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Synthesis of Oligopeptides with the Sequence SXWS and their Chemotactic Effects on a Ciliated Protozoan *Tetrahymena pyriformis*†

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Abstract: In this paper, the solid phase synthesis and chemical characterization of members of an SXWS sub-library (SAWS, SDWS and SKWS) as well as the comparison of their chemotactic properties with those of SEWS, which exhibits a prominent effect at 10^{-12} M on a ciliated protozoan, *Tetrahymena pyriformis*, are described. We found that the chemotaxis of cells induced with the SXWS peptides varied according to the nature of the amino acid residue (Ala, Asp, Lys) in position X. The chemotactic activity of SEWS was not surpassed by any of three new tetrapeptides, although SAWS was also chemoattractant. Interestingly, SDWS, with an acidic side chain at position X, could not elicit any chemotactic response. SKWS, however, showed mild but significant chemorepellent activity over a wide concentration range. Chemotactic selection studies showed that the two chemoattractant peptides (SAWS and SEWS) had an expressed ability to select high-responder offspring cell populations. Peptides with neutral (SDWS) or chemorepellent (SKWS) properties were not able to select such subpopulations from the mixed cultures of *Tetrahymena*, indicating that the chemotactic response elicited by SXWS peptides is ligand-specific. For ligand-binding experiments *N*-terminally labelled fluorescent derivatives of SXWS peptides were prepared, applying [4-[7-hydroxycoumaryl]]acetic acid (*Hca*-OH) or 4-ethoxymethylene-2-[1]-naphthyl-5(4*H*)-oxazolone (*naOx*-OEt) as markers. *Hca*-OH was introduced using an active ester technique as the last step of SPPS, or after cleavage in solution. The oxazolone *naOx*-OEt reacted with the amino group of the peptide by liberation of EtOH. The binding characteristics of fixed *Tetrahymena* cells with the *naOx*-labelled peptides showed good correlation between binding profiles and chemotactic responsiveness (SEWS > SAWS > SDWS ~ SKWS). A similar binding pattern was observed in the case of *Hca*-peptides (SEWS > SAWS > SDWS). *Hca*-SKWS, however, bound remarkably to the cell surface. The binding activity of the *Hca*-peptides was less pronounced than that of the *naOx*-peptides, indicating the importance of the fluorophores applied.

Keywords: chemoattractant properties; chemotactic peptides; chemotactic selection; fluorescent peptides; peptide synthesis; *Tetrahymena pyriformis*

Abbreviations: *Hca*-OH, [4-[7-hydroxycoumaryl]]acetic acid; *naOx*-OEt, 4-ethoxymethylene-2-[1]-naphthyl-5(4*H*)-oxazolone.

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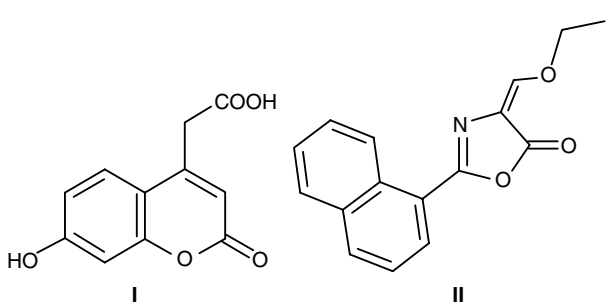
INTRODUCTION

The chemotactic response is considered to be one of the basic physiological activities of living cells. Ciliated protozoa (e.g. *Tetrahymena sp.*) have been established models for the investigation of chemotaxis and other biological phenomena for some 70 years [2, 3]. Various oligopeptides including formyltripeptide fMLF [4] possess chemoattractant activity towards this type of unicellular organism [5]. In a systematic study [6] we have investigated the chemotactic properties of 3 to 7-mer oligopeptides derived from the sequence of the extracellular part of the interleukin-6 receptor [7]. We found that depending on the length of the oligopeptides (EWS, SEWS, WSEWS, EWSEWS and GEWSEWS), all these compounds could have a significant chemoattractant effect on *Tetrahymena pyriformis*. The SEWS tetramer was the most potent ligand, inducing a 660% ± 21% increase in chemotaxis of the cells at a concentration of 10^{-12} M. Its shorter (EWS) and longer (WSEWS) derivatives had chemorepellent effects, while further elongated versions of EWS (EWSEWS and GEWSEWS) displayed moderate chemoattractant ability. Amidation of the *C*-terminal amino acid residue significantly modified the chemotactic character of these oligopeptides.

In order to obtain a better understanding of the structural background of the highly potent tetrapeptide SEWS, compounds in which Glu is replaced by an Asp (SDWS), Lys (SKWS) or Ala (SAWS) residue were prepared, and the chemotactic properties of these peptides were compared with those of SEWS. Two additional sets of peptides with *N*-terminal fluorescent moiety were also produced and the binding of these compounds was investigated. Considering the potential influence of the *N*-terminal modification on biological properties, two different reagents ([4-[7-hydroxycoumaryl]]acetic acid (**I**) and 4-ethoxymethylene-2-[1]-naphthyl-5(4*H*)-oxazolone (**II**)) were used for the introduction of fluorophores.



I **II**

In this paper we describe the synthesis, purification and chemical characterization of new SXWS peptides where X = D, K, A and fluorescent derivatives of SEWS, SDWS, SKWS and SAWS. The chemotactic activity and binding properties of these compounds are also reported.

