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

Synthesis of New Carrier Molecules Based on Repeated Tuftsin Sequences

Gábor Mezö[1], Adrián Kalászi[1], Judit Reményi[1], Nikolett Mihala[1], Zsuzsa Majer[2], Ágnes Hilbert[3], Orsolya Láng[4], László Kóhidai[4], Krisztina Barna[5] and Dezsö Gaál[5]

[1]Group of Peptide Chemistry, Hungarian Academy of Sciences, Eötvös L. University, Budapest 112, P.O. Box 32, H-1518 Hungary
[2]Department of Organic Chemistry, Eötvös University, Budapest
[3]Department of Immunology, Eötvös University, Göd
[4]Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest
[5]National Institute of Oncology, Budapest

Introduction

Epitope peptides identified from proteins are frequently attached to carriers and used extensively as immunogens in experimental animals to elicit antibody responses for the development of mono- or polyclonal antibodies with desired specificity or to provoke protective immune responses against microbial or parasitic diseases.

As carriers natural or synthetic molecules (e.g. branched polypeptides [1], lysine tree in MAP [2], SOCs [3], polytuftsin [4]) were applied. Tuftsin (Thr-Lys-Pro-Arg) based carriers may be an attractive choice as carriers, because of their tuftsin-like biological activity that increases the immune response against conjugated epitope peptides [4].

Our aim was the preparation of biodegradable and immunostimulant synthetic carriers that are available for attachment of epitopes and give well characterized conjugates.

Results and Discussion

To achieve this goal, oligo-tuftsin sequences based on canine tuftsin sequence (Thr-Lys-Pro-Lys) with different lengths were synthesized (H-[Thr-Lys-Pro-Lys-Gly]$_n$-NH_2; n=4 (T20), 6 (T30), 8 (T40)). All oligopeptides were prepared by step wise solid phase technique using the Boc/Bzl strategy. The yield of purified products was over 60% in all cases.

CD spectra of oligomers suggested that they have unordered structures in water that were not influenced by the change in pH or salt concentration of the solution. However in TFE slightly ordered conformation was observed independently of the length of the oligomers.

The toxicity of oligomers was studied on mouse spleen cells. Our results showed no toxic effect of oligo-tuftsin derivatives (till 50mg/mL concentration) even after 4 h incubation.

In Balb/c mice all three tuftsin oligomers induced a very low level of specific IgM after the first and second immunization. We could detect IgG response only after the second

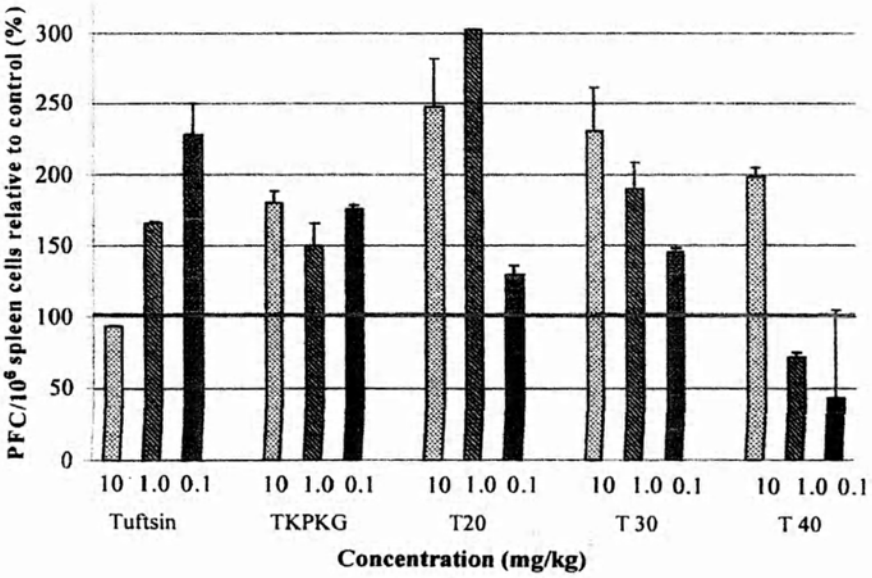

Figure 1. *Dose-dependent effect of tuftsin and oligo-tuftsin derivatives on the humoral immune response to sheep red blood cell antigens in BDF1 mice*

immunization with T30 and T40 antigens. In CBA mice no significant antibody response was observed.

All tuftsin, oligo-tuftsin derivatives and their basis pentapeptide (TKPKG) showed high immunostimulatory activity in PFC assay. Monomers have intensive stimulation at 0.1 mg/kg, while oligo-tuftsin derivatives showed high activity at 1.0 or 10 mg/kg concentration (Figure 1.).

All oligo-tuftsin derivatives have a chemotactic effect on *Tetrahymena pyriformis* cells. However, the elicited chemotactic responses are highly dependent upon the counter-ions of the applied ligands.

Acknowledgements

These studies are supported by Hungarian Research Fund OTKA (T 32425) and Foundation for Hungarian Peptide and Protein Research.

References

1. Hudecz, F., Szekerke, M.: *Coll. Czech. Chem. Commun.*, 50 (1985) 103
2. Tam, J.P., Lu, Y.A.: *Proc. Natl. Acad. Sci. U.S.A.*, 86 (1989) 9084
3. Tsikaris, V., Sakarellos, C., Cung, M.T., Marraud, M., Sakarellos-Daitsiotis, M.: *Biopolymers*, 38 (1996) 291
4. Trudelle, Y., Brack, A., Delmas, A., Pedoussaut, S., Rivaille, P.: *Int. J. Pept. Protein Res.*, 30, (1987) 54