*Cellular and Molecular Biology*™ **49**, Online, OLxxx-OLxxx ISSN 1165-158X
DOI 10.1170/xx 2003 Cell. Mol. Biol.™

# CHEMOTACTIC-RANGE-FITTING OF AMINO ACIDS AND ITS CORRELATIONS TO PHYSICOCHEMICAL PARAMETERS IN *TETRAHYMENA PYRIFORMIS* – EVOLUTIONARY CONSEQUENCES

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*Received July 2003; Accepted November 18, 2003*

**Abstract -** Amino acids are considered the oldest organic substances of the prebiotic evolution. Chemotactic effects of amino acid L-isomers investigated in the protozoan model *Tetrahymena* show that the chemotactic properties of amino acids are complex and depend on multiple physicochemical characteristics of the investigated ligands. The range of effectiveness is significantly wider for chemoattractant ligands than for chemorepellent ones. This phenomenon provides the basis of the "chemotactic-range-fitting" theory. The validity of this theory is supported by a decreased pK (-COOH), an increased pK ($-NH_2$), and a decrease in solvent exposed areas and hydropathy indexes in chemoattractant amino acids compared to chemorepellent ones. Chemotactic selection has proven the activity of long-term (I, H, T) and short-term (P, A, Q, S) selector amino acids and their characteristic diversities in values of the pK and SEA (surface exposed area). Comprehensive studies of the chemotaxis data with the results of consensus analysis of amino acids suggests that chemotactic activity was one of the most primordial physiological activities and had a prospective significance not only in the molecular evolution of ligands, but also in the evolution of signalling.

**Key words**: Chemotaxis, chemotactic-range-fitting, chemotactic selection, amino acid, phylogeny, *Tetrahymena*

## INTRODUCTION

Chemotaxis is one of the most basic cell-physiological activities. According to the most accepted theories (27), the origin of chemotaxis receptors –like other fundamental components of the signal recognition system– is derived from simple binding sites for nourishment on the surface membrane. On the unicellular level, a long process of consecutive selections of ligands and their appropriate receptors resulted in specific matching of binding site/ligand interactions and the induction of specific intracellular activities.

In this respect, amino acid-type ligands represent a fundamental level of phylogeny. Model experiments of Miller and Urey demonstrated that, among others, these relatively simple compounds were the first potentially synthesized organic molecules of the prebiotic soup (30). The biological significance of these amino acids and their derivatives was high as they provided not only nourishment for cells, but also acted as a series of potentially new signalling molecules (e.g. biogenic amines). In addition, the structural units (e.g. elements of cytoskeleton) were synthesized by dimerization or polymerization based on their structural characteristics.

Functional approaches of these processes of molecular phylogeny have a close relation with essential physiological cell activities like proliferation, migration or phagocytosis. Relatively simple amino acid or dipeptide type ligands are chemoattractant or chemorepellent molecules in prokaryotes. Their signalling, based on the presence and phosphorylation/dephosphorylation cascade of chemotaxis proteins (CheA-CheB-ChY-ChZ), is one of the most described networks. However, there are some points of continuity such as CheY-like proteins in eukaryotic cells (2) or the presence of actin-like peptides at the prokaryotic level (3). Elements of intracellular signalling at the eukaryotic unicellular level represent an altered and more complex form. At this higher level, the number of potential pathways and members of intracellular

**Abbreviations**: **LL**: Losina-Losinsky inorganic medium; **SEA**: solvent exposed area

signalling is increased (i.e. diversity of second messengers). In many regards, the ligand and receptor side of chemotactic signalling in eukaryotes is still unexplained. In this respect, investigations of amino acids and their role as chemotactic ligands have important significance. Previous work has presented that slight structural changes (D-L isomers) of these ligands possess diverse chemotactic potencies (1), while additional work has verified the role of essential amino acids (12) in protozoa.

Despite completion of multiple experiments and recognition of the decisive significance of amino acids in molecular evolution and chemotactic signalling, it is still unclear whether a) physicochemical characteristics or b) selective potencies have decisive roles in chemotaxis, and c) whether the chemotactic responsiveness elicited by amino acids has a short- and long-distance moiety?

