Peptides 2000. Jean Martinez and Jean-Alain Fehrentz (Eds.)
EDK, Paris, France © 2001

New Chemotactic Peptides

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Introduction

A large number of synthetic oligopeptides with different compositions and sequences have been reported to have chemotactic properties for different living cells [1]. Ciliated protozoa (e.g., *Tetrahymena sp.*) serve as established models for cell receptor research [2], especially for the analysis of chemotaxis [3]. We have studied the chemotactic properties of a new group of peptides with an SXWS sequence using the capillary method [4]. We found that, depending on the identity of X, some peptides exhibited pronounced chemoattractant characteristics.

Results and Discussion

Synthesis. We synthesized peptides corresponding to the SXWS sequence, where X = A, D, E, K. Wang resin (0.96 mmol/g) and Fmoc-technique were used, applying 1.5-fold molar excess of activated Fmoc-amino acids (HOBt/DIC). Two types of fluorescent derivatives of these peptides were also prepared for confocal laser scanning microscopy. For N-terminal fluorescent labelling we used 4-[7-hydroxycoumaryl]acetic acid (Hca) [5] or 4-ethoxymethylene-2-[1]-naphthyl-5(4H)-oxazolone (naOx) [6]. Hca was coupled to the N-terminus by a conventional HOBt/DIC procedure while naOx reacted with the N-terminal amino acid simply in DMF for 30 min. Peptides were purified and characterized by RP-HPLC, the correct structures were confirmed by ESI-MS.

Chemotactic response was evaluated in a two-chamber capillary chemotaxis assay [4]. In the set-up, an 8-channel-micropipette served as the inner chamber of the system filled with the test substance, while a microtitration plate filled with the model cells served as the outer chamber. At the end of the incubation time (20 min) positive responder cells were collected from the capillaries and measured with a Neubauer haemocytometer.

Chemotactic behaviour of model cells induced with the SXWS peptides varied with the nature of amino acid in position X. The presence of E in this position resulted in a highly

potent chemoattractant peptide (Ch_{max} 660%±21 at 10^{-12} M). The peptide possessing A as X was a less potent compound (Ch_{max} 174%±18.6 at 10^{-10} M). The peptide containing K had a negative, chemorepellent character in a wide concentration range (10^{-12}-10^{-6} M), while peptides with D had neither an attractant nor a repellent effect.

Chemotactic selection. Subpopulations of positive responder cells with chemotaxis were selected and used to follow long lasting, chemotaxis-receptor linked mechanisms in offspring generations [7]. The enhanced chemotactic responsiveness of subpopulations selected with SEWS (392.6% ±23.3) and SAWS (206.2% ±14.9) suggests that these peptides also have the ability to act *via* durable, receptor mediated mechanisms in the model protozoa.

Binding of fluorescent peptides. *Tetrahymena* cells fixed with 4% formaldehyde were incubated with naOx as well as Hca labelled peptides for 1, 5 and 15 min. Binding of labelled peptides was measured in a Labsystems ELISA plate reader at 340 and 460 nm. Our results show that there is a correlation between binding profiles and chemotactic responsiveness (E > A > D > K for naOx-SXWS and K > E > A > D for Hca-SXWS).

Phagocytosis. At lower and higher ranks of phylogeny, phagocytosis is one of the most essential target reactions of chemotaxis. Both time-course binding studies (1, 5, 15 min) with Hca and naOx labelled peptides and evaluation of phagocytic activity of selected subpopulations with Chinese ink test particles (the number of particles/cell being determined by flow-cytometer in 10,000 cells/sample) show that *Tetrahymena* cells prefer SAWS and SEWS over SDWS and SKWS. These results, which overlap with those of chemotactic activity (A- and E- vs. D- and K-containing peptides), raise the question whether there are common signaling mechanisms in chemotaxis and phagocytosis.

Conclusion. Our data show that in the tested group of synthetic SXWS peptides, the physicochemical character of the amino acid in the X position is significant in relation to its chemoattractant character or other associated surface membrane, chemotaxis linked cell-physiological activities.

Acknowledgements

This work was supported by grants from the Hungarian Ministry of Education (No. FKFP 0101/97 and 0229/99) and from the Hungarian Research Fund (OTKA, No.W 15598 and T 032533). Travel fund offered by EPS to one of us (E.I.) is highly acknowledged.

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