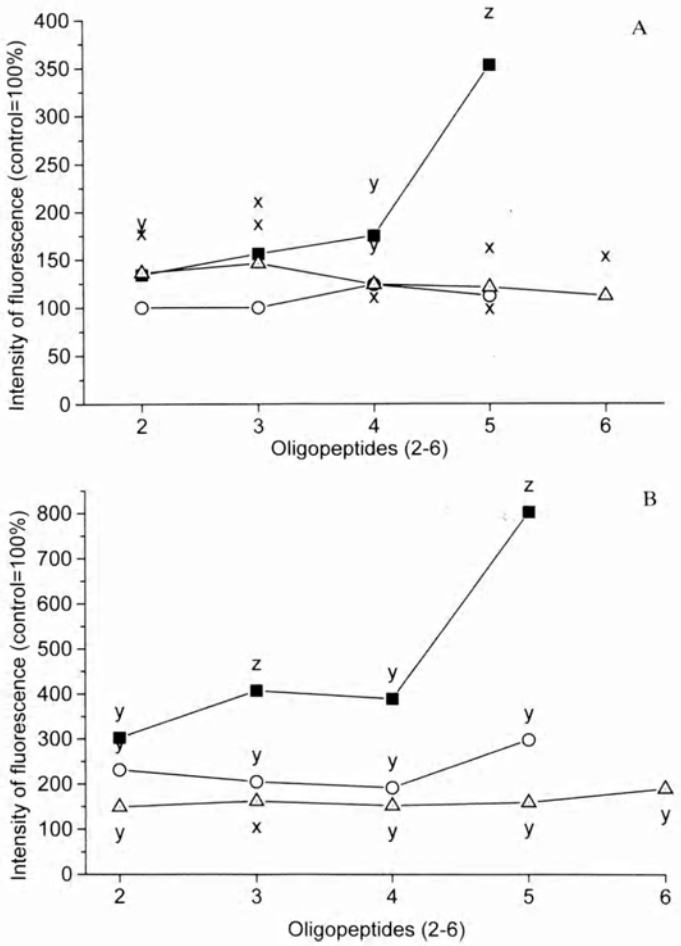

Fig. 2. Binding of FITC labelled bi-, tri-, tetra-, penta- and hexa peptides of glycine (circle), alanine (triangle) and lysine (square). Control = 100% (see in Materials and Methods) x = $p < 0.05$; y = $p < 0.01$; z = $p < 0.001$. Comparison of binding capacity of *Dunaliella* membrane (A) and *Tetrahymena* membrane (B) points to that food consumer cells have an increased number of binding sites for peptides of environment, but binding of longer chains is enhanced.

food intake and signaling at the early stages of evolution (3, 18). The two model-cells, the autotrophic *Dunaliella salina* and the heterotrophic *Tetrahymena* pyriformis embody the two different arms of phylogeny.

In the experimental setting, our main purpose was to evaluate such populations which live in so-called standard environmental conditions (see point ii). That was the reason why we chose inorganic conditions for maintaining cells for a longer time. This culture condition provided us theoretically no effects of

amino acids or peptides of axenic or synthetic cultures. Over this setting our cells were transferred periodically to fresh SW or LL solutions to minimize the effects of amino acids and other biologically active molecules produced by the cells in an autocrine or a paracrine way.

The different chemotactic responses elicited by the simple organic substances suggest that signaling mechanisms have divergent characteristics in unicellular algae and ciliates. The fact itself, that all the applied amino acids and their oligopeptides were chemotactic to the autotrophic, food-producer *Dunaliella* is worth noting as we have no other data about this activity in a unicellular motile alga, except *Chlamydomonas*, which is responsive to amino acids and inorganic salts (9). Over this, the relations of peptide length and the chemotaxis induced suggest that it was not a "simple" intake of food.

The amino acids tested represent a special grouping differing in the polarity of their side chains. Glycine with only a proton as side chain is more polar than alanine with its methyl group as side chain, while lysine has a side chain containing a basic group (1, 24). These properties of the molecules are decisive in α-helix formation. According to this, alanine is a promoter, while glycine and lysine are destabilizers in formation of the α-helix (23). The special profiles of the curves in the case of the glycine- and lysine-group suggest that increasing the number of the residues results in a loss of chemoattractant capacity of the molecule, however, the longest—five member—chain works still as an attractant. The reason for this decrease might be the increasing number of hydrophilic and polar side chain residues, especially in the lysine oligomers and slightly in glycine ones, too. In the case of lysine the consecutive decrease of membrane penetration with the increasing length of the molecule might also be a reason for the gradual decrease of the chemotactic activity. The periodicity described in case of odd and even subunit containing alanine oligopeptides requires more data about the three dimensional structure of the molecules. However when we check the criss-cross, strong and less strong chemotactic profile of the alanine-group, some structural background is available as alanine has a short and non-polar side-chain. This hydrophobic moiety of the molecule, and in the related oligopeptides, might serve as interacting or a non-interacting element with the surface membrane.