To answer the above described questions, the eukaryotic protozoon, *Tetrahymena* was used as a model. *Tetrahymena pyriformis* is a eukaryotic ciliate used frequently as a model organism for investigations in cell biology and phylogeny (5). Its homology with higher-ranked vertebrate models in membrane receptors [e.g. insulin (4), second messenger systems like cAMP (7), inositol lipids (14), $Ca^{2+}$-calmodulin (13) and metabolic processes (15)] provide a special role for this protozoa in model experiments. Chemotaxis of *Tetrahymena* is induced by the major chemoattractants of its natural environment. Several such molecules are derived from peptides or proteins which signal the presence of food in the environment (26). The ligand specific chemotactic responsiveness of *Tetrahymena* is elicited by short or longer chain peptides [e.g. amino acids (28) mirror variants of dipeptides (21), close derivatives of insulin (8), lectins (16) or chemokines (17)] as well as other types of ligands like volatile oils (19) or steroids (18). These all support the application of this low level eukaryotic cell as an ideal organism in studies of chemotactic signalling.

In the present work our objectives were:

• to characterize chemotactic responsiveness of *Tetrahymena* to the full amino acid library;

• to describe potential relationships of physicochemical properties of amino acids and the chemotactic responses elicited, and

• to apply chemotactic selection and describe durability of ligand-receptor relationships in the subpopulations gained.

## MATERIALS AND METHODS

*Tetrahymena pyriformis GL* cells, maintained in 0.1% yeast extract containing 1% Bacto tryptone (Difco, Michigan, USA) medium at 28ºC, were used in the logarithmic phase of growth. Due to the possibility of contamination of culture medium with simple amino acids to be tested the cultures were transferred into Losina-Losinsky minimal medium (hereafter LL) composed of only inorganic salts (29), 5 hr prior to the experiments. Density of samples was $10^4$ cell/ml.

*Chemicals*

The assayed twenty L amino acids were obtained from Sigma (St. Louis, USA). The amino acids were assayed in concentrations $10^{-12}$, $10^{-10}$, $10^{-8}$ and $10^{-6}$ M. Composition of LL medium was: 1% NaCl; 0.1% $MgCl_2$; 0.1% $CaCl_2$; 0.1% KCl and 0.2% $NaHCO_3$.

*Assay of chemotaxis*

The chemotactic ability of *Tetrahymena* cells was evaluated using a two-chamber, capillary chemotaxis assay (25) which we modified accordingly (19). Tips of an eight-channel-micropipette filled with the test substances served as the inner chamber of the system. The outer chamber consisted of a microtitration plate filled with the model cells. The incubation time was 20 min. This relatively short time facilitated the measuring of pure gradient, directed chemotactic responses and prevented the contamination of the samples from randomly running, chemokinetic responder cells (20). The concentration dependence of chemotactic response was determined in $10^{-12}$-$10^{-6}$ M range. In concurrent runs, pure LL medium served as control substance. The control samples were evaluated in parallel in each case to eliminate the undesirable disturbances elicited by spontaneous mutations. After incubation the samples were fixed in 4% formaldehyde containing LL solution. The number of cells was determined using a Neubauer haemocytometer.

*Chemotactic selection*

This technique deals with the chemotactic capacity of different signal molecules to form subpopulations of mixed cultures of cells (17). In this case, the chemotaxis assay described above was applied, but after incubations the positive responder cells were transferred to fresh culture medium. After one week cultivation the selected cultures were assayed again in the following combinations: responses of cultures selected with the amino acid (A) or the control substance (C) were tested in relation to the identical amino acid (A/A or C/A) or to the control (A/C or C/C). Maximal chemoattractant concentrations of identical amino acids were applied both in the selection and in the repeated chemotaxis assays.

*Statistical evaluation of data*

Each amino acid was tested in ten replica assays. Data of Table 1 demonstrate the averages of these results and ± SD values. The statistical analysis was done by ANOVA using Origin 4.0 software.

## RESULTS AND DISCUSSION

Amino acids constitute nourishment for protozoan organisms. However, simultaneously, these substances can also influence, attract or repel them. Lenhoff was the first who suggested that amino acid receptors could have been the ancestors of hormone receptors and he demonstrated this in *Hydra* (27). In earlier experiments, we supported this theory in *Tetrahymena* using tyrosine-diiodotyrosine and histidine-histamine model and studying the effect of these molecules to the growth of the protozoon (6). In other experiments the differing effects of four L and D amino acids (phenylalanine, valine, tryptophan and tyrosine) were studied on the growth of *Tetrahymena* and the selectivity of its receptor was demonstrated (10). Hauser *et al*. (?) studied the effect of tryptophan and phenylalanine on another unicellular organism (*Gyrodinium cohnii)*; however, the results were not convincing. It seems clear that amino acids are not neutral to the physiological reactions of

**Table 1** Chemotactic activity of L-amino acids tested in four concentrations ($10^{-12}$; $10^{-10}$; $10^{-8}$; $10^{-6}$ M) in *Tetrahymena*.

