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MccJ25 C-terminal is involved in RNA-polymerase inhibition but not in respiration inhibition

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Abstract

Microcin J25 appears to have two intracellular targets: (1) RNA polymerase, which was described in *Escherichia coli* and *Salmonella enterica* serovars, and (2) cell respiration in *Salmonella enterica* serovars. C-terminal glycine amidation of the threaded segment localized in the MccJ25 lariat ring region specifically blocked the RNA-polymerase inhibition, but not the cell respiration inhibition and peptide uptake. These results suggest that different regions of the molecule are responsible for each cellular effect, they are localized far away from the β-hairpin region and the C-terminal region is an important determinant for RNAP inhibition.
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Keywords: Microcin J25; RNA polymerase; MccJ25 amidated derivative; Oxygen consumption

Escherichia coli microcin J25 (MccJ25) is a plasmid-encoded antibiotic peptide consisting of 21 L-amino acid residues, primarily active on gram-negative bacteria related to the producer strain [1]. The structure of MccJ25 consists of an amino acid backbone chain (G^1-G-A-G-H^5-V-P-E-Y-F^{10}-V-G-I-G-T^{15}-P-I-S-F-Y^{20}-G), containing a lactam linkage between the α-amino group of Gly^1 and the γ-carboxyl of Glu^8, forming an 8-residue ring (Gly^1-Glu^8) denominated lariat ring [2–4]. The "tail" (Tyr^9-Gly^{21}) passes through the lariat ring, with Phe^{19} and Tyr^{20} straddling each side of the ring plane, remaining trapped by a non-covalent interaction (see Fig. 1). The amino acids F^{10}-V-G-I-G-T-P^{16} form a β-hairpin structure comprising two β-strands (F^{10}-V^{11} and T^{15}-P^{16}) and a β-turn (V^{11}-G-I-G^{14}).

MccJ25 disrupts the *Salmonella newport* inner membrane gradient, inhibiting several processes essential for cell viability such as oxygen consumption [5]; whereas in many *E. coli* strains, the peptide inhibits the RNA transcription [6] without affecting the oxygen consumption [5].

Previous studies from our laboratory demonstrated that the microcin J25 β-hairpin region is very important for antibiotic uptake but not for RNA polymerase (RNAP) and respiration inhibition [7]. In the present paper, we amidated the MccJ25 Gly^{21} residue to determine the role of the threaded segment in the lariat ring region in each one of the mechanisms.

Materials and methods

Luria broth and M9 minimal salts were purchased from Sigma Chemical. Minimal medium was supplemented with 0.2% glucose, 1 μg/ml $MgSO_4$, and 1 μg/ml vitamin B_1. Solid media were prepared by adding agar to a final concentration of 1.5%. When required 50 μg/ml ampicillin or 25 μg/ml chloramphenicol were added. Plasmid DNA was isolated with the Wizard miniprep DNA purification system (Promega). Transformation of competent cells was carried out by electroporation procedure [8] The chemical derivation and isolation of

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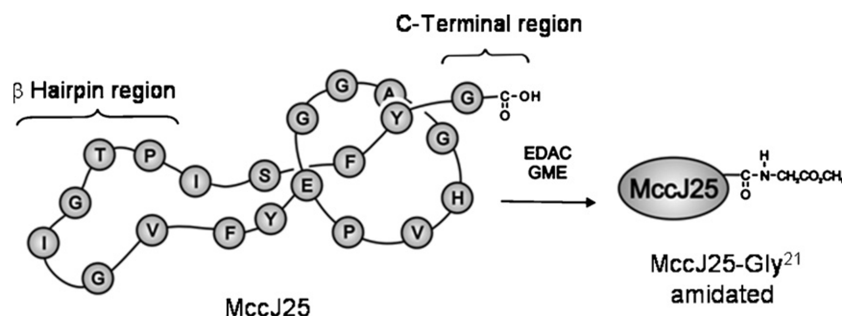

