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Chemotaxis induced by SXWS tetrapeptides in *Tetrahymena*—overlapping chemotactic effects of SXWS sequences and their identical amino acids

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The chemotactic potential of SXWS peptides and the components of the extracellular domain of cytokine receptors were investigated in *Tetrahymena* as a functional index of substitution with different amino acids in the position 'X' of the tetrapeptide. Data obtained demonstrate that position X plays a special determining role in the ligand, SEWS and STWS possess extremely strong chemoattractant ability, and aromatic amino acids result in chemorepellent ligands. Diverse effects of structurally related molecules, for example, SNWS–SDWS, demonstrate a highly sensitive discrimination potential in the applied model system. Physicochemical characteristics (hydropathy, residue size, and solvent-exposed area) of the amino acids were correlated with the chemotactic activity. Data obtained by computer-assisted conformation analysis of SXWS peptides and the highly overlapping chemotactic effects of the investigated SXWS peptides as well as the presence of the amino acids in the 'X' position indicate that member 'X' of the SXWS sequence performs a special role in interactions with the chemotaxis receptors in the membrane. Copyright © 2011 John Wiley & Sons, Ltd.

Keywords: chemotaxis; selection; conformation, SXWS; amino acid; *Tetrahymena*

INTRODUCTION

At different levels of phylogeny, a wide range of substances are acting as chemoattractant or chemorepellent ligands. The diversity of these compounds is rather high; small ligands like amino acids (Almagor *et al*., 1981) could be as effective as chemokines (e.g. interleukin 8 [IL-8]; Zachariae, 1993), peptide hormones (e.g. insulin; Csaba *et al*., 1994), or lipid mediators (e.g. leukotrienes; Scheja and Forsgren, 1986). The specificity of chemoattraction is based on the extremely high molecular discriminatory capacity of the responsible receptor complexes as demonstrated by variable effects of molecules like chemokines (IL-8 and derivatives; Auer *et al*., 1993) or bacterial peptides (fMXXX family; Freer *et al*., 1982). However, not only the sequence but also the physicochemical properties (p*K*, hydrophobicity, residual volumes, etc.) of the ligand as well as several physiological (cell cycle, pretreatments) and environmental factors can also modulate the chemotactic responses of the target cell (Mare *et al*., 1999).

The number of newly described chemotactic ligands is increasing very fast. The characterization of cell surface–bound molecules responsible for triggering haptotaxis—an alternative form of chemotaxis where the gradient of chemoattractant develops not in a fluid but on a semisolid phase—opened a new approach in chemotaxis research (Rot, 1993) and led to the recognition of a new group of molecules expressed in inflammation as chemoattractant ligands. Recent studies proved that peptides corresponding to the SXWS sequence, where X = any amino acid, represent one of the new group mentioned earlier (Kőhidai *et al*., 2003a). This sequence is part of the extracellular domain of several cytokine receptors (e.g. IL-6). Soluble forms of these receptor domains provide the possibility that SXWS sequences elicit their effects not only as cell surface–associated haptotactic ligands but also as classical chemotactic peptides. Data show that peptide SEWS possesses high chemotactic activity not only in monocytes or lymphocytes but also in the eukaryotic ciliate *Tetrahymena*. This observation indicates that the response to this peptide is phylogenetically conserved. The unicellular *Tetrahymena* is a widely used model for cell-physiological studies (Csaba, 1985). Homologies to vertebrate models in membrane receptors (e.g. insulin receptor; Christensen *et al*., 2003), second messenger systems (adenosine 3′,5′-cyclic monophosphate, Csaba and Nagy, 1976; IP3, Kovács and Csaba, 1994;

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Ca^{2+}-calmodulin, Kovács and Csaba, 1987) and in other physiological responses (e.g. growth, phagocytosis) established this ciliate as a useful organism in studies of phylogenetic conversation of signaling. The chemotactic responsiveness of *Tetrahymena* and other unicellular organisms is considered as one of the most ancient responses (Kőhidai, 1999). These cells are able to distinguish between derivatives of vertebrate hormones (insulin, bradykinin, endothelins, etc.) at a highly sensitive level (Csaba *et al*., 1994; Kőhidai *et al*., 2001; Kőhidai *et al*., 2002). Chemotactic ligands such as formylated peptides (N-formylmethionyl-leucyl-phenylalanine) (Leick, 1992) or chemokines (IL-8) (Kőhidai and Csaba, 1998) are active at similar concentrations in both unicellular organisms and in vertebrates.

These characteristics of *Tetrahymena* prompted us to investigate the chemotactic properties of a peptide library corresponding to the SXWS motif. We were interested in investigating (i) the effect of amino acid substitutions in position 'X' on the chemotactic character of SXWS peptide, and (ii) the possible correlation between the chemotactic abilities of SXWS peptides and the physicochemical character of the amino acid present at the position 'X'.