The experiments have disclosed significant differences of chemotaxis elicited in the unicellular, food-producer *Dunaliella* and the food-consumer *Tetrahymena*. In general we can say that the amino acids and their short-chain oligopeptides were repellent to *Tetrahymena*, and only longer-chain forms of the molecules were able to induce a chemotactic response. *Dunaliella* always responded positively. Curve-profiles in the lysine- and glycine-groups suggest that *Tetrahymena* itself or its membrane has different responsive mechanisms than *Dunaliella*, as increasing polarity (and hydrophilicity) of the molecule has a repellent character in *Dunaliella*. In *Tetrahymena* the increased chain length in 5-Lys turns the repellent character of the molecule into strong chemoattractant, and a similar change was found in 5-Gly. The decrease of chemoattraction parallel with the increase of chain length of glycine and lysine peptides in the case of *Dunaliella*, in contrast to the elevation of chemotaxis in the case of 5-Gly and 5-Lys in *Tetrahymena* could call attention to the signal-like character of polar

oligopeptides in *Tetrahymena* and food-like character of amino acids in *Dunaliella* (3). It suggests that not all of the available organic substances are inducers of chemotaxis at this level of phylogeny. However literature data shows, that there is a wide range of small molecules—histamine (13), amino acids (8), oxytocin (15)—which are able to induce chemotaxis in *Tetrahymena*.

Binding profiles do not correspond to the chemotactic activities elicited. In general, comparing the two model cells we could find that the binding capacity of the *Tetrahymena* membrane was higher than that of the *Dunaliella*. This fact might also point to an increased number of binding sites (receptors) in the *Tetrahymena* membrane. However, to state this requires further investigations of the membrane. The difference of the algal and ciliate membrane itself might provide a support to the basic theory about auxotrophic and heterotrophic organisms: metabolism of heterotrophics is dependent upon the organic components of the environment, therefore they have more binding sites for these ligands. Nevertheless according to the binding values of the membrane of *Dunaliella* or *Tetrahymena* we could conclude that there was a significant binding capacity to all ligands tested (short chain glycine peptides are exceptions). Trends of binding and chemotaxis suggest that presence of binding sites (receptors) in the membrane by itself is not enough to result in chemotaxis. Some other factors, e.g. the micro environment of the receptors in the membrane, intracellular cross-links and side effects of the ligand entering the cell by non-receptor mechanisms and interacting with the cytoskeletal elements, all might be responsible for the physiological response in chemotaxis. Previous data of the literature also demonstrated similar divergences of binding capacity and chemotaxis of *Tetrahymena* in the case of insulin, whth different species specificity (4). In those experiments the differences in binding had no dependence upon structure or origin of the hormone, just like hormonal imprinting elicited was also portrayed with an increased binding, however it was independent from chemotactic response.

ACKNOWLEDGMENTS

This work was supported by the Hungarian Research Fund (OTKA, T 013355), Hungary.

REFERENCES

1. Chou, P. Y. and Fasman, G. D. (1974) *Biochemistry* **13:**211–222.
2. Csaba, G. (1985) *Internat. Rev. Cytol.* **95:**327–377.
3. Csaba, G. (1994) *Internat. Rev. Cytol.* **155:**1–48.
4. Csaba, G., Kovács, P. and Kőhidai, L. (1994) *Microbios* **80:**215–221.
5. Druez, D., Marano, F., Calvayrac, R., Volochin, B. and Soufirm, J. C. (1989) *J. Submicr. Cytol. Pharmacol.* **21:**367–374.
6. Einsphar, K. J., Peeler, T. C. and Thompson, G. A. Jr. (1989) *Plant Physiol. (Bethesda)* **9:**1115–1120.
7. Hegyesi, H. and Csaba, G. (1994) *Cell Biochem. Function* **12:**221–226.

8. Hellung-Larsen, P., Leick, V. and Tommerup, N. (1986) *Biol. Bull.* **170:**357–367.
9. Hirschberg, R. and Rodgers, S. (1978) *J. Bacteriol.* **134:**671–673.
10. Karanov, E., Iliev, L., Tsankova, E., Tsolova, M. and Paunova, V. (1990) *Fiziologija Rastenijata (Sofia)* **15:**60–65.
11. Kovács, P. and Csaba, G. (1991) *acta Protozool.* **30:**107–113.
12. Kőhidai, L., Barsony, J., Roth, J. and Marx, S. J. (1992) *Experientia* **48:**476–481.
13. Kőhidai, L., Karsa, J. and Csaba, G. (1994) *Microbios* **77;**75–85.
14. Kőhidai, L., Kovács, P. and Csaba, G. (1994) *Cell Biol. Internat.* **18:**119–121.
15. Kőhidai, L. and Csaba, G. *Acta Microbiol.* Hung (*In press*).
16. Kőhidai, L., Lemberkovics, É. and Csaba, G. (1995) *Acta Protozool.* **34:**181–185.
17. Leick, V. and Helle, J. (1983) *Anal. Biochem.* **135:**466–469.
18. Lenhoff, H. M. (1974) In:Coelenterate Biology. (Ed. L. Muscatine and H. M. Lenhoff.) Academic Press, New York, 238–239.
19. Losiana-Losinsky, L. K. (1931) *Arch. Protistenk.* **74:**18–20.
20. Ohsawa, H., Nakayama, K. and Okada, M. (1992) *Botanical Mag. (Tokyo)* **105:**393–404.
21. Oren-Shamir, M., Pick, U. and Avron, M. (1990) *Plant Physiol. (Bethesda)* **93:**403–408.
22. Pauli, W. and Berger, S. (1992) *Bull. Environ. Contam. Toxicol.* **49:**892–899.
23. Rodwell, V. W. (1985) In: Harpers Review of Biochemistry. Maruzen, Tokyo, 14–40.
24. Taylor, W. R. (1986) *J. Theor. Biol.* **119:**205–218.
25. Wood, B. T., Thompson, S. H. and Goldstein, G. (1965) *J. Immunol.* **95:**225–229.
26. Zhang, X. Q., Dubacq, J. P. and Alfsen, A. (1993) *J. Phycology* **29** 203–209.