OCTAPEPTIDE DERIVATIVES OF TEICOPLANIN ANTIBIOTICS

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A series of octapeptide derivatives of teicoplanin-A2 component 2 (CTA/2), its aglycone (TD), and the L-lysyl derivatives of an amide of CTA/2 and TD, were prepared by condensation of the terminal amino group with *N*-hydroxysuccinimidyl esters of *tert*-butyloxycarbonyl (BOC) L- and D-amino acids, followed by acidic (TFA) removal of the BOC protecting function.

The antimicrobial properties of these compounds were compared with those of the corresponding unmodified antibiotics and their N^{15}-acetyl derivatives. The most active derivatives were the octapeptides with *N*-terminal glycine or lysine whose *in vitro* activity was comparable to that of the parent teicoplanins. The glycinyl and lysyl derivatives of CTA/2 showed better activity than CTA/2 against clinical isolates of *Staphylococcus epidermidis* and *S. haemolyticus* for which teicoplanin MICs were relatively high. No significant difference in their antibacterial activity was observed between octapeptides containing L- or D-lysine.

Teicoplanin[1] (Fig. 1) is a glycopeptide of the dalbaheptide group[2] of antibiotics. These agents inhibit the biosynthesis of bacterial cell walls by forming a complex with the terminal D-alanyl-D-alanine of peptidoglycan precursors.[3] The right-hand side of the molecule is directly involved in binding to the antibiotic's target peptide. In particular, in teicoplanin and its derivatives, the terminal amino group plays an important role as a base in the first step of complex formation, since *N*-acylation[4] and deamination[5] at C-15 lower the binding strength by approximately one order of magnitude, though this did not always result in a decrease of *in vitro* antimicrobial activity.† It had also been observed that in general the activity of teicoplanin antibiotics can be improved by increasing the lipophilicity of positively charged derivatives.[6]

To explore the effect of increasing lipophilicity and also, in some cases, of modifying the isoelectric point of a negatively charged glycopeptide, such as teicoplanin, by aminoacylating the terminal amino group,†† octapeptide derivatives of teicoplanin-A2 component 2 (CTA/2) and its aglycone (TD) were prepared.

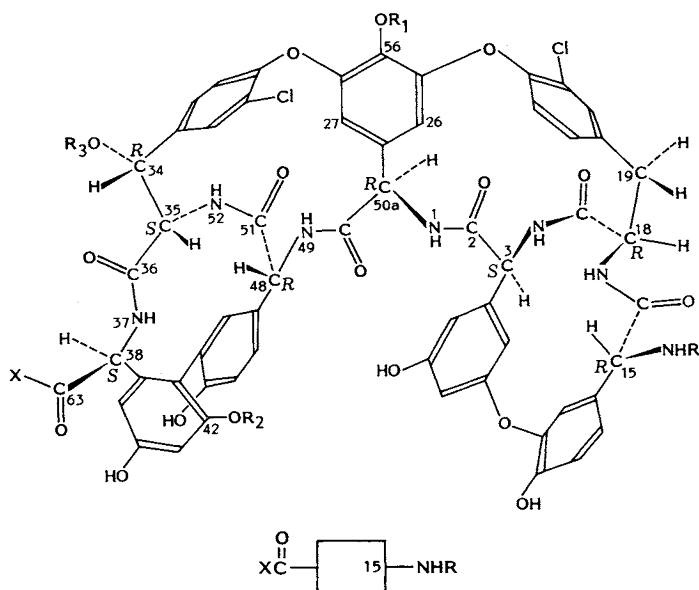
The present study also included the synthesis and evaluation of the L- and D-lysyl-octapeptides of CTA/2 and TD, in order to determine the effect of the chirality of the *N*-terminal amino acid on an hypothetical interaction between the carboxylate anion of the target peptide and the alpha or epsilon amino groups of lysine. The N^{15}-L-lysyl-octapeptides of the 63-carboxamides[7] of CTA/2 and TD with 3,3-dimethylamino-1-propylamine were also prepared to study the effect of this *N*-aminoacylation on the activity of teicoplanin derivatives with net positive charge.