Our previous experiments proved that the biological activity of SXWS peptides is highly sensible to the chemical structure of this short sequence. However, scrambled peptides were not prepared, and only the role of amino acid residue in position X was investigated by the relevant tetrapeptide with free C-terminal carboxylic group. Thus, the influence of the amino acid side chain (hydrophobic/hydrophilic, polar/apolar, etc.) of amino acid X was studied to evaluate the structure–function relationships. These experiments documented that the presence of the aromatic Trp (WSXWS peptides) or removal of the Ser (XWS) on the N terminus results a significant decrease/change in chemotactic activity of the peptides (Illyés *et al*., 2002). Chemical modification of the N terminus (e.g. amidation) is also very effective; in Glu containing members of the library, this modification results in increased (EWS), diminished (SEWS), and increased (WSEWS and EWSEWS) biological activities in the peptides (Kőhidai *et al*., 2003a).

Previous studies showed that the receptor pool responsible for chemotactic activity could be classified as either 'short-term' or 'long-term' receptors. Some chemotactic ligands, for example, vasoactive peptides, are acting on short-term receptors, which are inducible only at the time of exposition (perhaps the ligand itself induces the assembly of the receptor membrane components) (Kőhidai *et al*., 2001). The long-term receptors are expressed as constitutive functional units of the membrane and are responsible for enhanced chemotactic behavior of subpopulations obtained by chemotactic selection (Kőhidai *et al*., 2000). In view of these findings, we also studied the relationship between chemotactic selection and the identity of 'X' amino acid in SXWS. We also investigated whether the SXWS group of ligands requires constitutively expressed or ad hoc receptors in the membrane.

RESULTS AND DISCUSSION

Conformational analysis of SXWS peptides

According to our results, peptides of the SXWS sequence adopt very similar conformations in water (Figure 1). With the exception of Gly, Pro, and Arg at position 'X', the average RMS for backbone atoms of the peptide was found to be 0.29 Å with respect to the SWWS structure (the best mimic of the average conformation; see Table 1 for details). This conformation is held in place by a highly conservative H-bond pattern, consisting of five strong and specific H-bonds, which appear in all but two of the global minimum energy conformations of the SXWS peptides (Figure 2). The ACE carbonyl O...OG Ser1, NH Ser1...O Ser1, NH Trp3...O Trp3, O Trp3...OG Ser4, and NH Ser4...O Ser4 H-bonds provide a rigidified core structure from which the 'X' residue emerges and is unable to affect the overall conformation of the peptide. This is especially interesting because some 'X' residues participate in H-bond contacts, that is, proton donors Gln and Ser form H-bonds with the carbonyl of Ser1, His, and Lys with their own carbonyl group, whereas the proton acceptor carboxyl moiety of Glu ligates to the amide nitrogen of Ser1.

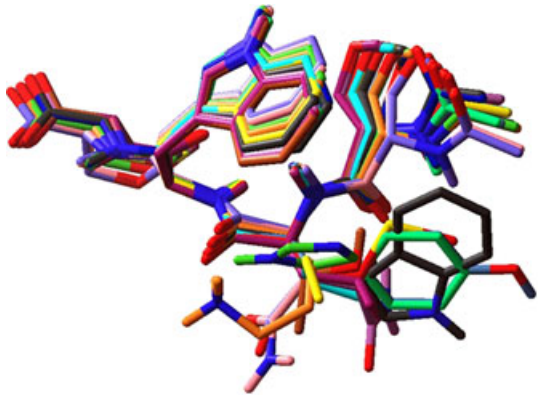

Figure 1. Superposition of the global minimum energy structure of the SXWS peptides (with the exception of SGWS, SPWS, and SRWS). Carbon and hydrogen atoms are colored uniformly within each molecule; nonpolar hydrogens were omitted for clarity.

Gly in position 'X', probably because of its small size, greatly enhances the conformational flexibility of the peptide, resulting in several low energy conformers. However, nearly all of them

Table 1. Calculated RMS deviation and maximum difference of atom pairs of backbone atoms of each SXWS peptide with respect to the structure of SWWS representing the average arrangement.

'X'	RMS [Å]	Maximum deviation [Å]
SIWS	0.09	0.22
SVWS	0.09	0.24
SYWS	0.15	0.47
SFWS	0.19	0.59
SMWS	0.20	0.36
SQWS	0.24	0.53
SHWS	0.24	0.48
SKWS	0.26	0.46
STWS	0.27	0.59
SLWS	0.30	0.64
SAWS	0.37	1.05
SSWS	0.38	1.10
SNWS	0.51	0.84
SDWS	0.54	0.90
SEWS	0.60	1.66
SGWS	1.01	1.77
SPWS	1.66	3.33
SRWS	2.19	4.25