| | AA | $10^{-12}$ [M] | $10^{-10}$ [M] | $10^{-8}$ [M] | $10^{-6}$ [M] | Present in "primordial soup" |
|---|---|---|---|---|---|---|
| Aromatic R group | Phe – F | 43,8 $^{y}$ | 64,34 $^{y}$ | 36,75 $^{y}$ | 36,75 | |
| | Trp – W | 62,22 $^{y}$ | 76,4 $^{x}$ | 73,51 $^{x}$ | 74,27 $^{x}$ | |
| | Tyr – Y | 67,81$^{y}$ | 65.62$^{y}$ | 55,12$^{z}$ | 77,29$^{x}$ | |
| Polar, uncharged R group | Ser – S | 136,59 $^{y}$ | 149,18 $^{y}$ | 93,44 | 63,34 $^{y}$ | + |
| | Thr – T | 107 | 130,46 $^{y}$ | 71,65 $^{x}$ | 78,7 $^{x}$ | + |
| | Met – M | 76,73 $^{x}$ | 143,23 $^{z}$ | 124,27 $^{x}$ | 86,34 | |
| | Cys – C | 206,67 $^{z}$ | 197,5 $^{z}$ | 174,16 $^{z}$ | 230 $^{z}$ | |
| | Asn – N | 101,31 | 121,27 | 89,47 | 104,38 | |
| | Gln – Q | 179,38 $^{z}$ | 171,69 $^{x}$ | 184,61 $^{y}$ | 128 $^{x}$ | |
| Negatively charged R group | Asp – D | 113,59 | 90,57 | 101,31 | 102,85 | + |
| | Glu –E | 207,69 $^{z}$ | 130,76$^{x}$ | 156,41 $^{y}$ | 125,64$^{x}$ | + |
| Positively charged R group | Lys – K | 57,97 $^{y}$ | 56,47 $^{y}$ | 42,35 $^{z}$ | 80 | |
| | Arg – R | 100 | 84,62 | 116,36 | 92,8 | |
| | His – H | 116,85 | 123,09 $^{x}$ | 144,57 $^{y}$ | 135,33 $^{x}$ | |
| Non-polar; aliphatic R group | Gly – G | 124,11$^{x}$ | 147,44$^{x}$ | 98,04 | 93,82 | + |
| | Ala – A | 124,27$^{x}$ | 131,9 $^{y}$ | 121,81 $^{x}$ | 107 | + |
| | Val – V | 65,88 $^{x}$ | 50,16 $^{x}$ | 39,15 $^{z}$ | 59,57 $^{y}$ | + |
| | Leu – L | 58,82 $^{y}$ | 59 $^{y}$ | 50,94 $^{y}$ | 39,17 $^{y}$ | + |
| | Ile – I | 121,52 | 62,7 $^{y}$ | 74,47 $^{y}$ | 62,7 $^{y}$ | + |
| | Pro - P | 102,27 | 165,9 $^{z}$ | 131,81 $^{x}$ | 152,27 $^{y}$ | + |

($^{x}p<0.05$; $^{y}p<0.01$; $^{z}p<0.001$). Data of primordial soup are from (30).

*Tetrahymena.* Considering the sparse data available, we determined to study all of the amino acids systematically in a model. We attempted to do this in a model of the most ancestral physiological reaction, chemotaxis and observe the results in light of organic evolution.

*Concentration dependence*

Chemotactic responsiveness elicited by the L isomers of 20 amino acids demonstrates that *Tetrahymena* possess highly developed discriminatory potency for these relatively small and structurally closely-related molecules (Table 1). Data show that the presence of aromatic R groups (F, W, Y) results in a uniform, strong, wide range chemorepellent responsiveness, while amino acids with R groups representing other basic chemical characters possess less similar properties in respect to chemotaxis. Despite the above mentioned transient (mixed) features, polar amino acids with uncharged R groups (S, T, M, C) had a wide (C) or narrow range (S, T, M) chemoattractant character. Biphasic, attractant and repellent shifts were also recorded (S, T, M) in this group of molecules. Pairs of the amino acids sets (E-Q and D-N) demonstrated high chemotactic sensibility and discriminator ability for *Tetrahymena*. While the E with negatively charged R group and Q with uncharged R group could work as strong and wide range chemoattractant ligands, their identical relatives, D and N, were neutral. Diversities in chemotactic properties of these four ligands suggest that minor changes in the size of the R group (3 *vs* 2 member C tails) even has significance in biological activity.

Amino acids with positively charged R groups elicited diverse effects. Arg, with the longest R group and two amino terminal residues, was neutral. Lys, expressing only one $–NH_3$, appears to be repellent in a wide range. In contrast, the presence of a non-aromatic imidazole ring in H results in a wide range, chemoattractant character.

