NOTES

Primary Structure of Colicin M, an Inhibitor of Murein Biosynthesis

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The DNA sequence of the colicin M activity gene cma was determined. A polypeptide consisting of 271 amino acids was deduced from the nucleotide sequence. The amino acid sequence agreed with the peptide sequences determined from the isolated colicin. The molecular weight of active colicin M was 29,453. The primary translation product was not processed. In the domain required for uptake into cells, colicin M contained the pentapeptide Glu-Thr-Leu-Thr-Val. A similar sequence was found in all colicins which are taken up by a TonB-dependent mechanism and in outer membrane receptor proteins which are constituents of TonB-dependent transport systems. The structure of colicin M in the carboxy-terminal activity domain had no resemblance to the pore-forming colicins or colicins with endonuclease activity. Instead, the activity domain contained a sequence which exhibited homology to the sequence around the serine residue in the active site of penicillin-binding proteins of Escherichia coli. The colicin M activity gene was regulated from an SOS box upstream of the adjacent colicin B activity gene on the natural plasmid pColBM-C1139.

Colicin M causes lysis of sensitive cells, with the formation of osmotically sensitive spheroplasts, and thus resembles β-lactam antibiotics (3, 23). It has been demonstrated by pulse-labeling with radioactive diaminopimelic acid that murein (peptidoglycan) biosynthesis of cells is inhibited shortly after addition of colicin M and that lysis accompanied by murein degradation starts 20 min later (24). During this period, colicin M has to be continuously present (23). Reversal of colicin M action can be achieved by addition of glycol-bis-(β-aminoethyl ether)-N,N′-tetraacetic acid, which binds the Ca2+ ions required for the activity of colicin M (23). Colicin M interferes late in murein biosynthesis, which is catalyzed by enzymes located in the cytoplasmic membrane. Therefore, it is possible that the target site of colicin M, like the pore-forming colicins, is the cytoplasmic membrane. In fact, the activity and immunity genes of colicin M are adjacent and are transcribed in opposite directions (20), as is the case for the genes of the pore-forming colicins (17, 19, 27, 29). Moreover, the immunity protein of colicin M was found in the cytoplasmic membrane (T. Ölschläger and V. Braun, unpublished). Although pore formation by colicin M could not be detected, it was of interest to determine whether colicin M exhibited any amino acid sequence homology to the pore-forming colicins.

Cells of Escherichia coli AB2847 araB tsx malT thi transformed with plasmid pTO4 produced colicin M and were immune to colicin M added to the culture (20). Plasmid pTO4 contains, on a 2.3-kilobase (kb) EcoRI-SauI fragment, the cma and cmi genes (Fig. 1). The sequence of cma was determined by cleaving the isolated 2.3-kb fragment with Sau3A, HpaII, and TaqI. The fragments were inserted into phages M13 mp18 and mp19 (Fig. 1) and sequenced by established methods (2, 8, 16, 22, 25, 30).

The nucleotide sequence determined from the NaeI to the DraI restriction sites contained one large open reading frame comprising 813 nucleotides (Fig. 2). The amino acid sequence deduced from the nucleotide sequence agreed with the sequence of the first 39 amino-terminal amino acids determined by Edman degradation and with the carboxy-terminal Lys-Arg sequence released by carboxypeptidase B from colicin M (7), showing that colicin M was not synthesized as a precursor. Other colicins are also synthesized without an amino-terminal signal sequence (6). Colicin M is the only colicin that retains the amino-terminal methionine residue in the mature form.

To obtain independent data on the amino acid sequence of colicin M, peptides were isolated and their amino acid sequence was determined. The peptides obtained after cy-
anogen bromide cleavage were separated on a large-pore-size reverse-phase high-pressure liquid chromatography Vydac C4 column with volatile buffers that allowed direct sequencing in a solid-phase sequencer (14, 21). The amino acid sequences of six pure peptides comprising residues 2 to 20, 2 to 27, 52 to 75, 124 to 140, 124 to 150, and 259 to 269 agreed completely with the corresponding sequences deduced from the nucleotide sequence (Fig. 2). Thus, colicin M is composed of 271 amino acids and has a molecular weight of 29,435.