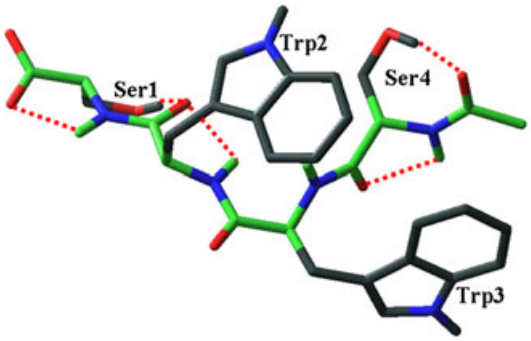

Figure 2. Characteristic H-bond pattern of the SXWS peptides shown on the example of the SWWS (Ac-SWWS-OH) structure (backbone C and H atoms are colored green, in hard copy in light gray.

retain the characteristic H-bond pattern. One conformer with energy of only 0.28 kJ/mol, less favorable than that of the global minimum, is quite similar to the average SWWS structure with a backbone RMS of 0.44 Å. In case of SRWS, the characteristic H-bond pattern is broken by the strong association (derived from three H-bonds) between the guanidino nitrogens of Arg and the carboxyl oxygens of Ser4. However, even SKSW can adopt a conformation similar to that of SWWS (backbone RMS of 0.37 Å) with 3.23 kJ/mol extra energy (the cost of losing one H-bond) by coordinating to the carbonyl oxygen of Ser1 instead of Ser4, in which case the H-bond pattern is restored to that described earlier. In SPWS, the presence of Pro induces a flip of the Ser1 carbonyl, which results in its coordinating with the amide nitrogen of Trp3 instead its own NH group. However, even SPWS keeps the basic topology seen with the other peptides (Figure 3); the backbone RMS calculated without the backbone atoms of Pro was 0.52 Å, reflecting the similarity.

Concentration course study: physicochemical characteristics and chemotaxis

Previous studies of the concentration dependence of chemotactic responsiveness showed that peptide ligands could have different, characteristic profiles. Besides the classical 'single-peak' profile of chemoattractant ligand, two chemotactically effective concentrations were detectable. Data in the literature (e.g. bradykinin, Leeb *et al*., 1997; endothelin, Kőhidai *et al*., 2003b) support the notion that diverse receptor pools are responsible for the multipeaked profile. A distinctive pattern can also be detected in the case of chemorepellents, with the curves presenting only one negative peak.

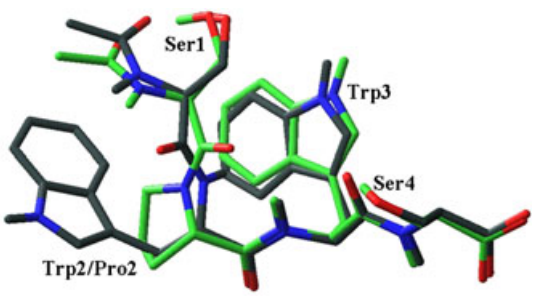

Figure 3. Comparison of the calculated SWWS structure (gray) with that of SPWS (green). (Please consider that the numbering of the residues is opposite to that used in Figure 2.)

In the case of the SXWS library, we also observed the variation mentioned earlier with respect to concentration dependence (Table 2).

Results in Table 1 show that two SXWS peptides (SEWS and STWS) have a strong chemoattractant character. Both peptides elicited maximal effect at a rather low concentration (10^{-12} M). The amplitude of the chemoattractant effect was extremely high in the case of SEWS (660%), although this peptide induced a pronounced chemorepellent response at the highest tested concentration. This is an intriguing observation because the chemoattraction observed at 10^{-12} and 10^{-11} M was the highest among all of the molecules studied while SEWS exhibited the strongest chemorepellent effect between 10^{-10} and 10^{-6} M.

Chemoattractant SXWS ligands can be distinguished by their optimal effective concentration. The concentration was relatively low (10^{-10} M) in the case of two ligands (SAWS and SMWS), with similar chemoattractant activity (SAWS = 170% and SMWS = 150%), whereas three other ligands (SSWS, SPWS, and SNWS) were active only at higher doses (10^{-8}, 10^{-7}, and 10^{-8} M, respectively).

Three peptides (SHWS, SIWS, and SQWS) acted as chemoattractants either at low (10^{-12}–10^{-10} M) or high (10^{-8}–10^{-6} M) concentrations.

Five SXWS peptides proved to be chemorepellent peptides. Interestingly, three peptides contain aromatic amino acid in position 'X' (SWWS, SYWS, and SFWS). In addition, Lys and Val containing ligands (SKWS and SVWS) also had a repellent effect. A characteristic negative peak was found only in response to SWWS (10^{-10} M), SVWS (10^{-10} M), and SYWS (10^{-9} M) peptides, whereas other two peptides had less marked chemorepellent effect at higher concentrations: SFWS (10^{-7} M) or over a wide concentration range SKWS (10^{-12} –10^{-7} M).

