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Synthesis of SXWS peptides and their chemotactic activity on a ciliated protozoan *Tetrahymena pyriformis*

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Introduction

Ciliated protozoa (e.g., *Tetrahymena sp.*) have been established models for investigation of chemotaxis and other biological phenomena for some 70 years [1]. Previously we have shown that tetrapeptide SEWS (this motif occurs in vertebrate cytokin receptor sequences) exhibits pronounced chemoattractant effect on *Tetrahymena pyriformis*. It has also been demonstrated that the chemotactic character of some other members of SXWS peptide sub-library (X=Ala, Lys, Glu or Asp) depends on the identity of amino acid residue X [2,3]. Here the synthesis and the chemotactic response of 19 SXWS peptides are presented and discussed.

Results and Discussion

Synthesis. We have synthesized peptides corresponding to SXWS sequence, where X = one of 19 proteinogen amino acids (Cys was omitted). Four peptides (SAWS, SDWS, SEWS and SKWS [3]) were synthesized by Fmoc/^{t}Bu method [4] starting from Wang resin. The other 15 tetrapeptides were prepared by Boc/Bzl technique [5] using Boc-Ser(^{t}Bu)-resin ester. The acylating protected amino acids were activated by DIC/HOBt in both cases. Cleavage was carried out with TFA/EDT and TFMSA/TFA/indole, respectively. Peptides were purified and characterized by RP-HPLC, the correct structures were confirmed by ESI-MS (Table 1).

Table 1. Chemical Characteristics of SXWS peptides [6]

Peptide	Relative Molecular Mass		R_t (min)[a]
	Calculated	Observed	
SAWS	449.2	449.8[b]	20.1
SDWS	493.2	493.8[b]	20.2
SEWS	507.2	507.9[b]	21.0
SFWS	525.2	526.3[c]	26.4
SGWS	435.2	436.3[c]	20.7
SHWS	515.2	516.1[c]	23.8
SIWS	491.2	492.1[c]	26.1
SKWS	506.2	506.9[b]	19.3
SLWS	491.2	492.4[c]	25.4
SMWS	509.2	510.2[c]	26.5
SNWS	492.2	493.3[c]	20.0
SPWS	475.2	476.2[c]	26.4
SQWS	506.2	507.4[c]	20.2
SRWS	534.2	535.3[c]	20.9
SSWS	465.2	466.3[c]	20.9
STWS	479.2	480.4[c]	20.0
SVWS	477.2	478.3[c]	24.6
SWWS	564.2	565.3[c]	29.0
SYWS	541.2	542.2[c]	24.7

Chemotactic response was evaluated in a two-chamber capillary chemotaxis assay [7] in a wide concentration range (10^{-16}-10^{-6} M). The chemotactic activity of peptides was compared at concentration where the maximum response was observed. Results of these studies are expressed as chemotaxis index values and presented in Figure 1. According to these data, SEWS had the strongest chemoattractant activity (660%). Nine peptides (STWS, SSWS, SIWS, SAWS, SNWS, SHWS, SMWS, SPWS and SQWS) were moderately chemoattractant (chemotaxis index: between 300 and 150%). There was no chemotactic effect in the case of peptides SRWS, SDWS, SGWS, SLWS, SVWS and SFWS. Three peptides (SKWS, SWWS and SYWS) exhibited some repellent character (chemotaxis index: 90 and 75%).

The chemotactic properties of SXWS peptides containing acidic amino acid at position X (SEWS and SDWS) were found to be very much dependent on the side chain length of the amino acid X. While peptide SEWS induced an intense chemoattractant response, in sharp contrast, peptide SDWS, also with COOH in the side chain did not elicit any significant chemotactic response of *Tetrahymena* cells.
It is also interesting to note that peptides with hydroxy amino acid at position X (Thr or Ser) were chemoattractant, while amino acids with aromatic side chain (X = Phe, Trp, Tyr) proved to be inactive or sligthly chemorepellent.

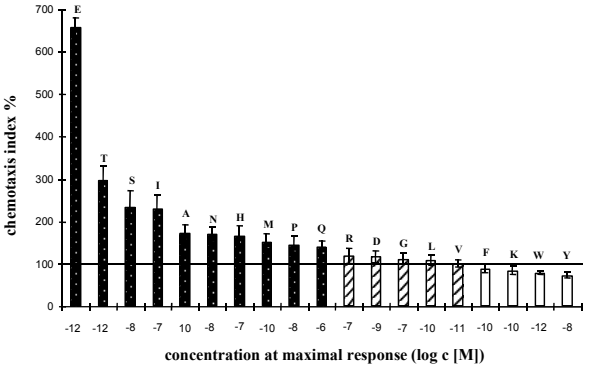

Figure 1. Maximal chemotaxis indexes of SXWS peptides

It seems difficult to draw conclusions from these data without published results on the receptor structure involved. The nature of the side chain of amino acid residue in position X, however, must have possessed a functional importance in the induction of chemosensory response in *Tetrahymena*.

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6. [a] HPLC was performed on a Phenomenex Jupiter C18 (250 x 4.6 mm) column (300 Å, 5 µm) with a flow rate of 1.0 ml/min at RT, detection at λ = 220 and 280 nm. Eluents: A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile/water (80:20, v/v). B 5 to 55% in 25 min; Relative molar mass was determined by [b] ES or [c] FAB MS.
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