MATERIALS AND METHODS

Materials

p-Alkoxybenzylalkohol resin (Wang-resin, 0.96 mmol/g) was obtained from Bachem, Bubendorf, Switzerland (Cat. No. D 1250; lot 504143). Fmoc-L-amino acids (Fmoc-Ala, Fmoc-Asp(O^{t}Bu), Fmoc-Glu(O^{t}Bu), Fmoc-Lys(Boc), Fmoc-(^{t}Bu)Ser and Fmoc-Trp) were purchased from Fluka AG, Buchs, Switzerland. Reagents (DIC, DMAP, EDIA, HOBt, piperidine, TFA) and solvents (DMF and MeOH) were Fluka products of analytical grade. [4-[7-Hydroxycoumaryl]]acetic acid (*Hca*-OH) was prepared according to Baker *et al.* [8], 4-ethoxymethylene-2-[1]-naphthyl-5(4*H*)-oxazolone (*naOx*-OEt) was synthesized by the method of Kóczán *et al.* [9].

Synthesis

Synthesis of peptides. The coupling of Fmoc-(^{t}Bu)Ser to the Wang resin was performed with DIC using DMAP as the acylating catalyst [10]. Wang resin (1 g, 0.96 mmol) was swollen in 10 ml DMF then Fmoc-(^{t}Bu)Ser (1.15 g, 3 mmol), DIC (0.47 ml, 3 mmol) and DMAP (37 mg, 0.3 mmol) were added. The mixture was stirred for 3 h, washed with DMF, EtOH and DMF. The whole coupling procedure was then repeated.

In a typical synthetic procedure, 50–100 mg of Fmoc-(^{t}Bu)Ser-Wang resin was treated with 3–5 ml of reagent solution or washing solvent. To start a coupling cycle, the resin was swollen with DMF (3 × 2 min). Deprotection was achieved by reacting the resin three times with a mixture of piperidine and DMF (1 : 1, v/v; 1, 9 and 1 min), followed by washing with DMF (3 × 1 min), MeOH (2 × 1 min) and DMF (4 × 1 min). For coupling, a 200% molar excess of Fmoc-amino acid and HOBt [11] and DIC [12] were dissolved in 2.0–2.5 ml DMF. The solution was allowed to stand for 5 min, added to the resin and the slurry was shaken for 90 min. The mixture was then washed with DMF (4 × 2 min) and MeOH (2 × 2 min). The conversion was checked with the ninhydrin test [13].

Cleavage. After a final deprotection step followed by washing and drying, the peptidyl resin (50–100 mg) was stirred with 3–5 ml of a mixture of TFA (95%), ethanedithiol (2.5%) and water (2.5%) for 3 h. The mixture was filtered, and the filtrate was precipitated with ether, centrifuged in a sealed tube, the precipitate was washed three times with ether, dissolved in water or dilute AcOH, freeze dried and purified by HPLC.

Labelling of peptides. The fluorophore moiety was introduced on to the SXWS sequence either by reacting the N^{α}-deprotected resin-bound peptide or the free peptide in solution with the appropriate reagent.

N-terminal labelling of resin-bound peptide. With *Hca*-OH was carried out as a normal coupling cycle using the fluorescent carboxylic acid instead of an Fmoc-amino acid [14]. The coupling was repeated to achieve complete acylation. For the reaction of the *N*-terminus with *naOx*-OEt [9] the fluorescent reagent was dissolved in DMF (2–3 ml) and the resin was shaken for 30 min. It was then washed with DMF and MeOH.

Coupling of Hca-OH in solution. Peptide (10 μmol), purified by HPLC, was dissolved in 1 ml DMF, then 20 μmol of *Hca*-OH, DIC and HOBt (1 : 1 : 1 mol/mol/mol) were each added. The mixture was allowed to stand for 1 h at room temperature, diluted with water, evaporated *in vacuo*; the oily residue was diluted and evaporated again, and purified by HPLC.

Coupling of naOx-OEt in solution. HPLC purified synthetic peptide (10 μmol) and *naOx*-OEt (11 μmol) were dissolved in 2 ml DMF, and 20 μl EDIA was added. The solution was kept in the dark for 1 h. Processing and purification were performed as described above for *Hca*-peptides.

RP-HPLC. 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 semi-preparative Phenomenex Jupiter C18 (250 × 10.0 mm) column (300 Å, 10 μm; flow rate: 4.0 ml/min). A Waters HPLC system composed of a No. 600 pump, a No. 600 controller and a No. 490 programmable multiwave length detector was used, with a linear gradient of 80% acetonitrile in 0.1% aqueous TFA.

Mass spectra. Were recorded on a Perkin Elmer Sciex API2000 tandem mass spectrometer equipped with an ionspray source. Samples were dissolved in a mixture of MeOH and water (1 : 1) containing 0.05% AcOH.

Cells and Culturing

Tetrahymena pyriformis GL cells were cultured in 0.1% yeast extract containing 1% Bacto tryptone (Difco, Michigan, USA) medium at 28 °C. The cells were used in the logarithmic phase of growth. The density of samples was 10^4 cell/ml.