Four peptides (SDWS, SGWS, SLWS, and SRWS) showed no chemotactic activity in *Tetrahymena*. Previous studies showed that the chemotactic character of amino acid or oligopeptide-type ligands was linked to their physicochemical properties (Kőhidai, 1999; Kőhidai *et al*., 2003b). In the case of amino acids, correlations were observed between p*K* values or solvent-exposed areas (SEAs) (Kőhidai *et al*., 2003c) and chemotactic activity, whereas in proline containing dipeptides, the size of the C-terminal amino acid was found to be decisive for chemotaxis (Kőhidai *et al*., 1997). Investigation of peptides with an EWS motive showed that the chemotactic ability of these ligands was sensitive to amidation of the C-terminal residue (Kőhidai *et al*., 2003a).

In the SXWS peptides studied, only amino acid 'X' was different, indicating the importance of this residue for inducing a chemotactic response. The problem is more emphasized in the case of the SXWS sequence, which is present in different cytokine receptors (X = E, D, etc.), indicating high polymorphism.

Next we performed a calculation to find common ground in the three groups of peptides studied. Evaluating the significance of amino acids used by substituting the investigated peptides is rather complex. A direct link between the basic physicochemical moiety and the chemotactic (chemorepellent) effect was found only in the case of the aromatic amino acids. However, the group of neutral peptides itself proved to be heterogeneous. Comparison of the effectiveness to the chemoattractant or chemorepellent ligands indicates that our model cell can distinguish slight molecular alterations of the chemotactic ligand. The Gly containing SGWS had a neutral effect, whereas SAWS with an additional methyl group was chemoattractant. The substitution of SXWS peptides with the related amino acids Asp (presence of $-$OH residue—SDWS) and Asn (presence of $-NH_2$ residue—SNWS) or Leu

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Table 2. Concentration dependence of chemotaxis elicited by SXWS peptides in *Tetrahymena pyriformis* GL

		Chemotactic activity (%) (control = 100%) / SD						
Type of response	SXWS	10^{-12} M	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M	10^{-6} M
Chemoattractant with optimum concentration $< 10^{-10}$ M	SEWS	$660^{z} \pm 17.67$	$430^{z} \pm 18.21$	70 ± 14.44	91 ± 15.39	45 ± 14.01	60 ± 12.74	$25^{x} \pm 11.94$
	STWS	$298^{z} \pm 12.38$	$160^{y} \pm 13.05$	$148^{y} \pm 13.84$	117 ± 15.56	110 ± 10.91	110 ± 10.24	120 ± 17.46
	SAWS	116 ± 14.62	122 ± 10.02	$174^{z} \pm 18.76$	$142^{x} \pm 14.08$	112 ± 12.48	114 ± 7.7	124 ± 13.53
	SMWS	118 ± 14.48	102 ± 12.59	$153^{y} \pm 17.4$	130 ± 16.33	97 ± 11.9	109 ± 13.88	95 ± 10.07
Chemoattractants with optimum concentration $> 10^{-9}$ M	SSWS	96 ± 8.64	133 ± 16.5	126 ± 14.07	143 ± 13.39	$235^{z} \pm 17.05$	$179^{y} \pm 14.56$	135 ± 17.96
	SPWS	96 ± 6.5	114 ± 10.93	107 ± 10.78	125 ± 11.17	$146^{x} \pm 18.66$	$162^{y} \pm 19.65$	107 ± 12.2
	SNWS	88 ± 11.5	111 ± 15.1	136 ± 12.02	132 ± 13.27	$172^{x} \pm 15.1$	$145^{x} \pm 16.85$	126 ± 16.86
Chemoattractants with two optimum concentrations	SHWS	$68^{x} \pm 13.39$	$122^{x} \pm 16.35$	90 ± 9.06	$61^{x} \pm 6.02$	114 ± 12.6	$166^{x} \pm 24.92$	$68^{x} \pm 10.22$
	SIWS	84 ± 6.71	109 ± 15.55	$169^{y} \pm 18.45$	130 ± 17.11	$171^{x} \pm 17.83$	$231^{z} \pm 21.79$	116 ± 9.63
	SQWS	$132^{x} \pm 8.83$	106 ± 8.51	100 ± 8.96	99 ± 8.71	116 ± 9.78	111 ± 8.34	$143^{y} \pm 12.57$
No effect	SDWS	95 ± 13.39	91 ± 15.37	84 ± 10.56	117 ± 14.57	88 ± 10.22	94 ± 12.35	117 ± 17.42
	SGWS	108 ± 13.24	91 ± 15.55	110 ± 12.2	93 ± 7.23	87 ± 11.63	112 ± 13.84	89 ± 8.85
	SLWS	104 ± 10.27	116 ± 15.35	109 ± 11.48	88 ± 9.07	112 ± 14.01	127 ± 12.59	104 ± 8.75
	SRWS	96 ± 13.18	98 ± 11.39	85 ± 9.23	103 ± 7.9	103 ± 7.9	120 ± 17.51	96 ± 9.07
Chemorepellent	SWWS	80 ± 4.44	68 ± 7.81	$57^{y} \pm 6.3$	66 ± 8.43	73 ± 9.16	74 ± 9.57	80 ± 8.19
	SFWS	96 ± 12	86 ± 10.33	89 ± 9.9	$79^{x} \pm 7.57$	87 ± 6.63	$69^{z} \pm 5.75$	76 ± 13.16
	SYWS	$55^{z} \pm 8.84$	$60^{z} \pm 9.2$	$71^{z} \pm 4.07$	$46^{z} \pm 5$	$75^{x} \pm 7.57$	$66^{y} \pm 6.95$	$67^{y} \pm 7.3$
	SVWS	$71^{x} \pm 6.18$	102 ± 8.03	$58^{y} \pm 5.68$	$71^{y} \pm 6.8$	86 ± 13.94	75 ± 12.73	78 ± 10.15
	SKWS	86 ± 12.48	$83^{x} \pm 7.6$	$85^{x} \pm 9.5$	$80^{x} \pm 8.56$	$85^{x} \pm 10.23$	$83^{x} \pm 12.21$	94 ± 9.09