Fig. 1. Schematic representation of MccJ25 and MccJ25-Gly^{21} amidated. The β-hairpin and C-terminal regions are indicated.

glycine methyl ester MccJ25 analogue (MccJ25-Gly^{21} amidated) were performed as described in Bellomio et al. [9] (see Fig. 1). Methanolic solutions of MccJ25 and MccJ25-Gly^{21}amidated analogue (2 mM) were prepared for antibiotic activity assay. Antibiotic activity determined as the minimal inhibitory concentration (MIC), the in vitro RNAP activity, and oxygen consumption were determined as described in Bellomio et al. [7].

Results and discussion

A previous report indicated that the amidated derivative did not show antibiotic activity on *E. coli* W3110. The loss of antibiotic activity correlated with the inhibition absence of an in vivo RNA transcription assay [9]. Table 1 shows that other *E. coli* strains are also totally resistant to MccJ25-Gly^{21} amidated derivative, namely MC4100 [*F*$^{-}$*ara D139Δ*(*argF-lac*)*205λ*$^{-}$ *rpsL150* (*Smr*) *flbB5301 relA deoC1 pstF25*] and AB259 [*HfrH supQ80λ*$^{-}$ *relA1 spoT1 thi-1*]. Since the lack of in vivo RNA synthesis inhibition could be due to an inability of the microcin variant to reach RNA polymerase [9], we now tested the effect of MccJ25-Gly^{21} amidated on in vitro RNA transcription. The results show that the amidated peptide is unable to inhibit in vitro RNA transcription, despite the fact that MccJ25 at the same concentration inhibited up to 50% (Fig. 2). Nevertheless, amidated MccJ25 derivative retained the MccJ25 ability to inhibit both, the growth (Table 1) and oxygen consumption of *S. newport* (Fig. 3).

Table 1
Effect of MccJ25 and MccJ25-Gly21 amidated derivative on *E. coli* and *Salmonella* strains

Strains	Sensitive spot-on-lawn test (MIC μM)	
	MccJ25	MccJ25-Gly21 amidated
E. coli MC4100	0.12	R[a]
E. coli AB259	0.48	R
S. newport	0.003	2.4
S. typhimurium	R	R
S. typhimurium (pGC01)	0.007	1.95

[a] No inhibition was observed in the presence of 2000 μM.

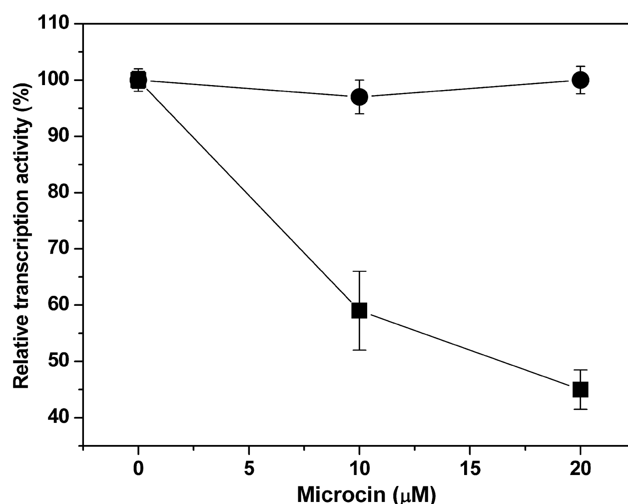

Fig. 2. Effect of MccJ25 (●) and MccJ25-Gly^{21} amidated (■) on in vitro RNA synthesis. Results are expressed as means ± SD ($n = 4$) of the relative transcription activity percentage with respect to the control (without peptide).