In the case of the non-polar amino acids with aliphatic R groups; only the two smallest ligands (G and A) could elicit positive chemotaxis in *Tetrahymena*. Besides these ligands, P, with its pyrrolidine ring, was also chemoattractant in a wide range, which supports the idea that bearing the ring structure is not accompanied by a negative, chemorepellent moiety. The aromatic character thus appears significant.

The three other members of the group (V, L, I) have a wide range chemorepellent effect, which has a good correlation with the similarities of the non-branched R groups.

*Theory of "chemotactic-range-fitting"*

Comprehensive study of the registered chemotactic activities and the ranges of amino acids (Fig. 1) show that the effective range of chemoattractant amino acids (e.g. C, E, Q, P, S, G) is significantly wider (66.1 ± 14.2) than the chemorepellent ones (24.66 ± 8.16) (e.g. K, Y, W, V, F, L). Range of effectiveness (36 ± 17,18) and ranking of chemotactic activity also has good correlation. Both represent intermediate position in evaluations. A possible explanation for the observed phenomenon is complex. A potential interpretation is that the responses induced by the two kinds of ligands (chemoattractants and chemorepellents) have fundamentally diverse functional significance. Responses induced by chemoattractants –basic nourishments– are based on a wide buffer capacity of the receptor repertoire, while detection of chemorepellent negative signals is more vital. Its signalling background, therefore, is a more exact one. Our above described, "*chemotactic-range-fitting*" theory of chemoattractants describes the extremities of the tested range. Transient ligands of the middle phase provide a supposed buffer capacity which should obtain high significance in

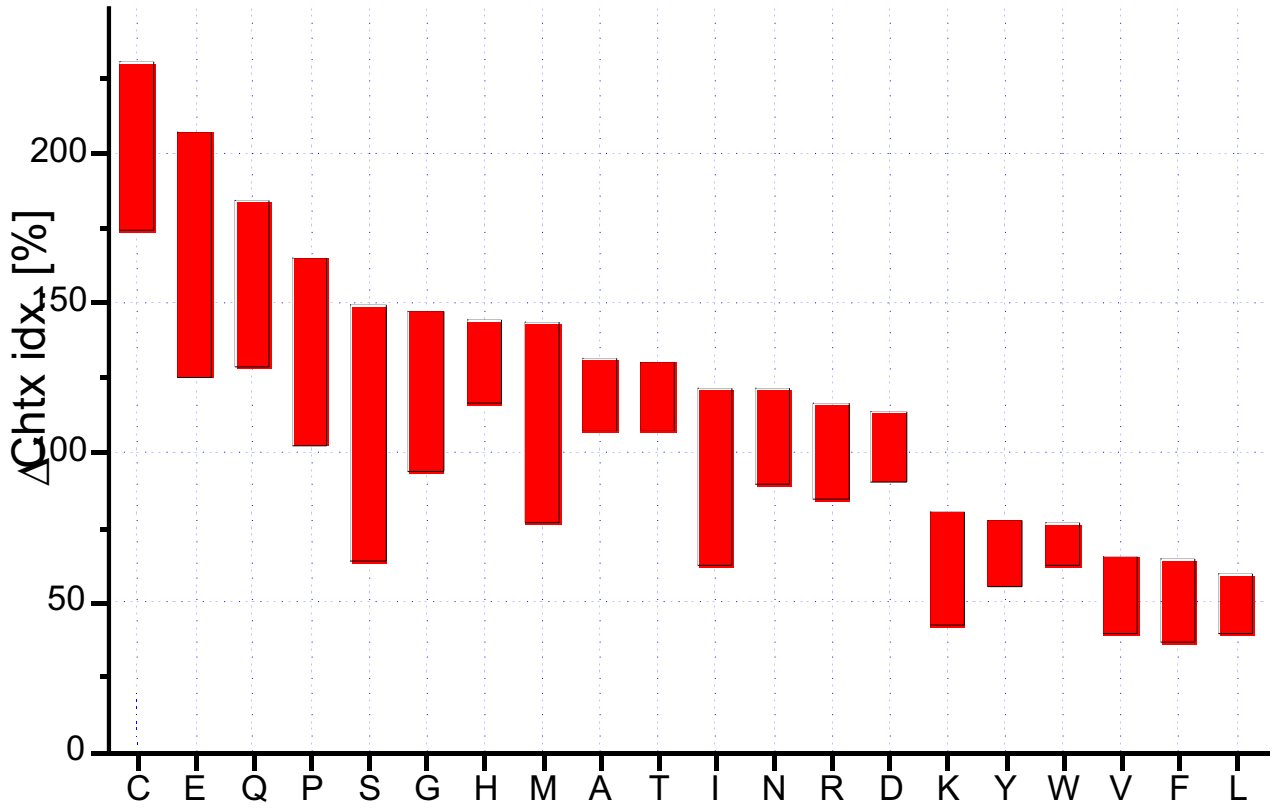


**Fig. 1** Range of effective chemotactic activity of amino acids in *Tetrahymena*

**Table 2** Physicochemical characteristics of amino acids working as chemoattractant, chemorepellent and intermediate ones in *Tetrahymena*