(presence of $-CH_3$ residue—SLWS) and Ile (absence of the $-CH_3$ residue—SIWS) resulted also in different biological effectiveness. The presence of long side chains was not as important as the nature of the terminal part of these chains, which affected the chemotactic potency of the ligands; the Arg containing SRWS (with $-NH_2$ and =NH) was neutral, whereas the Lys containing SKWS (with only one $-NH_2$) was chemorepellent.

Partial agreements were also present in the case of other physicochemical characters (Table 3). Such attributes are the changing hydropathy values of the amino acids. In the three main groups of the SXWS ligands (chemoattractants, neutrals, and chemorepellents), we found that a significant negative value (−1.15) results in a neutral chemotactic moiety, whereas positive values characterize the group of chemorepellent peptides (0.18). With respect to hydropathy chemoattractants, these are featured with an intermediate, albeit negative value (−0.56), which suggests that there is a fine adjustment in formation of SXWS ligand–receptor complex, and the matching physicochemical condition of the residue in position 'X' is determined.

The special, chemorepellent character of 'X' residue is supported by its dependence upon residual sizes. The average residual size is significantly higher in the chemorepellent group (162.6), whereas neutral and chemoattractant ligands show no diversity in this respect.

The availability of the ligand in biological systems is characterized not only by the total or partial size of the molecule but also by the calculations of SEAs or mass–SEA ratio of work to evaluate accessibility of the ligand. In the present case, we can see that values of mass–SEA ratio might be considered as a sensible index to distinguish chemotactically diverse peptides. The data on residual size show that low values of the mass–SEA ratio for 'X' amino acids are preferred in chemoattractants, whereas high values were found in the chemorepellent group (neutral ligands possess intermediate ratio).

Phylogenetical approach: occurrence of amino acids and chemotaxis

In addition to the discussed relationships on the chemotaxis and physicochemical characteristics of peptides or their amino acid constituents, some more general observations were also noted. Reference data on the basis of the analysis of several thousands of peptides proved that the frequency of amino acids composing

Table 3. Physicochemical characteristics and frequencies of amino acids used to substitute SXWS peptides

	Hydropathy	Residual size	Mass–SEA ratio	Occurrence in proteins%	Occurrence in primordial soup
Chemoattractant X = E, T, A, M, S, P, N, H, I, Q	-0.56 ± 0.93	128.8 ± 6.80	177.3 ± 29	5.04 ± 0.571	6/1060%
Neutral X = D, R, G, L	-1.15 ± 1.86	128.2 ± 10.32	213.8 ± 42	6.67 ± 0.936	3/475%
Chemorepellent X = W, F, Y, V, K	0.18 ± 1.46	162.6 ± 14.85	306.5 ± 22	4.2 ± 0.937	1/425%

Values represent averages calculated for the three chemotactically diverse groups of ligands.

peptides is diverse (Dagan *et al.*, 2002). In respect of these frequencies, we suppose that the composition of chemoattractant or chemorepellent ligands, as extracellular messenger molecules of a most ancient cell-physiological response, shows some correlation with the uneven distribution of amino acids. As Table 2 shows, there is a good match between the calculated frequencies in general and the chemotactic abilities of the SXWS peptides substituted with the different amino acids. The relatively high frequency of amino acids of neutral SXWS peptides (6.67), in contrast to the depressed values of chemorepellent (4.2) and chemoattractant (5.04) ligands, suggests that both groups represent biased signal molecule quality in contrast to the chemotactically noneffective substitutions.

Special, phylogenetical backgrounds of alarming significance of chemorepellent and beneficial moieties of chemoattractant ligands are revealed by their appearance in the primordial soup. Amino acids yielded first have a significantly reduced frequency in the chemorepellent SXWS derivatives (Table 3). This suggests that in motives present in eukaryotic systems (i.e. membrane receptors or soluble ligands—SXWS fulfils both roles), there is still a good correlation between the abovementioned early occurrence in evolution and the biological effectiveness of a signal peptide substituted with the different amino acids.