Chemotaxis Assay and Chemotactic Selection

Chemotaxis assay. The chemotactic responsiveness of *Tetrahymena* cells was evaluated in a two-chamber capillary assay [15] as modified by us [16]. In this setup, the tips of a multi-8-channel automatic pipette served as the inner chamber to minimize the standard error of sampling. The wells of the microtitration plates were used as the outer chambers. The outer chamber was filled with the cells to be tested, the inner one contained the solution of test substance (SAWS, SDWS, SEWS, SKWS; Glu, Asp) at various concentrations (10^{-12}–10^{-6} M). The compounds were dissolved in the culture medium described above. In control experiments, the fresh culture medium was applied as a test substance. After 15 min incubation the samples of the inner chambers, containing the chemotactically positive responder cells, were fixed after transferring the content of the inner chambers into PBS (0.05 M phosphate buffer, pH 7.2; 0.9 M NaCl) containing 4% formaldehyde. The number of cells in the samples was evaluated by counting in a Neubauer haemocytometer. The average of ten replica assays of each ligand is presented in the Figures. The statistical analysis was performed by ANOVA of Origin 4.0.

Chemotactic selection and re-exposure of subpopulations. The chemotaxis assay outlined above was used to select positive responder cell subpopulations. This test had two steps: the first was for the chemotactic selection with the optimal chemotactic concentrations of each peptide in the dose-response study. In parallel there was a control group, in which fresh culture medium was used as the 'chemoattractant'. At the end of selection, the samples were transferred into fresh medium and the cells were cultured with consecutive transfers for 168 h. In the second step the chemotactic activity of the samples selected with SXWS peptides and control substances was measured in four

combinations: C/C, subpopulations selected with the culture medium — responsiveness tested with culture medium; C/S, subpopulations selected with the culture medium–responsiveness tested with the actual SXWS peptide; S/C, subpopulations selected with the SXWS peptide–responsiveness tested with culture medium; S/S, subpopulations selected with the SXWS peptide–responsiveness tested with the identical SXWS peptide. Based on four possible combinations of the chemotactic selection described above (control vs control, control vs peptide, peptide vs control and peptide vs peptide) and re-exposures, the chemotactic selection quotient $(Ch_{sel} = (C/C \times S/S)/(S/C \times C/S))$ was calculated. This value was used as a measure of the intensity of chemoattractant ($Ch_{sel} > 1$) or chemorepellent ($Ch_{sel} < 1$) activity.

Binding Experiments

For binding experiments *Hca*- or *naOx*-labelled fluorescent SXWS peptides were used. The *Tetrahymena* cells were fixed with PBS containing 4% formaldehyde for 5 min at room temperature, then washed three times with PBS. The *Hca*- or *naOx*-labelled SXWS derivatives were added at a concentration of 10^{-6} M to the samples (cell density 5×10^4 cell/ml), and incubated for 60 min at room temperature. After that, the samples were washed three times with PBS to remove the excess of labelled peptides and the fluorescent activity was measured with a 96-well ELISA plate reader (Labsystems Multiscan MS, using λ_{ex} 340 nm, λ_{em} 460 nm). The average of eight replica assays of each ligand is represented in the figures. The statistical analysis was performed by ANOVA of Origin 4.0.

RESULTS AND DISCUSSION

Synthesis of SXWS Peptides and Their Fluorescent Derivatives

In order to analyse the effect of the amino acid side chain in position X of the SXWS sequence on the chemoattractant character, four peptides were prepared containing the non-charged alanine (SAWS), acidic amino acids (aspartic acid, SDWS; glutamic acid, SEWS) and a basic amino acid (lysine, SKWS). This study was initiated by the discovery of the highly chemoattractant effect of SEWS [6]. In this work the chemotactic properties of SAWS, SDWS and SKWS were investigated. In addition, our analysis was extended by the study of chemotactic selection and cell-binding properties of all four compounds. For the latter experiments, two sets of fluorescent derivatives of peptides were prepared.

For solid phase synthesis the Fmoc/^{t}butyl technique [17, 18] was used, with an *in situ* active ester (HOBt/DIC) coupling. The synthetic protocol is described in the experimental section. The use of Fmoc-Trp without any protecting group on the indole ring of its side chain gave satisfactory results. The HPLC retention times and MS data for the SXWS peptides, and chromatogram of purified SAWS are shown in Table 1 and in Figure 1, respectively.

The fluorescent labelling of the *N*-termini of the tetrapeptides was performed either as the last step of SPPS on resin-bound peptide by reaction with *Hca*-OH [14] or *naOx*-OEt [9] or by treatment with the appropriate reagents with tetrapeptides in solution. Analytical data for the fluorescent peptide derivatives are provided in Table 1, while HPLC of purified *Hca*-SEWS and *naOx*-SEWS are given in Figure 2.