The antimicrobial and physico-chemical properties of the teicoplanin octapeptides were compared

† For example, the N^{15}-acetyl derivatives (N^{15}-Ac-CTA/2 and N^{15}-Ac-TD, Fig. 1) of teicoplanin-A2 component 2 and its aglycone, which are also described in this paper the first time, were almost as active as the unmodified antibiotics (see Tables 4 and 5).

†† Recently, it was reported [Kobrin, *et al.*, J. Antibiotics 42: 1441 ~ 1442, 1989] that the aglycone of ristocetin, which is structurally related to deglucoteicoplanin but is positively charged, did not lose antimicrobial activity after acylation of the *N*-terminus with L-amino acids.

Fig. 1. Structures of teicoplanin A2-2 (CTA/2) and deglucoteicoplanin (TD): X=OH, R=H; of CTA/2- (CTA/2-A) and TD- (TD-A) amide: X=$NH(CH_2)_3N(CH_3)_2$, R=H; and of their N^{15}-acetyl (Ac) and octapeptide (OP) derivatives (R in Tables 1~3).



Contracted formula used in Schemes 1, 2.

CTA/2 R_1=*N*-isodecanoyl-β-D-glucosaminyl,
R_2=α-D-mannosyl, R_3=*N*-acetyl-β-D-glucosaminyl
TD $R_1=R_2=R_3=H$

Table 1. Octapeptide (OP) and N^{15}-acetyl derivatives of CTA/2 (Fig. 1).

Compound (*N*-terminal AA)	R	Yield (%)	HPLC[a] t_R (minutes)	E.W.[b] (found)	MW	Formula
CTA/2-OP-1 (Gly)	CO-CH(NH_2)-H, i.e. CO-CH-NH_2 / H	78	7.7	950	1,936.8	$C_{90}H_{100}N_{10}Cl_2O_{34}$
CTA/2-OP-2 (L-Lys)	CO-CH-NH_2 / $(CH_2)_4$-NH_2	65	8.5	645	2,007.9	$C_{94}H_{109}N_{11}Cl_2O_{34}$
CTA/2-OP-3 (D-Lys)	CO-CH-NH_2 / $(CH_2)_4$-NH_2	67	8.5	651	2,007.9	$C_{94}H_{109}N_{11}Cl_2O_{34}$
CTA/2-OP-4 (L-Gln)	CO-CH-NH_2 / $(CH_2)_2$-$CONH_2$	83	6.8	993	2,007.9	$C_{93}H_{105}N_{11}Cl_2O_{35}$
N^{15}-Ac-CTA/2	CO-CH_3	91	7.6	1,930	1,921.8	$C_{90}H_{99}N_9Cl_2O_{34}$

[a] See Experimental section.
[b] E.W.: Equivalent weight obtained from acid-base titration.

with those of the corresponding unmodified antibiotics and their N^{15}-acetyl derivatives.

Chemistry

Teicoplanin octapeptide (OP) derivatives (Tables 1~3) were prepared by reacting CTA/2, TD, or their 63-(3,3-dimethylaminopropyl)amides (CTA/2-A, TD-A)[7)] with *N*-hydroxysuccinimidyl esters of *N*-BOC (*N*α,*N*ε-di-BOC in the case of L- and D-lysine) amino acids in DMF at room temperature (Scheme 1). The reactions were completed within 2~3 hours using equimolecular amounts of the reacting compounds.

Table 2. Octapeptide and N^{15}-acetyl derivatives of TD (Fig. 1).

Compound (*N*-terminal AA)	R	Yield (%)	HPLC[a] t_R (minutes)	E.W.[b] (found)	MW	Formula
TD-OP-1 (Gly)	CO-CH(H)-NH_2	83	6.2	625	1,256.0	$C_{60}H_{48}N_8Cl_2O_{19}$
TD-OP-2 (L-Lys)	CO-CH($(CH_2)_4$-NH_2)-NH_2	71	6.9	443	1,327.1	$C_{64}H_{57}N_9Cl_2O_{19}$
TD-OP-3 (D-Lys)	CO-CH($(CH_2)_4$-NH_2)-NH_2	63	6.9	451	1,327.1	$C_{64}H_{57}N_9Cl_2O_{19}$
TD-OP-4 (L-Gln)	CO-CH($(CH_2)_2$-$CONH_2$)-NH_2	73	5.5	677	1,327.1	$C_{63}H_{53}N_9Cl_2O_{20}$
N^{15}-Ac-TD	CO-CH_3	85	6.1	1,250	1,241.0	$C_{60}H_{47}N_7Cl_2O_{19}$