Chemotactic range fitting of SXWS

Previous studies introduced *chemotactic range fitting* as a new aspect in the characterization of chemotactic ligands (Kőhidai *et al.*, 2003b). This phenomenon describes close relationships between the chemotactic ability of peptides and the range of their effectiveness. In this respect, chemoattractants can elicit their effects over a wide range; the amplitude of chemotactic responses cover a wide scale in the concentration course study. Although chemorepellents have a narrow amplitude, they result in similar responses in the whole range of concentrations tested.

The characteristics of chemotactic range fitting were evaluated for SXWS peptides (Figure 4). Data of chemotactic range fitting for SXWS peptides agree with the abovementioned reference data for amino acid–type ligands. The two strongest chemoattractant ligands (SEWS and STWS) proved to have the widest range (30%–660%), and other chemoattractant peptides (SSWS, SIWS, SAWS, SNWS, SHWS, SPWS, and SMWS) have also wide or moderately wide range (60%–230%) of effectiveness. A good correlation was found also on the other boundary of the scale, as the narrowest effective chemotactic ranges belong to chemorepellent peptides (SYWS, SWWS, SKWS, SFWS, and SVWS; 10%–50%).

Overlapping of chemotactic ability of SXWS and amino acids

The good correlation of biological effectiveness and concentration fitting as well as that of the chemotactic range fitting works in the same manner in amino acid and SXWS-type ligands suggests that the X position of SXWS peptides probably possess unique role in respect of biological abilities of these molecules. To validate the hypothesis mentioned earlier, we have compared chemotactic ability of SXWS peptides and the respective individual amino acid corresponding to the X position. The data (Table 4) show that a high level (84%) of overlapping was found between the chemotactic abilities of SXWS peptides and the identical amino acids.

As the overlapping was found in all of the three main groups of ligands (chemoattractant, neutral, and repellent), and only 3 of the 19 SXWS ligands were not matching with the respective amino acid results, it is proposed that the second amino acid of the SXWS sequence has a special, more exposed position in the peptide than would be considered. The abovementioned relationship of the cell-physiological character of the ligand and its structure is supported by our data gained by conformational analysis. SXWS seems constructed as a transport vehicle of 'X', therefore explaining why the chemotactic activity of 'X' is not disturbed by inclusion into the motif. We have to consider also the potential interactions of the ligand with the cell, which can also influence expression (or burial) residues, side chains, or major portions of residues. Considering our findings and calculations shown earlier, we suggest that the receptor–ligand interaction occurs according to the mechanism depicted in Figure 5.

Chemotactic selection

On the basis of the results on chemotactic properties of SXWS peptides described earlier, we performed chemotactic selection experiments to obtain information about the dynamics of chemotaxis-related receptors involved in the signaling of SXWS peptides.

The term *chemotactic selection* covers a phenomenon that has both practical and theoretical consequences (Kőhidai, 1999). These experiments are also suitable to isolate subpopulations of cells with high chemotactic responsiveness toward the ligand in question. Following consecutive transfers of subpopulations, the chemotactic ability is tested in the offsprings (70th–100th generations) of the selected cells. In this manner, the results

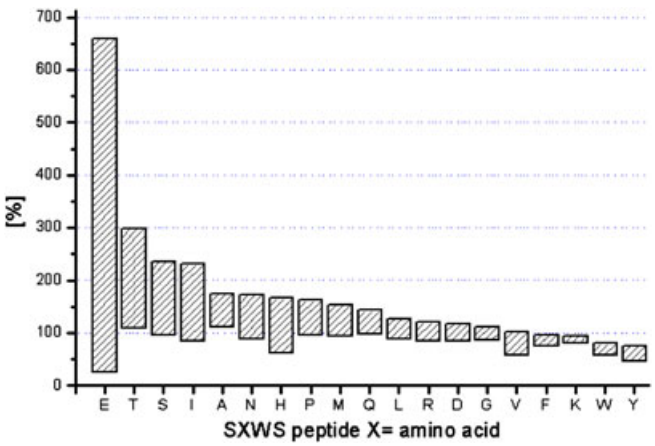

Figure 4. Chemotactic range fitting of SXWS peptides in *Tetrahymena*.

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Table 4. Comparative study on chemotaxis elicited by SXWS peptides and amino acids

Effect	Amino acid 'X'	Peptide SXWS	Amino acid 'X'
Chemoattractant	A	+	+
	S	+	+
	M	+	+
	P	+	+
	H	+	+
	T	+	+
	E	+	+
	Q	+	+
Neutral	G	0	0
	R	0	0
	D	0	0
Chemorepellent	W	−	−
	F	−	−
	Y	−	−
	V	−	−
	K	−	−
Diverse effect	N	+	0
	L	0	−
	I	+	−

Trends of chemotactic ability of amino acids in *Tetrahymena* were given according to our previous work (Kőhidai *et al*., 2002). +, chemoattractant; 0, neutral; −, chemorepellent.

