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Structure-Activity Relationship of Tuftsin, a Phagocytosis-Stimulating Peptide, and Its Analogs

The structure-activity relationship of tuftsin, a phagocytosis-stimulating peptide, has been investigated using its analogs newly synthesized. Two basic amino acids on tuftsin could be replaced with each other without activity loss.

Keywords——tuftsin; peptide; phagocytosis-stimulating-activity; granulocyte; *Staphylococcus aureus*

Fidalgo and Najjar showed that a specific cell bound leucophilic γ-globulin fraction, leucokinin, is essential for maximal stimulation of the phagocytic activity of the blood neutrophilic leucocyte.[1] Soon thereafter it was found that the whole stimulatory effect of leucokinin can be ascribed to a single peptide fragment liberated by a specific enzyme, leucokininase.[2] This natural phagocytosis-stimulating peptide has been referred to as tuftsin,[3] and its primary structure has been determined to be H–Thr–Lys–Pro–Arg–OH.[2a]

In order to investigate the structure–activity relationship of tuftsin, in particular, the contribution of two basic amino acids in this peptide to the activity, the following analogs were synthesized by a conventional liquid phase method as was tuftsin[4] and then examined for their phagocytosis-stimulating activity: H–Thr–Arg–Pro–Arg–OH, H–Thr–Lys–Pro–Lys–OH, H–Thr–Arg–Pro–Lys–OH, and H–Arg–Pro–Lys–Thr–OH.

The phagocytosis-stimulating activity of the tuftsin analogs was assayed essentially according to the description by Najjar and Constantopoulos.[5] Granulocytes were prepared from guinea pig exudates. Two intraperitoneal injection of 30 ml of 0.1% glycogen in sterile saline were given to the guinea pig, the first 24 hr and the second 4 hr before harvesting the peritoneal exudates. The reaction mixture consisted of (a) 100 μl of the guinea pig granulocyte suspension containing 2×10^6 cells (b) 100 μl of opsonized *Staphylococcus aureus* 209P suspension containing 4×10^6 bacteria and (c) 800 μl of sample solution containing various amounts of tuftsin or its analogs. Hanks' solution was used as buffer and diluent. The mixture, in siliconized glass stoppered tubes, and control blanks were incubated at 37° for 30 min in a vertical circular rotor at 8 cycles per minute. Smears were then stained and cells containing one or more bacterial particles were recorded per 100 cells as phagocytic activity (%). 300 to 900 cells were usually observed. All samples were run in duplicate or triplicate. Phagocytic index represents the phagocytic activity obtained with tuftsin or its analogs minus the phagocytic activity of the control blanks.

Table I shows the phagocytosis-stimulating activity of the tuftsin analogs in comparison with tuftsin. A stimulatory effect on phagocytosis was definitely observed with three of the analogs, *i.e.*, H–Thr–Arg–Pro–Arg–OH, H–Thr–Lys–Pro–Lys–OH, and H–Thr–Arg–Pro–Lys–OH. Up to a concentration of 6 nM a dose response and a rapid increase in the stimulation

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3) K. Nishioka, A. Constantopoulos, P.S. Satoh, W.M. Mitchell, and V.A. Najjar, *Biochem. Biophys. Acta*, **310**, 217.
4) S. Nozaki, K. Hisatsune, and I. Muramatsu, *Bull. Chem. Soc. Japan*, **50**, 422; S. Nozaki, K. Kobayashi, K. Hisatsune, and I. Muramatsu, "Proceeding of the 14th Symposium of Peptide Chemistry," ed. by T. Nakajima, Protein Research Foundation, Osaka, 1977, pp. 131—134.
5) V.A. Najjar and A. Constantopoulos, *J. Reticuloendothel. Soc.*, **12**, 197 (1972).

Table I. Phagocytosis-stimulating Activity of Tuftsin and Its Analogs

Compound	Concentration nM/ml	Phagocytic index[a)]
H-Thr-Lys-Pro-Arg-OH (tuftsin)	2	10.3
	6	13.5
	30	17.6
H-Thr-Arg-Pro-Arg-OH	2	11.2
	6	14.8
	30	17.3
H-Thr-Lys-Pro-Lys-OH	2	11.9
	6	16.5
	30	19.4
H-Thr-Lys-Pro-Arg-OH (tuftsin)	2	4.2
	6	15.9
	30	16.1
H-Thr-Arg-Pro-Lys-OH	2	11.3
	6	16.9
	30	20.0
H-Arg-Pro-Lys-Thr-OH	2	—
	6	0.0
	30	0.7

a) Phagocytic index represents the phagocytosis-stimulating activity obtained with tuftsin or its analogs minus the phagocytic activity of the control blanks.

rate were clearly observed, both of which were comparable with those obtained by tuftsin. In contrast, practically no stimulatory effect was observed with H-Arg-Pro-Lys-Thr-OH which possesses the inverse sequence of tuftsin.

Thus it is preliminarily indicated that either or both of the two basic amino acids, lysine and arginine, situated at positions 2 and 4 on tuftsin, can be replaced by each other without affecting the original activity.

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