| | pK (-COOH) | pK (-$NH_2$) | SEA [sqÅ] | SEA of Side Chains [sqÅ] | Buried Residues [%] | Hydropathy Index |
|---|---|---|---|---|---|---|
| Chemoattractants in wide-concentration range | -2.14 ± 0.05 | 9.79 ± 0.28 | 148.36 ± 15.73 | 61.95 ± 8.38 | 36.3 ± 9.34 | -1.21 |
| Intermediate | 2.12 ± 0.07 | 9.35 ± 0.12 | 179.7 ± 14.68 | 73.12 ± 9.57 | 34.87 ± 7.14 | -0.9 |
| Chemorepellents- in narrow concentration range | 2.21 ± 0.08 | 9.3 ± 0.11 | 218.21$^{y}$ ± 14.43 | 102.25$^{y}$ ± 10.52 | 43.00 ± 9.91 | 0.78 |

($^{x}p<0.05$)

changes of environment overriding the threshold limits.

Existence of the above-described "chemotactic-range-fitting" is supported by the characteristic mean values of physicochemical properties of the tested amino acids (Table 2). Hydropathy of amino acids is a good index for description of the developed relation between the amino acid and the phospholipid bilayer of the surface membrane. The average values of these indices are portrayed by a gradual increase: -1.21 (± 0.91); -0.9 (± 1.16); 0.78 (± 1.34) and were calculated for the wide-range chemoattractants, the intermediate phase and the narrow range chemorepellents, respectively (24). The pKa values are determinant physicochemical properties of amino acids as well. Our data show a close relationship between the chemoattractant character (average of pK –COOH) which is low (2.14 ± 0.05), while pK $–NH_2$ is increased (9.79 ± 0.28) as well as chemorepellent one (average pK –COOH is increased (2.21 ± 0.08) and pK $–NH_2$ is depressed (9.3 ± 0.11). This suggests that beyond hydropathy, minor deviations of intramolecular charges are also responsible for the chemotactic ability of even relatively small ligands, like amino acids.

In the case of the classical chemoattractant family, fMLP, and its derivatives or WSXWS peptides we observed that a third point of characterization, the size of accessible solvent area exposed (SEA), also determines ligand-receptor interactions in chemotaxis (23). Table 2 demonstrates the calculated surface areas and related values in the three, chemotactically diverse, groups of amino acids.

We can observe that there is a close relationship between the measured chemotactic activity and ranges of amino acids and their accessible surface areas or the surface areas of accessible side-chains. It is suggested that low surface values are preferred for being a chemoattractant amino acid, whereas increasing values are accompanied (responsible?) by a chemorepellent trend. On the other hand, we can also observe that there is no clear correlation between the ratios of buried – not solvent-available-residues.

*Chemotactic selection*

The new technique of chemotactic selection (17) deals with the diverse functional state of receptors in subpopulations of the investigated model cells. Application of this technique allows us to select chemotactic responder cells and subsequent chemotaxis of the selected subpopulations assay with the selector ligand. This provides the opportunity to analyse whether the receptors responsible for signalling of the particular ligand are expressed with short-term or long-term characteristics. As in our prior studies, four combinations of assays are taken (see Materials and Methods). Better perspicuity required the introduction of coefficient chemotactic selection ($Ch_{sel}$), an index of which is calculated from the chemotactic responsiveness of selected (*S*) and non-selected (*C*) subpopulations to the selector ligand (*s*) and control medium (*c*), $Ch_{sel}=\frac{S/s \cdot C/c}{S/c \cdot C/s}$ (20). In this part of our investigations chemoattractant amino acids (including the two border-line members, Arg and Ile) of the concentration course were assayed. Data of chemotactic selection (Fig. 2) show that only three amino acids (I, H and T) selected *Tetrahymena* subpopulations with a long-term enhanced chemotactic responsiveness ($Ch_{sel}$>1.4). In the case of the other four amino acids (E, M, C, G and R) the $Ch_{sel}$ had a borderline positive value. There were four amino acids (P, A, G and S) which did not elicit positive chemotactic responses in the subpopulations selected. Their $Ch_{sel}$ indexes were below 1.0 significantly.

