

## Antibacterial Properties of Tuftsin and Its Analogs

DANUTA BLOK-PERKOWSKA,<sup>1</sup> FELIKS MUZALEWSKI,<sup>1</sup> AND DANUTA KONOPIŃSKA<sup>2\*</sup>

*Institute of Chemistry, Gdańsk University, Gdańsk,<sup>1</sup> and Institute of Chemistry, Wrocław University, Wrocław, 50383,<sup>2</sup> Poland*

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The antibacterial properties of tuftsin and its 11 analogs on 20 bacterial strains were investigated. Tuftsin showed a definite antibacterial effect at a minimal effective concentration of 62.5 µg/ml. All analogs were either less effective or lacked any activity. The bacteria used included some highly pathogenic organisms.

The tetrapeptide tuftsin (Thr-Lys-Pro-Arg) was discovered and isolated by Najjar et al. (11, 15, 16) in 1973 on the basis of its ability to stimulate the phagocytic activity of polymorphonuclear granulocytes and macrophages. The broad spectrum of the biological activities of tuftsin has been presented in several communications (5, 10, 12-14, 17). Besides phagocytosis stimulation, other activities include stimulation of (i) motility of granulocytes, (ii) humoral antibody formation, and (iii) tumoricidal activity of phagocytic cells as well as bacterial killing properties. It also exhibits analgesic properties.

In this communication, we reported an investigation of the antibacterial activities of tuftsin and its analogs that were modified in position 3 of the peptide chain: Thr-Lys-His-Arg, Thr-Lys-Gly-Arg, Thr-Lys-Ser-Arg, Thr-Lys-Phe-Arg, Thr-Lys-Asp-Arg, Thr-Lys-Ile-Arg, Thr-Lys-Sar-Arg, Thr-Lys-Tyr-Arg, Thr-Lys-Thr-Arg, Thr-Lys-Arg-Arg, and Thr-Lys-Gln-Arg (Table 1).

We have shown earlier (7, 8) that most of these tetrapeptides stimulated phagocytosis to a high degree. In particular [Thr<sup>3</sup>]tuftsin (7) and [Arg<sup>3</sup>]tuftsin (8) displayed activities comparable to that of tuftsin (7, 8). Martinez et al. (10) have reported that macrophages incubated in the presence of tuftsin exhibit bactericidal activity on several bacteria, including *Listeria monocytogenes*. It is to be noted that tuftsin and its analogs used here possess strongly basic characters. In this connection, it is known that basic peptides such as di- and tripeptide derivatives of L-lysine (2, 4), L-ornithine (1), and the pentapeptide Lys-Lys-Thr-Lys-D-Leu and its *N*-decanoyl derivative (9) show very strong activities against various bacterial species. On this basis, our objective was to ascertain whether tuftsin and its analogs could display any antibacterial activity directly in bacteria cultures.

### MATERIALS AND METHODS

All tetrapeptides were synthesized as previously reported (6-8). The activities of the compounds were investigated against the following bacterial strains: *Micrococcus glutamicus* IAW 26, *Staphylococcus aureus* IW 35 strain 209P, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* subsp. *mycoides* SWRO 10, *Sarcina flava* IAW 37, *Streptococcus faecalis* subsp. *liquefaciens* PCM 1947, *Gaffkya tetragena* OCM 509, *Diplococcus pneumoniae* PCM 1823, *Flavobacterium flavescens* ATCC 8315, *Citrobacter freundii* PCM 1412, *Pasteurella pseudotuberculosis*, *Alcaligenes faecalis* PCM 551, *Salmonella paratyphi* B KOS 3, *Shigella flexnerii* type 1a PCM 112, *Klebsiella pneumoniae* PCM 1, *Proteus vulgaris* NCTC 4175, *Escherichia coli* IAW 21, *Pseudomo-*

*nas aeruginosa* WSRO 109, *Bordetella bronchoseptica* NCTC 8344, and *Serratia marcescens* PCM 501. The culture medium was a ready-to-use Difco broth. Preparation of the culture medium was carried out as described before (2, 4).

The antibacterial activities of tuftsin and its 11 analogs were assessed by a standard dilution method in an appropriate test medium. The initial concentration of standard solution in water was 10 mg/ml. The concentrations of working solutions prepared in the test media were 1,000, 500, 250, 125, 62.5, 31.25, 15.6, and 3.9 µg/ml. To the geometrical series of the solutions, 1 ml of each standardized suspension of microorganisms was added. Samples with bacteria were incubated for 24 h at 37°C. After incubation, the minimal inhibitory concentrations (MICs) were determined in terms of concentration of the compound at which the sample became turbid. For each compound and microorganism, three series of determinations were run (Table 1).