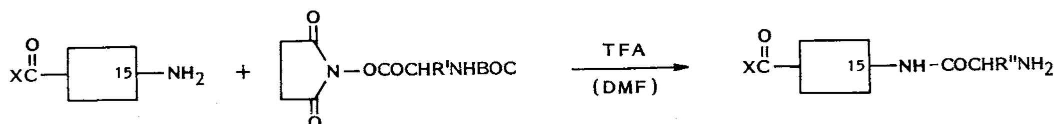
[a] See Experimental section.
[b] E.W.: Equivalent weight obtained from acid-base titration.

Table 3. L-Lys-Octapeptide and N^{15}-acetyl derivatives of CTA/2-A and TD-A (Fig. 1).

Compound (*N*-terminal AA)	R	Yield (%)	HPLC[a] t_R (minutes)	E.W.[b] (found)	MW	Formula
CTA/2-A-OP (L-Lys)	CO-CH($(CH_2)_4$-NH_2)-NH_2	45	15.0	699	2,092.0	$C_{99}H_{121}N_{13}Cl_2O_{33}$
TD-A-OP (L-Lys)	CO-CH($(CH_2)_4$-NH_2)-NH_2	39	13.8	475	1,411.3	$C_{69}H_{69}N_{11}Cl_2O_{18}$
N^{15}-Ac-CTA/2-A	CO-CH_3	77	12.8	1,981	2,005.9	$C_{95}H_{111}N_{11}Cl_2O_{33}$
N^{15}-Ac-TD-A	CO-CH_3	95	10.9	1,350	1,325.2	$C_{65}H_{59}N_9Cl_2O_{18}$

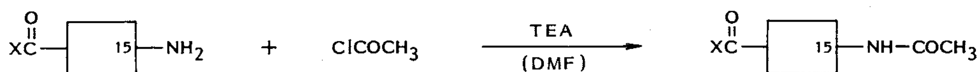
[a] See Experimental section.
[b] E.W.: Equivalent weight obtained from acid-base titration.

Scheme 1.



X, see Fig. 1.
CTA/2- and TD-OP-1,4: R′=R″ (in Tables 1 and 2: R=CO-CHR″-NH_2).
CTA/2- and TD-OP-2,3, CTA/2-A- and TD-A-OP: R′=$(CH_2)_4$NHBOC, R″=$(CH_2)_4NH_2$.

Scheme 2.



The BOC protective groups were removed by acidolysis of the resulting *N*-BOC (or *N*α,*N*ε-di-BOC) octapeptides with dry TFA at room temperature under controlled conditions. The removal of the BOC groups from CTA/2 derivatives was a critical step since reaction times longer than two minutes led to the loss of the *N*-acylglucosamine at C-56.[8)]

The N^{15}-acetyl (Ac) derivatives of CTA/2, TD, CTA/2-A, and TD-A were prepared (Scheme 2) by

reaction with acetyl chloride in DMF at room temperature in the presence of triethylamine (TEA).

The ^{1}H NMR spectra of teicoplanin octapeptides and acetyl derivatives, obtained at 500 MHz, show the signals of the protons of the new terminal amino acid or acetyl residue and the typical pattern of the teicoplanin structure.[1)] The acylation of the terminal amino group causes a downfield shift of the signal due to the 15-CH††† from 1.0 to 1.5 ppm, or from 0.1 to 0.3 ppm, with respect of the chemical shift of the 15-CH-NH_2, or 15-CH-NH_3^+, respectively, in unmodified CTA/2 and TD.†††† The α-CH (in Lys- or Gln-octapeptides), and CH_2 (in Gly-octapeptides) protons belonging to the new terminal amino acid resonate at δ 3.3 ppm and δ 3.6 ppm, respectively, while the CH_3-CO in the N^{15}-acetyl derivatives resonates at δ 1.9 ppm.

Acid-base titrations confirm the presence of one additional basic ionizable function in lysyl-octapeptides and the absence of basic groups in the acetyl derivatives of CTA/2 and TD. One basic function, *i.e.*, the dimethylamino group of the amide chain, is titrated in the acetyl derivatives of CTA/2-A and TD-A.