As data of chemotactic selection demonstrates, there are deviations in the chemotactic ability of amino acids and their efficiency for selection in the long-term. The best "selectors" Ile, His and Thr belonged not to the strongest chemoattractant amino acids, but rather have the capacity to select subpopulations with a characteristic chemoattractant responsiveness. Our data support the observations of previous studies (22) that chemoattractant

**Table 3** Physicochemical characteristics of amino acids working as short- and long-term or border-line selectors in *Tetrahymena*

| | pK (-COOH) | pK ($-NH_2$) | SEA [sqÅ] | SEA of Side Chains [sqÅ] | Buried Residues [%] | Hydropathy Index |
|---|---|---|---|---|---|---|
| Long-term selectors (I, H, T) | 2.09 ± 0.15 | 9.49 ± 0.16 | 178.66 ± 14.48 | 75.75 ± 12.85 | 43.33 ± 11.39 | 0.2 |
| Border-line selectors (E, M, C, G, R) | 2.19 ± 0.06 | 9.56 ± 0.20 | 175.9 ± 28.17 | 84.83 ± 11.47 | 39.80 ± 12.73 | -0.8 |
| Short-term selectors (P, A, Q, S) | 2.18 ± 0.07 | 9.73 ± 0.38 | 146.97 ± 16.49 | 49.97 ± 11.98 | 31.75 ± 7.49 | -1.025 |

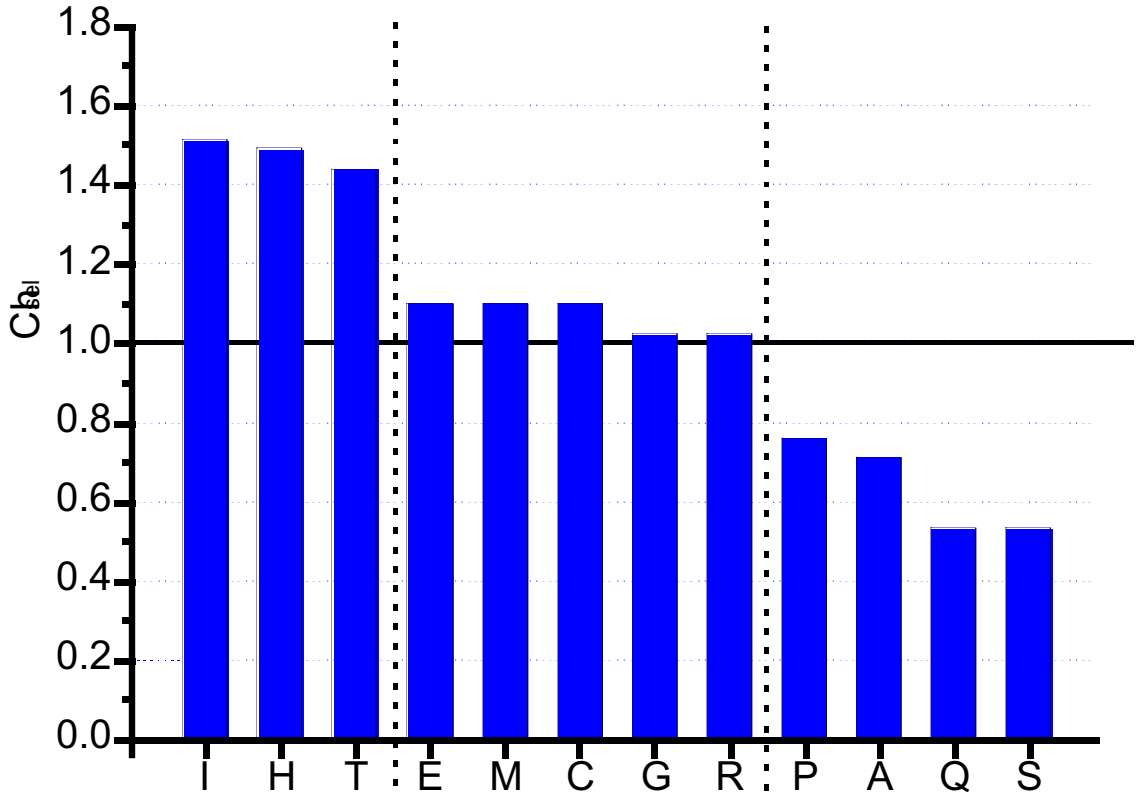


**Fig. 2** Chemotactic selection of *Tetrahymena* with amino acids. Responsiveness of subpopulations is characterized by the values of $Ch_{sel}$ coefficient

ligands do not unambiguously work on receptors expressed permanently in the target cell. In respect to the lifetime of receptors, part of the ligands induce chemotaxis via transient receptors induced by the ligand. This kind of responsiveness is not coded genetically but has a "short-term" feature, in contrast to those in which receptors are permanent components of the surface membrane and expressed in the selected subpopulations long-term.