Table 1 Chemical Characterization of SXWS Peptides and Their Fluorescent Derivatives

Peptide and peptide derivative	Relative Molecular Mass[a]		R_t[b]
	Calculated	MH^+ observed	
SAWS	449.2	449.8	20.2[c]
SDWS	493.2	493.8	20.0[c]
SEWS	507.2	507.9	21.0[c]
SKWS	506.2	506.9	19.3[c]
Hca-SAWS	651.2	652.0	25.2[c]
Hca-SDWS	695.2	696.1	25.0[c]
Hca-SEWS	709.2	710.2	24.6[c]
Hca-SKWS	708.2	709.0	24.0[c]
naOx-SAWS	670.2	670.9	37.0[d]
naOx-SDWS	714.2	714.7	35.5[d]
naOx-SEWS	728.2	728.5	34.8[d]
naOx-SKWS	727.3	728.2	41.1[d]
naOx-WSK(*naOx*)WS	1134.4	1135.4	57.5[e]

[a] Relative molar mass was determined by ES mass spectrometry.
[b] HPLC was performed on a Phenomenex Jupiter C18 (250 × 4.6 mm) column (300 Å, 5 μm) with a flow rate of 1.0 ml/min at, detection at $\lambda = 220$, 280 and 360 nm. Eluents: A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile/water (80 : 20, v/v).
[c] B 5 to 55% in 25 min.
[d] B 20% to 70% in 45 min.
[e] B 20% to 90% in 60 min.

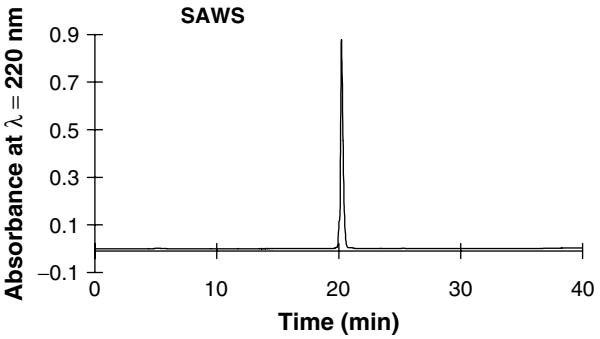


Figure 1 HPLC chromatogram of purified peptide SAWS. Phenomenex Jupiter C18 (250 × 4.6 mm) column (300 Å, 5 μm); flow rate, 1.0 ml/min. Eluent B 5% to 55% in 25 min. See Table 1 for further details.

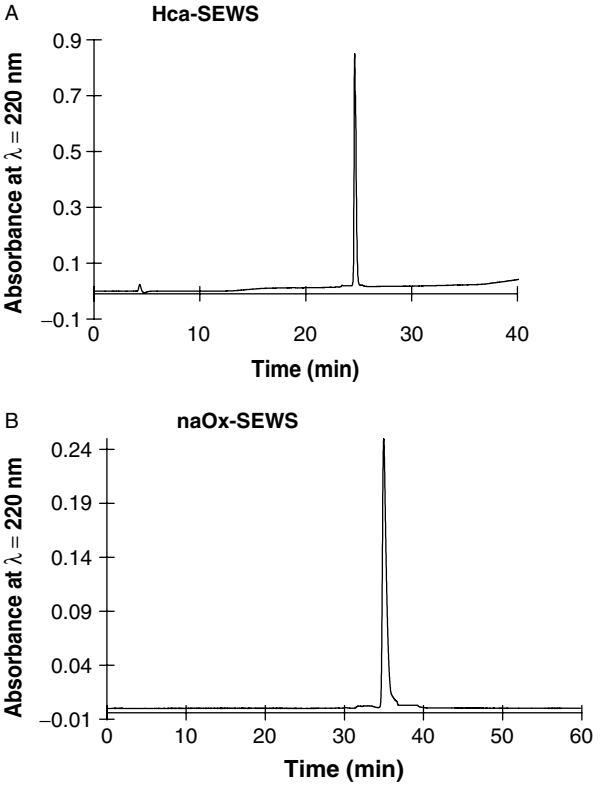


Figure 2 HPLC chromatograms of purified *Hca*-SEWS and *naOx*-SEWS derivatives. Eluent B 5% to 55% in 25 min and 20% to 70% in 45 min, respectively. See Table 1 for further details.

The comparison of labelling strategies with *naOx*-derivatives clearly showed that the coupling performed in solution using HPLC purified oligopeptides resulted in a crude product with one predominant peak. In contrast, the coupling of *naOx*-OEt to partially protected peptides bound to the resin led to a more complex mixture of products after cleavage. Differences in the composition of crude *naOx*-SEWS preparations can be appreciated in Figure 3. A similar observation was made with *Hca*-labelled peptide pairs (data not shown).

The chromatographic properties of free and *naOx*- or *Hca*-conjugated peptides were also studied by comparison of the retention times of these three compounds. Figure 4 shows the HPLC chromatogram of co-injected samples of SAWS, *Hca*-SAWS and *naOx*-SAWS obtained under identical conditions. Significant differences were found between the three compounds. The retention time for free SAWS was the shortest (21 min), while *naOx*-SAWS possessed the longest R_t value. A similar tendency was observed with all oligopeptide series tested (Table 1), and the following order of R_t values was established: R_t(*naOx*-peptide) > R_t(*Hca*-peptide) > R_t (free peptide). This relative order of R_t values could reflect the hydrophobic-hydrophilic character of the compounds. In fact we found that *naOx*-derivatives have the lowest water solubility of the three variants (data not shown).