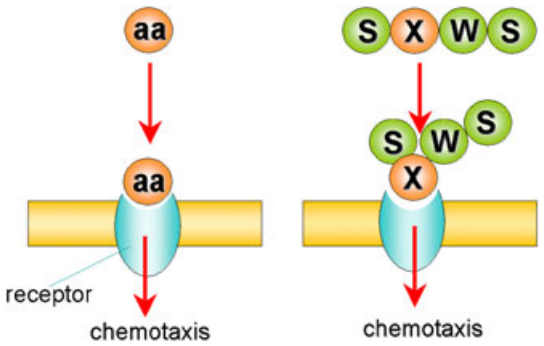

Figure 5. Proposed receptor-level background of overlapping effects amino acids and their identical SXWS peptides (based on data shown Table 3).

obtained will provide information about the expression of chemotaxis receptors in the surface membrane as constitutive, long-term receptors or as transient, short-term components, which are inducible by the presence of the ligand; however, they are not characteristic, constant constituents of the membrane (Kovács and Csaba, 1994). To characterize chemotactic selection, we have to evaluate the chemotactic responsiveness of cultures in four combinations (see Materials and methods section). Chemotactic selection coefficient (Ch_{sel}) was also introduced as a numerical index to distinguish long-term ($Ch_{sel} > 1.25$) and short-term ($Ch_{sel} < 0.75$) chemotactic responsiveness (Kőhidai *et al*., 2000).

The results (summarized in Table 5) show that SXWS peptides can exhibit long- or short-term effect. Peptides with His (SHWS), Asn (SNWS), or Thr (STWS) in position 'X' acted on receptors expressed permanently, whereas peptides containing Ile (SIWS)

Table 5. Chemotactic selection with SXWS peptides

Effect	Peptide	Chsel	Hydrophobicity of 'X' amino acid
Long term	SHWS	1.97	0.87
	SNWS	1.59	0.09
	STWS	1.39	0.07
Short term	SIWS	0.72	3.15
	SPWS	0.75	2.77
Mixed	SEWS	0.80	0.67
	SAWS	0.93	0.87

Effectiveness of responsiveness of cells was calculated according to the following formula: $Ch_{sel} = \frac{S/S \cdot C/C}{S/C \cdot C/S}$ (Kőhidai *et al*., 2000). Values of hydrophobicity were calculated according to Csaba (1985).

or Pro (SPWS) induced the formation of ad hoc receptors of the ciliate, *Tetrahymena*. It is worthwhile to mention that the two extremely chemoattractant peptides (SEWS and STWS) are different in this respect; peptide STWS has a long-term character, but SEWS proved to have mixed pool of receptors.

Given the ability of the peptides to select subpopulations, we found some diversities in hydrophobicity of amino acid 'X' (Jones, 1975). In the short-term acting SXWS ligands, this factor was high, whereas the low values are characteristic to the long-term acting tetrapeptides. This suggests that the physicochemical properties of amino acid in position 'X' could influence the process of chemotactic selection as well.

CONCLUSIONS

In summary, our investigations on chemotaxis demonstrate that substitutions of the position 'X' in SXWS–peptides result in characteristic and significant changes in the chemotactic potency. There was also good correlation between the biological effectiveness (attractant–neutral–repellent) of the peptides and the values of mass–SEA ratios of the varied amino acids of the tetrapeptides. The overlapping chemotactic effects of single amino acids and their SXWS pair molecules draw the attention to the second amino acid of these peptides as it plays a significant role in the interaction between the SXWS peptide and the chemotaxis receptor. Chemotactic selection by the investigated chemoattractant SXWS peptides indicates that the investigated peptides elicit their effects via individual, long- or short-term expressed chemotaxis receptors in the ciliate *Tetrahymena*.

MATERIALS AND METHODS

Cells and cultures

Populations of *Tetrahymena pyriformis* GL in the logarithmic phase of growth were cultured in 1% tryptone medium (Difco, Michigan USA) containing 0.1% yeast extract.

Chemicals

Trifluoroacetic acid (TFA), 1-hydroxibenztriazole (HOBt), diisopropylcarbodiimide (DIC), and 4-dimethylamino-pyridine were

obtained from Fluka. Dichloromethane, dimethylformamide, acetonitrile, MeCN, and Fmoc-L-amino acid derivatives were obtained from Reanal (Budapest, Hungary) and *p*-alkoxybenzylalcohol resin (Wang resin, 0.96 mmol/g) from Bachem (Bubendorf, Switzerland).

Synthesis of SXWS peptides

Peptides with free C-terminal carboxylic group were synthetized by manual solid phase methodology, using the conventional Fmoc/^{t}Bu strategy with DIC/HOBt coupling protocol (Stewart and Young, 1984). Activating and coupling were performed using the DIC/HOBt method (3 equivalent volumes of each). The coupling efficacy was monitored by the ninhydrin assay (Kaiser *et al*., 1970). The crude peptide were cleaved from the resin with TFA containing 2.5% EDT and 2.5% water. The mixture was filtered and precipitated with ether, centrifuged, and dissolved in water or diluted in AcOH, then freeze dried. The crude products were analyzed and purified by reverse phase high-performance liquid chromatography as described in the next section.