Results and Discussion

Tables 4~6 compare the *in vitro* antimicrobial activities of the octapeptides of CTA/2, TD, CTA/2-A, and TD-A with those of the corresponding unmodified antibiotics and their N^{15}-acetyl derivatives. Most of the octapeptides had activity similar to that of CTA/2 against Streptococci and *Enterococcus faecalis*. Against Staphylococci, the octapeptide and N^{15}-acetyl derivatives of TD, CTA/2-A and TD-A had activities similar to those of the parent compounds; they were more active than CTA/2. The lysyl-octapeptides (CTA/2-OP-2 and -3) of CTA/2 were more active than CTA/2 against some strains of coagulase-negative Staphylococci (*S. epidermidis* and *S. haemolyticus*); the glycinyl-derivative (CTA/2-OP-1) was significantly more active only against the *S. haemolyticus* isolate, which was the least sensitive to CTA/2 (MIC 32 μg/ml). The only significant difference in activity between the L- (CTA/2-OP-2) and D- (CTA/2-OP-3) lysyl derivatives of CTA/2 was for *S. epidermidis*. Thus it is unlikely that these isomers differ in their affinity for the target peptide. The glutaminyl-octapeptide (CTA/2-OP-4) of CTA/2 was less active than CTA/2 against all strains of Staphylococci tested.

Table 4. *In vitro* antimicrobial activity of CTA/2 and its N^{15}-acyl-derivatives.

Organism	MIC (μg/ml)					
	CTA/2	CTA/2-OP-1	CTA/2-OP-2	CTA/2-OP-3	CTA/2-OP-4	*N*-Ac-CTA/2
Staphylococcus aureus L 165	0.25	0.25	0.5	0.5	1	0.5
S. aureus L 561	8	8	8	8	32	8
S. epidermidis ATCC 12228	1	0.5	0.25	0.25	4	2
S. epidermidis L 533	8	4	1	4	32	8
S. haemolyticus L 602	32	4	4	8	64	8
Streptococcus pyogenes L 49	0.13	0.06	0.06	0.06	0.13	0.13
S. pneumoniae L 44	0.13	0.13	0.13	0.13	0.25	0.25
Enterococcus faecalis ATCC 7080	0.13	0.25	0.25	0.25	0.5	1
Escherichia coli L 47	>128	>128	>128	>128	>128	>128

††† The 15-CH (a doublet) resonates at δ 5.78~5.77 ppm in N^{15}-glycinyloctapeptides, at δ 5.72 ppm in lysyl- and glutaminyl-octapeptides, and at δ 5.62 ppm in the acetyl derivatives.

†††† The 15-CH proton in unmodified teicoplanins resonates at δ 4.62 ppm when the terminal NH_2 is present as the free base, or at δ 5.47 ppm when the terminal amino group is in the protonated NH_3^+ form.

Table 5. *In vitro* antimicrobial activity of TD and its N^{15}-acyl-derivatives.

Organism	MIC (μg/ml)					
	TD	TD-OP-1	TD-OP-2	TD-OP-3	TD-OP-4	*N*-Ac-TD
Staphylococcus aureus L 165	0.13	0.25	0.13	0.13	0.25	0.13
S. aureus L 561	0.13	0.13	0.06	0.13	0.25	0.13
S. epidermidis ATCC 12228	0.06	0.13	0.06	0.06	0.13	0.13
S. epidermidis L 533	0.06	0.13	0.06	0.13	0.13	0.13
S. haemolyticus L 602	0.25	0.5	0.25	0.5	1	1
Streptococcus pyogenes L 49	0.13	0.5	0.13	0.13	0.25	0.13
S. pneumoniae L 44	0.13	0.13	0.13	0.13	0.06	0.13
Enterococcus faecalis ATCC 7080	0.13	0.25	0.13	0.25	0.5	0.5
Escherichia coli L 47	64	>128	32	64	>128	>128

Table 6. *In vitro* antimicrobial activity of CTA/2-A, TD-A and their N^{15}-acyl-derivatives.