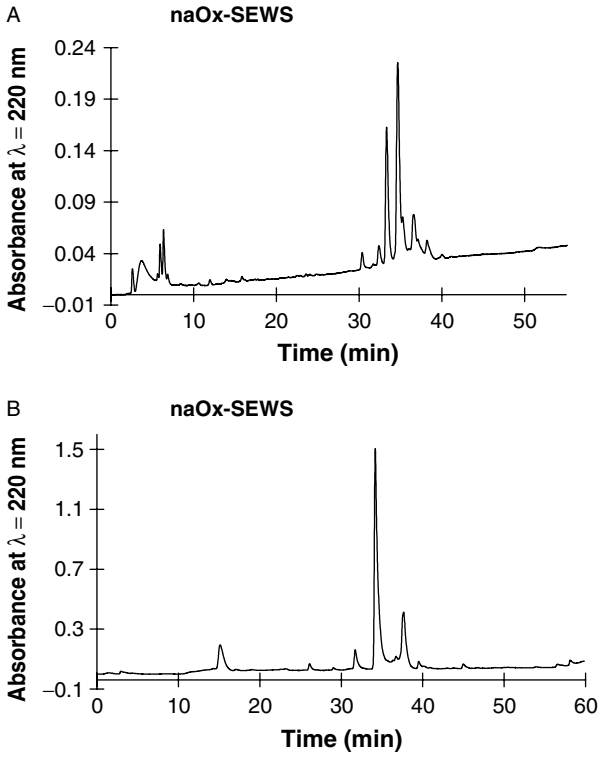


Figure 3 HPLC chromatogram of crude *naOx*-SEWS prepared by A: solid phase labelling, B: labelling in solution. Eluent B 20% to 70% in 45 min. See Table 1 for further details.

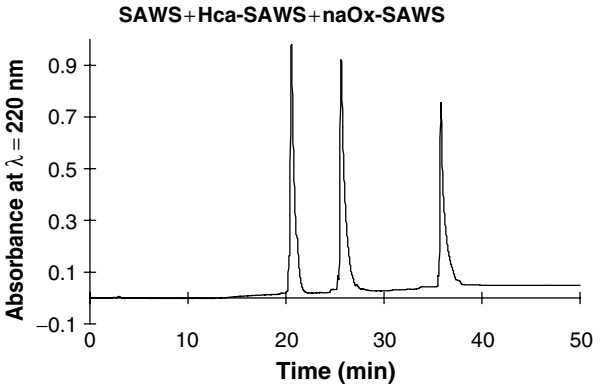


Figure 4 HPLC chromatogram of co-injected samples of peptides SEWS, *Hca*-SEWS and *naOx*-SEWS. Eluent B 5% to 55% in 25 min, 55% to 70% in 20 min. See Table 1 for further details.

It is interesting to note that the introduction of two *naOx*-groups into an oligopeptide (e.g. WSKWS) having both α- and ε-amino groups resulted in a highly hydrophobic, but still water soluble, derivative (Table 1).

The spectroscopic properties of labelled peptides were characterized by measurement of their absorption and emission spectra [19]. We found that the absorption maxima of all peptide derivatives, regardless of the presence of *naOx*- or *Hca*-groups, were at $\lambda = 340$ nm in 0.14 M phosphate buffer, pH = 7.4. Under the same conditions the emission maximum for *Hca*-peptides was at $\lambda = 466$ nm, and that of *naOx*-derivatives at $\lambda = 445$–480 nm. These values are in the range of published spectral characteristics of *Hca*-[14] or *naOx*-peptides [9] of different length and composition.

Chemotaxis Induced by SXWS Peptides

The concentration dependent, vectorial migration of cells — chemotaxis — is a fundamental and ligand specific physiological response of uni- and multicellular systems. Several types of ligand (e.g. amino acids, lectins, chemokines) have been described. Their biological functions vary from the nutritive to the immune responses and paracrine activities, etc [20, 21]. Some SXWS peptides (X = Glu or Asp) also represent a special sequence which is a constitutive component of the extracellular domains of cytokine receptors (IL2, IL4, IL6) [22]. In this way their accessibility on the cellular membrane can potentially elicit a special, surface linked form of chemotaxis, haptotaxis. In addition, soluble cytokine receptors with exposed SXWS sequences can also be considered as inducers of chemotaxis. In a previous study, we have reported on the pronounced chemoattractant effect of the tetrapeptide SEWS on *Tetrahymena pyriformis* cells. In order to gain a better understanding of this phenomenon and to establish a structure–function correlation we have extended our studies to the analysis of the chemotactic properties of further SXWS tetrapeptides containing amino acid residues with acidic (Asp), basic (Lys) or aliphatic (Ala) side chains at position X. The chemoattractant/chemorepellent properties of these compounds were studied in a chemotaxis assay used previously [6], as a function of peptide concentration. The results are summarized in Figure 5. The four tested tetrapeptides elicited different, but characteristic, chemotactic responses.

SAWS was chemoattractant in a narrow concentration range (10^{-10}–10^{-9} M): the maximal effect was 174% ± 20% at 10^{-10} M. Neither chemoattractant, nor chemorepellent, activity could be observed at concentrations of 10^{-12}–10^{-11} M or 10^{-8}–10^{-6} M (Figure 5a).

The chemotactic properties of SXWS peptides containing Glu (SEWS) or Asp (SDWS) were found to be very much dependent on the nature of the amino acid X. The peptide SEWS induced an intense chemoattractant response in a low and narrow concentration range peaking with 660% ± 21% at $c = 10^{-12}$ M. At a higher peptide concentration ($c = 10^{-8}$–10^{-6} M), this compound exhibited significant chemorepellent character (25%–70%) [6] (Figure 5b). In sharp contrast, peptide SDWS, also containing a side chain with COOH at position X did not elicit any significant chemotactic response of the *Tetrahymena* cells: its character was neutral in all the concentrations tested, although a slight increase in chemotactic index was observed at 10^{-9} M (Figure 5c).