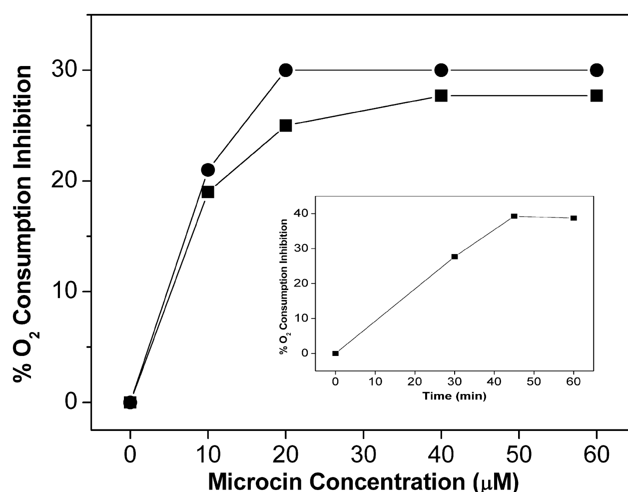

Fig. 3. Effect of different concentrations of MccJ25 (●) and MccJ25-Gly^{21} amidated (■) on *S. newport* oxygen consumption. The inset shows the time-dependent *S. newport* oxygen consumption inhibition of 40 μM MccJ25-Gly^{21} amidated. The results are expressed as percent of the control measured in the absence of peptides.

The uptake of MccJ25 by *E. coli* was dependent upon the outer cell membrane receptor, FhuA, and the inner membrane proteins TonB, ExbD, ExbB, and SbmA [10,11]. The SbmA protein receptor transports the MccJ25 through the inner cell membrane. We reported that *Salmonella typhimurium*, *Salmonella derby*, and several *Salmonella enteritidis* strains were completely resistant to MccJ25. These strains became MccJ25 hypersensitive when transformed with a plasmid pGC01, that is the pBR322 plasmid carrying *E. coli fhuA* [8]. Similarly, *S. typhimurium* LT2 strain becomes sensitive to amidated MccJ25 derivative when the bacteria were transformed with pGC01 (Table 1). These results indicate that the MccJ25 derivative can access into the cell through the FhuA and SbmA receptors, just like how the native peptide does [8].

Recently, we reported that extensive treatment of MccJ25 with thermolysin hydrolyzed the β-hairpin region giving a two-chain peptide: (Gly^{1}-Phe^{10}) and (Ile^{13}-Gly^{21}) of 10 and 9 amino acid residues, respectively. This deep modification affected the MccJ25 transportation into the cell but conserved the capacity to inhibit *E. coli* RNAP activity and *S. newport* respiration [7].

The results reported in the present paper indicate that the chemical modification of the C-terminal (Gly^{21}) of threaded segment in the lariat ring zone resulted in the loss of ability to inhibit the RNAP activity. However, the modification did not induce changes either in the capability to inhibit oxygen consumption or in the peptide transportation. It was suggested that MccJ25 inhibits transcription by binding within and obstructing RNAP secondary channel [6,12–14]. Biochemical and genetic results demonstrated that inhibition of transcription requires an extensive RNAP determinant, comprising more than fifty amino acid residues; on the other hand, they suggested that MccJ25 acts essentially as a "cork in a bottle" in the RNAP secondary channel [13]. Afterward, addition of glycine methyl ester group to MccJ25 C-terminal could either: (a) perturb its approximation to RNAP MccJ25-binding site, or (b) cancel a possible electrostatic interaction between the unique carboxyl group of MccJ25 with a positive amino acid residues of RNAP. It is interesting to note that in the RNAP MccJ25 binding site model proposed by Mukhopadhyay et al. [13] the Arg^{780} is around the site that binds MccJ25, and an *E. coli* strain in which this RNAP positive amino acid was mutated by Cys was highly resistant to MccJ25 [13].

Concluding remarks

In this paper, we are providing data regarding the role of another region (C-terminal) of MccJ25 and its relationship with the antibiotic activity. The results presented here taken together with our previous studies [7,9] let us suggest three important conclusions: (a) the determinants for respiration and RNAP inhibition are different, (b) they are localized away from the β-hairpin region which is important for the MccJ25 uptake, and (c) the C-terminal region is an important determinant for RNAP inhibition (Fig. 1).

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