Organism	MIC (μg/ml)					
	CTA/2-A	CTA/2-A-OP	*N*-Ac-CTA/2-A	TD-A	TD-A-OP	*N*-Ac-TD-A
Staphylococcus aureus L 165	0.25	0.25	0.5	0.13	0.25	0.13
S. aureus L 561	2	1	2	0.13	0.13	0.13
S. epidermidis ATCC 12228	0.25	0.13	0.13	0.06	0.13	0.06
S. epidermidis L 533	0.25	0.13	0.5	0.06	0.13	0.13
S. haemolyticus L 602	0.5	0.25	0.25	0.13	0.13	0.13
Streptococcus pyogenes L 49	0.06	0.06	0.06	0.06	0.13	0.06
S. pneumoniae L 44	0.13	0.06	0.25	0.13	0.25	0.13
Enterococcus faecalis ATCC 7080	0.13	0.25	0.25	0.13	0.25	0.13
Escherichia coli L 47	>128	>128	>128	8	8	8

Preliminary studies of the ability of the teicoplanin octapeptides to complex with the antibiotic's target D-Ala-D-Ala have been done by measuring their binding to the synthetic analogue Ac_2-L-Lys-D-Ala-D-Ala according to the differential UV assay.[9] The experiments, carried out in comparison with CTA/2, TD, CTA/2-A, TD-A, and their N^{15}-acetyl derivatives, showed that teicoplanin octapeptides possess a binding strength to the tripeptide which is substantially the same as that of parent N^{15}-acetylated compounds, and 5 to 10 times lower than that of the respective unmodified antibiotics.†††††

Further studies of the rates and equilibria of peptide binding of teicoplanin octapeptide and N^{15}-acetyl derivatives, using the fluorescent reporter peptide ε-*N*-acetyl-α-*N*-dansyl-L-Lys-D-Ala-D-Ala,[10] are planned to better understand the mechanism of the dinamic (K_{ON}, K_{OFF}) binding interaction between these N^{15}-aminoacyl and acetyl derivatives and the target peptide. We hope this would help us to establish new SARs to justify the differences in their antimicrobial activity between these structurally related derivatives of teicoplanin.

Conclusions

In general, the aminoacylation of terminal amino group of teicoplanin antibiotics had a marginal effect on the activity of the resulting octapeptide derivatives. The better activity of glycinyl- and lysyl-CTA/2 than that of CTA/2 against coagulase-negative Staphylococci may be ascribed to the slightly increased lipophilicity of these octapeptides rather than to an improvement of their affinity for the target peptide.

††††† Unpublished results; these laboratories.

The modification of the isoelectic point in positively charged lysyl-derivatives of CTA/2 and TD, as well as the increased lipophilic character in the lysyl-octapeptides of positively charged N^{63}-teicoplanin amides, had no effect on the activity of corresponding teicoplanin octapeptide derivatives.

The chirality of the new terminal amino acid seems to play no particular role in binding to target D-Ala-D-Ala considering that no significant difference in their antibacterial activity was observed between octapeptides containing L- or D-lysine.

Experimental

^{1}H NMR spectra were recorded at 500 MHz on a Bruker AM 500 NMR-spectrometer equipped with an Aspect 3000 computer. The spectra were obtained at 40°C in DMSO-d_6 solution, using Me_4Si (δ 0.00 ppm) as internal reference.

Acid-base titrations were carried out under the following conditions: The sample was dissolved in methylcellosolve - water (4 : 1). After adding an excess of 0.01 N HCl in the same solvent mixture, the resulting solution was titrated with 0.01 N NaOH.

The products were purified by reversed-phase column chromatography on silanized silica gel (0.063 ~ 0.2 mm; Merck). Reactions, column eluates, and final products were checked by HPLC analyses, which were performed on a column Hibar (120 × 4.5 mm; Merck) prepacked with LiChrosorb RP-8 (10 μm), using a Varian Model 5500 LC pump equipped with a 20-μl loop injector Rheodyne Model 7125 and a Varian Model 2050 UV variable detector. Chromatograms were recorded at 254 nm, using CTA, component A2-2, or TD as internal references. Elutions were carried out at a flow rate of 2 ml/minute according to a linear step gradient from 20 to 60% of CH_3CN in 0.2% aqueous HCO_2NH_4 in 30 minutes. Under these conditions, the retention times (t_R's) of CTA/2 and TD were 7.4 and 5.3 minutes, respectively.