Peptide SKWS, however, showed small but statistically significant (80%–83%) chemorepellent activity over a wide concentration range (10^{-11}–10^{-8} M) (Figure 5d). The interesting difference in chemotactic properties of SEWS and SDWS peptides prompted us to compare the chemotactic responses elicited by the respective free amino acids. Similar studies with various amino acids have also been reported [20].

The results of concentration dependent chemotactic experiments show that glutamic acid induced the movement of *Tetrahymena* cells (130%–207%) in the 10^{-12}–10^{-8} M range (Figure 6A), while aspartic acid, differing only in one CH_2 unit, did not elicit chemotactic responses in the range (10^{-12}–10^{-6} M) tested

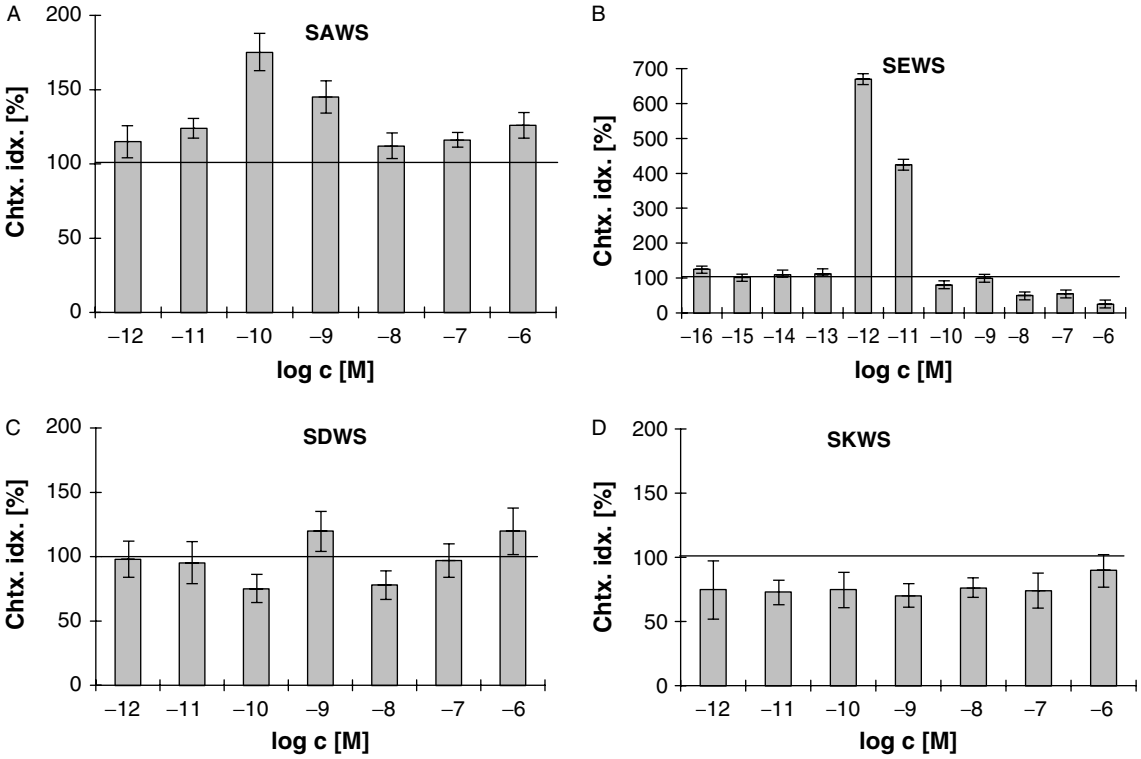


Figure 5 Dose-response correlation of chemosensory responses induced with SXWS peptides in *Tetrahymena pyriformis*. (SD of control = ±8.25; x – $p < 0.05$; y – $p < 0.01$; z – $p < 0.001$).

(Figure 6B). It should be mentioned that this difference in chemotactic properties between Glu and Asp does not necessarily explain the much more pronounced change in the activity of SEWS when E is replaced by D. However, these results might indicate the functional significance of the amino acids in position X of SXWS peptides in relation to the chemoattractant character of the peptide.

Chemotactic selection of Tetrahymena cell subpopulation. Since chemotactic responses are considered to be receptor mediated mechanisms, there is a possibility of separating subpopulations of cells possessing identical receptors [23]. These responder cells could operate signalling mechanisms on a higher functional level than other, non-responder, cells. It has been demonstrated that in the case of several ligands (e.g. insulin, histamine) chemotactic properties are transmitted also to offspring generations (long-term character) [24]. However, there are also some chemoattractant ligands (i.e. endothelins [25]) which act only on a short-term basis: in long-term experiments with such ligands no transfer of chemotactic properties is detected from generation to generation.