High-performance liquid chromatography

Analytical experiments were made on a Phenomenex Jupiter C18 (250 × 4.6 mm) column (300 Å, 5 μm) with a flow rate of 1.0 ml/min. The peptides were purified by using a semipreparative Phenomenex Jupiter C18 (250 × 10.0 mm) column (300 Å, 10 μm) with a flow rate of 4.0 ml/min. A Waters high-performance liquid chromatography system composed of No. 600 pump, No. 600 controller, and No. 490 programmable multiwavelength detector was used, with a linear gradient of 80% acetonitrile in 0.1% aqueous TFA.

Mass spectrometry

Mass spectra (electrospray ionization-ESI) were recorded on a Perkin Elmer Sciex API2000 or a Finnigan MAT 95SQ tandem mass spectrometer equipped with an ionspray source. Samples were dissolved in a mixture of MeOH and water (1:1) containing 0.05% AcOH. fast atom bombardment mass spectroscopy – FAB-MS experiments were performed with a Fisons VG ZAB-2SEQ hybrid tandem mass spectrometer of BEQQ configuration (Loughborough, UK) equipped with a liquid secondary ion mass spectrometer source (Cs^{+} ion gun used at 30 keV) and coupled to an OPUS 2000 data system. The samples dissolved in 0.05 M NH_4HCO_3 buffer containing 0.1% TFA were mixed with glycerol matrix before subjected to FAB-MS analysis.

Computer-assisted conformational analysis

Conformational analysis of the SXWS peptides was carried out by performing MCMM (Monte Carlo Multiple Minimum) searches (Chang *et al*., 1989)—as implemented in MacroModel (Mohamadi *et al*., 1990). MCMM steps involved the random variation (within the range of 0°–180°) of a randomly selected subset of all torsional angles. The perturbed structures were minimized using the TNCG algorithm. The resulting minimum energy complex structures were sorted by energy, and the unique structures within a 50-kJ/mol energy window above the global minimum were stored. In each case, 30 000 monte carlo multiple minima carried out. In calculations where the energy separation of the global minimum structure and the next conformer did not exceed 1 kJ/mol, an additional, more local, low mode conformational search (Kolossvary and Guida, 1996) was performed to map the low energy region of the potential energy surface. In drawing the conclusions, structures of all low energy conformers were considered.

Calculations were carried out using the AMBER* force field (Weiner *et al*., 1984). Solvent effects were modeled by the GB/SA algorithm (using water as solvent) (Still *et al*., 1990).

Chemotaxis assay

The chemotactic responsiveness of *Tetrahymena* cells was evaluated in a two-chamber capillary assay (Leick and Helle, 1983) as modified by Kőhidai *et al*. (1995). In this setup, tips of a multi-8-channel automatic pipette served as inner chamber to minimize the standard error of sampling. The wells of the microtitration plates were used as outer chambers. The outer chamber was filled with the cells to be tested; the inner one contained the solution of test peptide with various concentrations (10^{-12}–10^{-6} M). Compounds were dissolved in the culturing medium described earlier. In control experiments, the fresh culture medium was applied as a test substance. After 15 min of incubation, the samples of inner chambers, containing the chemotactically positive responder cells, were fixed after transferring the content of the inner chambers into phosphate-buffered saline (0.05 M phosphate buffer, pH 7.2; 0.9 M NaCl) containing 4% formaldehyde. The number of cells in samples was evaluated occulometrically in a Neubauer hemocytometer. An average of 10 replica assays for each ligand is presented in Figures 1–5.

Chemotactic selection

The chemotaxis assay described earlier was used to select chemotactically high previously identified responder subpopulations from cultures of logarithmic phase of growth. In this study, only chemotactic SXWS peptides were applied. The following analogues were used: SEWS (10^{-12} M), STWS (10^{-12} M), SSWS (10^{-8} M), SIWS (10^{-7} M), SAWS (10^{-10} M), SNWS (10^{-8} M), SHWS (10^{-7} M), SMWS (10^{-10} M), SPWS (10^{-8} M), and SQWS (10^{-6} M). Culture medium was used as negative control. After chemotaxis assay with the optimal concentrations of the substances, the positive responder cells were transferred into fresh culture medium, and these cultures were consecutively transferred every 48 h for 7 days. The cultures were tested in an identical chemotaxis assay. The theoretical groups of samples were as follows: S/S—cells selected with the SXWS derivative in the first run and assayed to the same SXWS derivative in the second run; S/C—cells selected with the SXWS derivative in the first run and assayed to the control substance in the second run; C/S—cells selected with the control substance in the first run and assayed to the SXWS derivative in the second run; and C/C—cells selected with the control substance in the first run and assayed to the control substance in the second run C/C.

Statistical analysis

Statistical evaluation of data was analyzed using ANOVA of Origin Pro 8.0.

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