### RESULTS AND DISCUSSION

It is clear from the data shown in Table 1 that tuftsin possesses the strongest antibacterial activity of all the analogs tested. Thus, tuftsin inhibited the growth of eight gram-positive bacteria. More specifically, *Micrococcus glutamicus* IAW 26 and *Staphylococcus aureus* IW 35 strain 209 P were inhibited by a concentration of 62.5 µg/ml (0.12 µmol/ml), whereas the remaining ones were inhibited by a concentration of 125 µg/ml (0.24 to 0.96 µmol/ml). Of the remaining 11 tetrapeptides, [His<sup>3</sup>]tuftsin, [Gly<sup>3</sup>]tuftsin, and [Ser<sup>3</sup>]tuftsin showed antibacterial activities mainly against gram-positive bacteria at concentrations of 125 to 500 µg/ml and against some gram-negative organisms. The rest of the peptides, with the exception of [Phe<sup>3</sup>]tuftsin and [Asp<sup>3</sup>]tuftsin, which inhibited the growth of some bacteria at a concentration of 500 µg/ml, were inactive.

Tuftsin, which among this series of analogs possesses the strongest antibacterial activity, is also the most potent stimulator of phagocytosis. [Arg<sup>3</sup>]tuftsin and [Thr<sup>3</sup>]tuftsin, which stimulate phagocytosis to a degree comparable to that of tuftsin, do not possess antibacterial activity. Furthermore, [Gly<sup>3</sup>]tuftsin, [His<sup>3</sup>]tuftsin, and [Ser<sup>3</sup>]tuftsin, which possess selective activity against gram-positive bacteria, do not stimulate phagocytosis.

From the data presented above, it is difficult to draw a meaningful correlation between the antibacterial activity of tuftsin and its phagocytosis-stimulating effect. However, the concentrations of tuftsin which are active in vitro in the phagocytic process are comparable to the MICs for the bacterial strains. The maximal stimulation of phagocytosis by tuftsin in vitro was obtained at concentrations above 0.2 µmol/ml (14), and we found the MIC of tuftsin to be 62.5 to

\* Corresponding author.

TABLE 1. Antibacterial activities of tuftsin and its analogs expressed as MICs.

Bacterial strain	MIC ( $\mu\text{g/ml}$ ) of following compound <sup>a</sup> :											
	Tuftsin (Thr-Lys-Pro-Arg (tuftsin))	Thr-Lys- His-Arg	Thr-Lys- Gly-Arg	Thr-Lys- Ser-Arg	Thr-Lys- Phe-Arg	Thr-Lys- Asp-Arg	Thr-Lys- Ile-Arg	Thr-Lys- Sar-Arg	Thr-Lys- Tyr-Arg	Thr-Lys- Thr-Arg	Thr-Lys- Arg-Arg	Thr-Lys- Gln-Arg
<b>Gram-positive</b>												
<i>Micrococcus glutamicus</i> IAW 26	62.5	250	125		500							
<i>Staphylococcus aureus</i> IAW 35 strain 209	62.5				500							
<i>Bacillus subtilis</i> A TCC 6633	125	500	125									
<i>Bacillus cereus</i> subsp. <i>mycoides</i> WSR0 10	125	500										
<i>Sarcina flava</i> IAW 37	125	250	125		125							
<i>Streptococcus faecalis</i> subsp. <i>liquefaciens</i> PCM 1947	125	250			500							
<i>Gaffnya tetragena</i> PCM 509	125	250										
<i>Diplococcus pneumoniae</i> PCM 1823	125	250			500							
<b>Gram-negative</b>												
<i>Flavobacterium flavescens</i> ATCC 9315	125	250	125		125							
<i>Citrobacter freundii</i> PCM 1412												
<i>Pasteurella pseudotuberculosis</i>	125				125							
<i>Alcaligenes faecalis</i> PCM 551												
<i>Salmonella paratyphi</i> B KOS 3												
<i>Shigella flexneri</i> type 1a PCM 112			500									1,000
<i>Klebsiella pneumoniae</i> PCM 1												
<i>Proteus vulgaris</i> NCTC 4175					250							
<i>Escherichia coli</i> IAW 21												
<i>Pseudomonas aeruginosa</i> SRO 109												
<i>Bordetella bronchiseptica</i> NCTC 8344												
<i>Serratia marcescens</i> PCM 501					500							

<sup>a</sup> >1,000  $\mu\text{g/ml}$  = Inactive; blank space indicates no activity.

125 µg/ml (0.12 to 0.24 µmol/ml). However, these concentrations exceed the average levels of tuftsin in the blood (255 ng/ml) of normal humans (3, 14).

It is difficult to draw meaningful conclusions about the mechanism of action of tuftsin against susceptible organisms. The degree of basic charge of the compound, as postulated above, did not correlate with its antibacterial properties. It is of interest to point out that analogs possessing a more basic charge than tuftsin are either inactive (Thr-Lys-Arg-Arg) or less active (Thr-Lys-His-Arg) than tuftsin, suggesting that, at least in this series, the basic charge is not a determinant of activity.

In this paper, we have presented an investigation of the antibacterial activities of tuftsin and its analogs that were modified in the 3 position of the peptide chain by amino acid residues other than L-proline. Only tuftsin showed substantial antibacterial activity, whereas all analogs were either less effective or lacked any activity. These results substantiate the fact that the presence of L-proline in the 3 position of the tuftsin chain is necessary for antibacterial activity. It is of interest to point out that L-proline in the 3 position of the peptide chain of tuftsin plays an important role in the stabilization of the biologically active conformation for this peptide (7, 17).

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