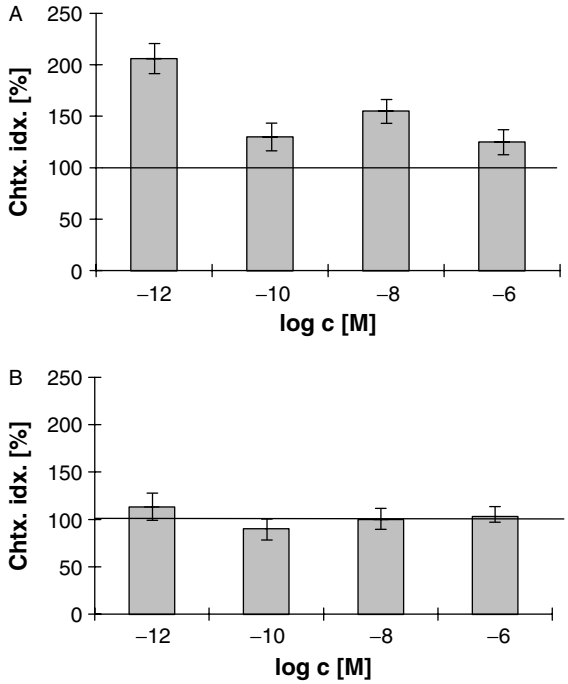


Figure 6 Dose-response correlation of chemosensory responses induced with (A) Glu, (B) Asp in *Tetrahymena pyriformis*. (y – $p < 0.01$; z – $p < 0.001$).

The chemotactic properties of SXWS peptides were further studied on selected cell subpopulations. The chemotactic selection quotient (Chsel) as a measure of chemoattractant (Chsel > 1) or chemorepellent (Chsel < 1) activity provides a sensitive criterion for distinguishing short- and long-term selections [24]. The two chemoattractant peptides possessing aliphatic (SAWS) or acidic (SEWS) side chains, had the ability to select high-responder subpopulations, which points to these peptides having the capacity to induce the signalling mechanisms of unicellular protozoa which are required for positive chemotactic responses (Figure 7A,B). This long-term feature of the chemotaxis elicited by SAWS or SEWS is well characterized by the high (>1) values of chemotactic selection quotients (Ch_{sel}). Ch_{sel} for SAWS is 1.34, while Ch_{sel} for SEWS is 1.15. In the case of the other two SXWS peptides, the selection experiment had also good correlations with the concentration-course studies performed on non-selected cell populations. Peptides SDWS and SKWS with neutral or chemorepellent properties were not able to select positive responder subpopulations from the mixed cultures of *Tetrahymena* (Figure 7C,D). This kind of responsiveness was demonstrated by the reduced values of Ch_{sel}, which was 0.67 for SDWS and 0.66 for SKWS.

The results of the chemotactic selection studies showed that the elicited chemotactic responses of the four SXWS peptides are ligand-specific, in agreement with the results of concentration course study performed with a non-selected cell population.

Binding of Fluorescent SXWS Peptides to Fixed *Tetrahymena* Cells

Chemotaxis is a membrane receptor linked process in prokaryotes and eukaryotes alike. The integrity