Diversities of physicochemical characteristics of the long- and short-term selector amino acids are summarized in Table 3.

As we can see in the case of pK (-COOH) values, there was only a slight correlation, or none at all, between the net chemoattractant character and the long-term selector capacity of the amino acids. On the other hand, a reciprocal relation was found in relation to pK ($-NH_2$) values: amino acids with low values are preferred for the long-term selection, while high values are favoured in the short-term chemoattractant amino acids.

In respect to the solvent exposed areas, there was a positive correlation between the chemoattractant behaviour of the amino acid ligands and the short-term responsiveness. In both cases the low surface areas were optimal. We have to mention that SA discrepancies of short and long-term selectors seems to be only a comparative one, as high SA values of chemorepellents (see Table 2) were not found for either short- or long-term selector ligands. Similar observations were noted for the SA of the side-chains as the characteristic high values of chemorepellents (see Table 2) were not found for any group of selector amino acids.

*Phylogenetical significance of chemoattractant amino acids*

As chemotaxis is one of the most basic cell-physiological responses, our objective was to study the results in relation to the phylogeny of signal molecules. Phylogenetically, amino acids were "pioneer" organic molecules synthesized in the special environment of the prebiotic stage of phylogeny. Several experiments calculated and proved the contingency of these chemical processes. Some of them possess basic theoretical significance (30), while others were approved as practical descriptions of the primordial phase of molecular phylogeny (11). Among the derivatives of amino acids, G, A, V, L, I, P, D, E, S and T were detected in model systems as the first organic molecules (30).

Our chemotaxis studies provide further functional evidence to the phylogenetical story. Comparison of the listed amino acids and the chemotactically active,