All derivatives were analyzed for N and Cl on samples previously dried at 140°C under N_2 atmosphere. Weight loss was determined by thermogravimetry (TG), at 140°C. Inorganic residue was determined after heating the samples at 900°C in O_2 atmosphere. The analytical results obtained for N and Cl were within ±0.4% of the theoretical values. Solvent content (in general H_2O, with traces of BuOH) and inorganic residue were always less than 10% and 0.3%, respectively.

Preparation of CTA/2-octapeptides

General Procedure

A solution of 5.5 g (~3 mmol) of CTA/2 and 3.3 mmol of the appropriate BOC (or di-BOC) amino acid *N*-hydroxysuccinimidyl ester in 50 ml of DMF was stirred at room temperature for 4 hours, afterwards it was poured into 150 ml of H_2O. The resulting cloudy solution (or suspension) was adjusted at pH 3 with 1 N HCl and extracted with 300 ml of a mixture of BuOH - EtOAc (1 : 3). The organic layer was separated and concentrated at 40°C under reduced pressure to a small volume. By adding Et_2O a solid separated which was collected and re-dissolved at room temperature (or at 0°C) in 70 ml of TFA. The resulting solution was stirred for 2 minutes at room temperature (or 0°C), then it was poured into 300 ml of Et_2O previously cooled at 0°C. The precipitated solid was collected, washed with Et_2O and re-dissolved in a minimum amount of a mixture of H_2O - MeCN (9 : 1). The resulting solution was loaded on a column of 700 g silanized silica gel in H_2O and elution was performed by a linear gradient from 10 to 50% of MeCN in 0.01 N AcOH in 20 hours at the flow-rate of 200 ml/hour, while collecting 15 ml-fractions. Those containing pure title compounds were pooled and the resulting solution was concentrated at 40°C under reduced pressure in the presence of enough BuOH to obtain a concentrated (~25 ml) butanolic suspension which was poured into Et_2O (200 ml) under stirring. The precipitated solid was collected, washed several times with Et_2O and then dried at room temperature *in vacuo* for 3 ~ 5 days, to yield pure title compounds (see Table 1).

Preparation of TD-octapeptides

General Procedure

A solution of 3.6 g (3 mmol) of TD and 4.5 mmol of the appropriate BOC (or di-BOC) amino acid *N*-hydroxysuccinimidyl ester in 50 ml of a mixture of DMF - DMSO (1 : 1) was stirred at room temperature

for 5 hours. Then, reaction work-up, treatment of crude intermediate products with TFA (in this case, a 30 minutes reaction at room temperature was more suitable), recovery and purification of the final title compounds (see Table 2) were carried out according to the same overall procedure described above.

Preparation of CTA/2-A-OP and TD-A-OP

These compounds were prepared from the corresponding 3,3-dimethylamino-1-propylamide[7] (CTA/2-A or TD-A) of CTA/2 and TD, respectively, according to the appropriate above procedure (See Table 3).

Preparation of the N^{15}-Acetyl Derivatives of CTA/2, TD, CTA/2-A and TD-A

To a stirred solution of 1 mmol of the appropriate teicoplanin antibiotic, or its amide with the 3,3-dimethylamino-1-propylamine, and 0.5 g of $NaHCO_3$ in 40 ml of a mixture of Me_2CO - H_2O (1 : 1), a solution of 0.3 ml of AcCl in 15 ml of dry Me_2CO was added dropwise at 0~5°C over 30 minutes. Afterwards, 100 ml of H_2O was added and the resulting solution was extracted with 200 ml of BuOH. The organic layer was washed with H_2O (100 ml) and then it was concentrated at 45°C under reduced pressure to a small volume (~10 ml). On adding Et_2O (100 ml) the precipitated solid was collected, washed several times with Et_2O, and then it was dried at room temperature *in vacuo* overnight, yielding pure title compounds (See Tables 1~3).

Determination of Antibacterial Activity

MICs were determined by the microbroth dilution method in Difco Todd-Hewitt broth (Streptococci) or Oxoid Iso-Sensitest broth (other bacteria). The inoculum was 5×10^5 cfu/ml. Incubation was for 24 hours at 37°C.

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