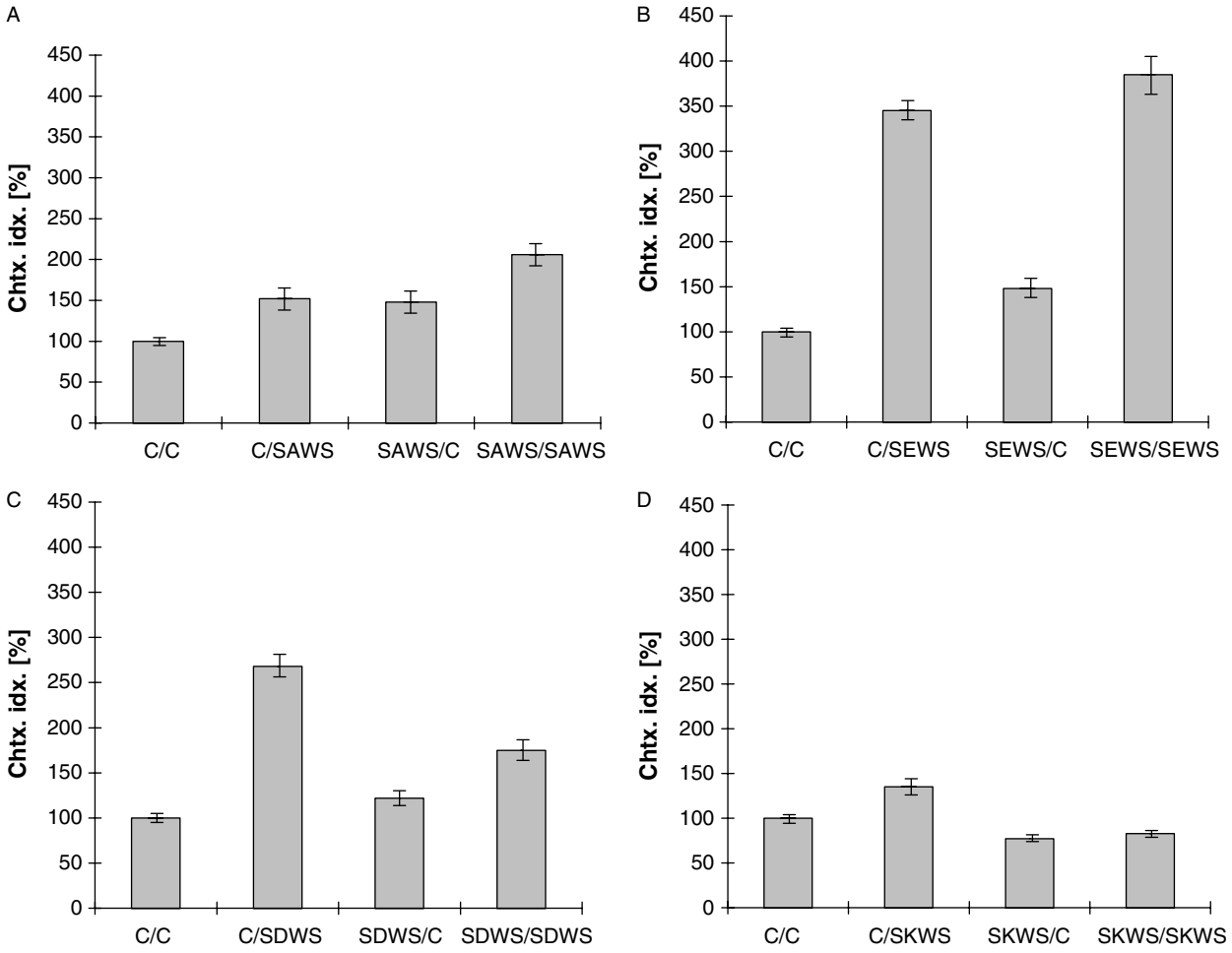


Figure 7 Chemotactic responsiveness of *Tetrahymena* cells selected with SXWS peptides (A) SAWS, (B) SEWS, (C) SDWS, (D) SKWS. (x – $p < 0.05$; y – $p < 0.01$; z – $p < 0.001$).

of these structures in the surface membrane (e.g. aspartate [26] or dipeptide receptors of *E. coli* and fMLF or chemokine receptors of PMN cells [27, 28]) is responsible for the specificity of chemotactic responses. It should also be noted that the afferent and efferent pathways induced by the chemoattractant or chemorepellent ligands could be also different on the two levels of phylogeny. Considering this receptor-mediated chemotactic signalling, the detection of ligand binding properties of target cells are important complementary measures of functional studies of chemotaxis.

For binding experiments, we have synthesized two new groups of labelled *Hca*- and *naOx*-SXWS peptides and used them as fluorescent derivatives of the potential peptide ligands. The binding of labelled peptides to fixed *Tetrahymena* cells was studied by measuring the fluorescent activity of cell samples. Based on values expressed as the percentage binding compared with the control (100%), we found that both *Hca*- and *naOx*-modified peptides showed significant differences. The binding of *Hca*-peptides was less pronounced, while *naOx*-SXWS peptides showed significantly higher activity. This might be due to the composition and structure of the two chromophores.

The binding characteristics of fixed *Tetrahymena* cells with the *naOx*-labelled peptides showed a good correlation between binding profiles and the chemotactic responsiveness (SEWS > SAWS > SDWS ~ SKWS) (Figure 8A). A similar order was shown for the *Hca*-labelled derivatives of three peptides (SEWS > SAWS > SDWS). However, in this case *Hca*-SKWS also exhibited pronounced binding (Figure 8B). The overlapping results of binding assays gained with *naOx*- and *Hca*-labelled SXWS peptides and the detected functional activity of *Tetrahymena* suggest that the studied chemotactic response is based on a receptor-linked signalling pathway. On the other hand, the good correlation of physiological responses and binding characteristics underlines the suitability of the applied fluorophores — especially the *naOx*-group — to follow ligand–binding site interactions, even in the case of relatively small ligands.

CONCLUSIONS

Our data suggest that in the group of synthetic SXWS peptides tested the nature of X influences markedly the chemoattractant/chemorepellent effect on *Tetrahymena pyriformis* cells. The concordant

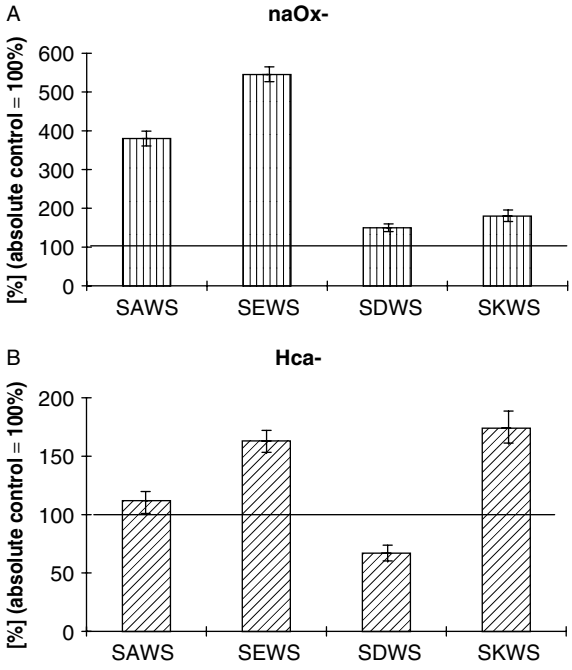


Figure 8 Binding of (A) *naOx*- and (B) *Hca*- labelled SXWS peptides to fixed *Tetrahymena* cells.

results of chemotactic responsiveness elicited by the peptides (SAWS, SEWS, SDWS) and their binding characteristics indicate that chemotactic activity is a surface-membrane associated event, and also point to the SXWS sequences of cytokine receptors having functional importance of phylogenetic significance.

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