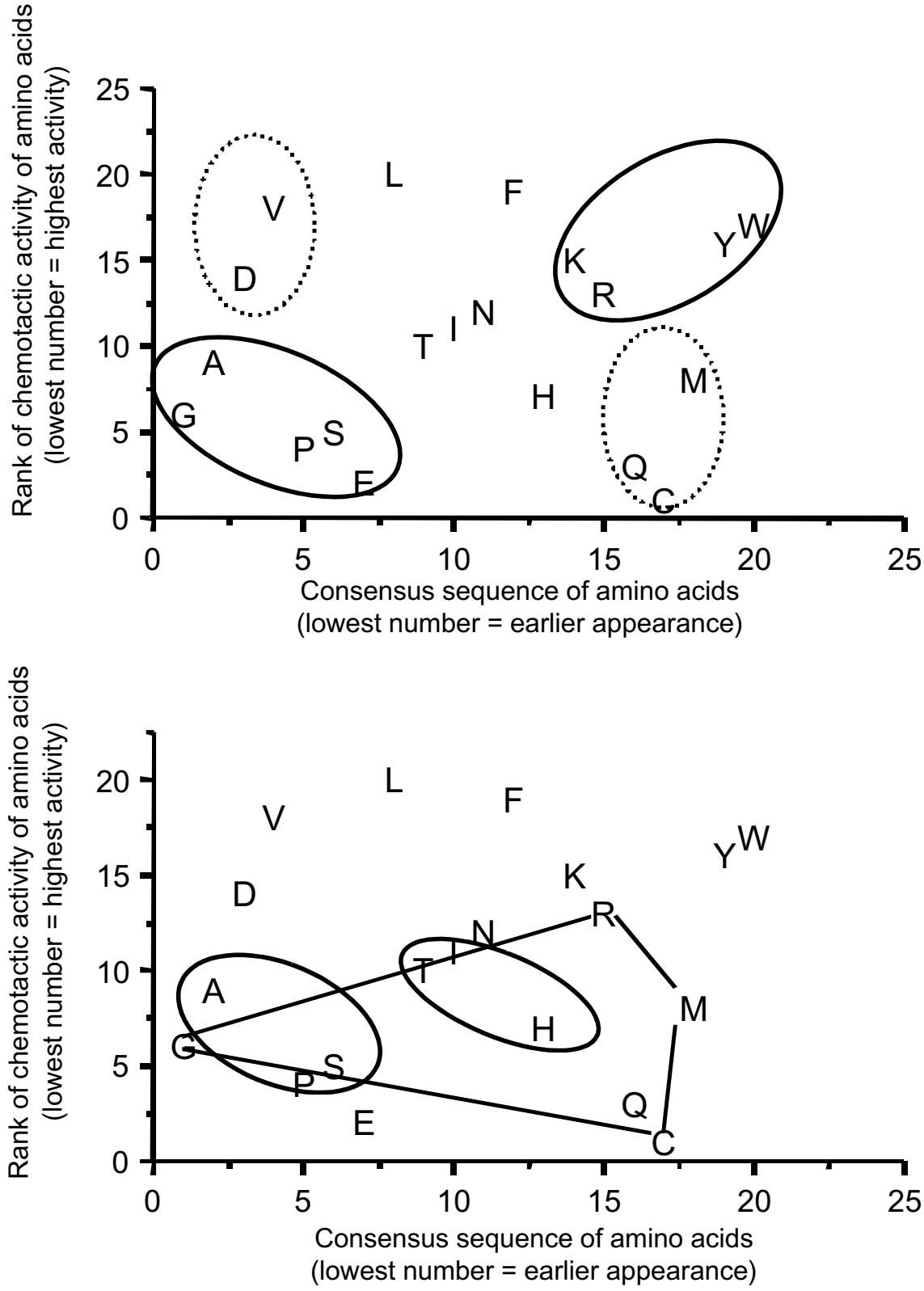


**Fig. 3** Relation of chemotactic ability of amino acids in *Tetrahymena* and the consensus chronology of amino acids in molecular phylogeny. **a) top** Represents preferred groups of amino acids in chemotactic responsiveness; **b) bottom** shows relation of chemotactic selector capacities to the investigated matrix.

chemoattractant ones (Table 1) shows a 90% overlap, which suggests that these amino acids were not only present in the primordial environment, but that their appropriate signalling mechanisms were conserved by means of a special selection. Although the present day eukaryotic protozoan *Tetrahymena* is not identical with the ancestral ones, the results of this selection are still detectable in the current model. Our selection studies demonstrated that the appropriate receptors are highly specific and that their induction requires a fine matching of physicochemical properties of the ligand/receptor relation. Nevertheless, we can also observe that million years of phylogeny could also modify this recognition. Histidine is not considered a starting amino acid but its selector capacity is significant. Also, almost all of the works mentioned above demonstrated a rank order for the appearance of all amino acids. One of the most accepted ranking is that of Trifonov (31). The consensus chronology he proposed provides a descending order, starting with the earliest amino acid: G, A, D, V, P, S, E, L, T, I, N, F, H, K, R, Q, C, M, Y, W (31). By taking a ratio of his ranking and the ranking of maximal chemotactic efficiency of amino acids in *Tetrahymena*, we can draw a matrix which describes a proposed relation between the chemotactic activity and appearance of amino acids on Earth (Fig. 3). As we can see, there is a good correlation between the net chemotactic activity of chemoattractant amino acids and their early appearance in the prebiotic systems, while chemorepellent amino acids turned up significantly later. As our figure shows, the proposed relations during the phylogeny did not always run parallel: some amino acids with poor chemoattractant capacities were also in the starting set (e.g. V or D), while a new set of chemotactic amino acids (M, C and Q) appeared in the late period.

In contrast, the above described results and trends gained by chemotactic selection shows that short-term selectors (A, P, S) were among the pioneer amino acids. A distinct but intermediary-position group of long-term selectors raises the possibility that the required time for the development of signalling mechanisms is responsible for the special position in the matrix. Wide range appearance of practically neutral selectors indicates that in the background of chemotactic selection distinct molecular characters are required for short- or the long-term activity. Evolutionary significance of the above-mentioned amino acids ranked on the basis of their chemotactic activity provides further potential significance. Evaluation of amino acid frequencies in different compositions shows that long-term selector amino acids, or border-line ones, are expressed in membrane and extracellular matrix proteins, while the short-term working ligands have dominance in nuclear proteins (9). The diverse distribution of amino acids correlates the above-described phenomenon with higher levels of peptide phylogeny and suggests that basic functional characteristics of amino acids are potentially determinants for higher grades of molecular and cellular levels of evolution.

The significant matching of trends in physiochemical properties of amino acids and their cell-physiological activities, as well as the phylogenetical approaches of chemotaxis, support our previous theory concerning the ancestral and exclusive role of chemoresponsive behaviour as a fundamental targeting of the cell physiological activity from the primordial phases of phylogeny.

**Acknowledgment** – This work was supported by grants from the Hungarian Scientific Research Fund (OTKA T17773, T32533 and T37303)

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