Colicin M contained 17 negatively and 21 positively charged amino acids and nine histidine residues, which at pH 7 were partially positive. Therefore, colicin M had a net positive charge, in agreement with the isoelectric point of pH 9.5 determined previously by electrofocusing (23).

The activity of colicin M was found to be stabilized by addition of 0.1% Triton X-100 (23). This finding suggests that a hydrophobic sequence exposed at the surface of the protein destabilizes the protein in an aqueous environment and is shielded by binding of the detergent. In fact, the hydrophathy profile (13) of colicin M revealed more hydrophobic regions along the entire sequence than in most other colicins (data not shown) but did not disclose whether any of these sequences are exposed to water.

Colicin M bears no obvious sequence resemblance to the pore-forming colicins A, B, E1, Ia, or Ib (17, 19, 27, 29) or to
the endonuclease colicins E2 and E3 or colicin DF13 (4, 18, 28).

However, colicin M had a homologous pentapeptide at the amino-terminal end with colicins B, 1A, and Ib (25) whose uptake into cells required the TonB function. The same pentapeptide homology is contained in receptor proteins close to the amino terminus which are constituents of iron and vitamin B12 transport systems (5, 9, 12, 15; M. Sauer, K. Hantke, and V. Braun, submitted for publication). The pentapeptide in colicin M is Glu-Thr-Leu-Thr-Val, in colicin B is Asp-Thr-Met-Val-Val, and in colicins 1A and Ib is Glu-Ile-Met-Ala-Val. It is proposed that this sequence is involved in the interaction with the TonB protein.

Since colicin M inhibits murein biosynthesis, the amino acid sequence was compared with sequences of known penicillin-binding proteins which are involved in late steps of murein synthesis and turnover (10). Homology was found in the region around the serine residue which is covalently esterified by penicillin (Fig. 3). There were differences in the conserved penicillin-binding sites in that, for example, colicin M contained a histidine adjacent to the serine residue, in contrast to the penicillin-binding proteins, which contained either isoleucine, valine, alanine, or threonine residues there. Otherwise, the amino acid replacements were conservative in that amino acids with similar physicochemical properties were exchanged. The number of differences between colicin M and the penicillin-binding proteins was similar to the differences among the latter. Computer-assisted comparisons did not reveal additional homologous regions. Preliminary experiments indicated covalent binding of tritium-labeled benzylpenicillin to isolated colicin M. However, the specific activity obtained was much weaker than that of the penicillin-binding proteins. Labeling of the penicillin-binding proteins was not affected by preincubating cells with colicin M (E. Fischer, unpublished). The activity of colicin M was also not inhibited by ampicillin. On the contrary, ampicillin and colicin M administered at concentrations at which one inhibitor did not induce cell lysis caused rapid lysis when supplied together (24). At present it cannot be decided whether the homology to the active-site sequence of penicillin-binding proteins indicates the target site of colicin M, whether its activity is an evolutionary relic, assuming that colicin M is derived from a penicillin-binding protein, or whether it is a mere accident.

The nucleotide sequence upstream of cma contained a nearly ideal Shine-Dalgarno ribosome-binding site (26) with only one nucleotide deviation from the consensus sequence TAAGGAGGT (Fig. 2). The distance from the translation initiation codon to the AGG sequence was 8 nucleotides, which follows the rule.

The most interesting observation regarding transcription regulation was the lack of an SOS box in front of cma, although synthesis of colicin M can be induced 1,000-fold by treatment with mitomycin C (20, 23). The naturally occurring plasmid pColBM-CI139 typically carries not only the colicin M activity and immunity genes cma and cmi, respectively, but in addition carries directly adjacent to them the colicin B activity and immunity genes cba and cbi in the order cmi-cma-cba-cbi (20). cba and cma are transcribed in the same and cbi and cmi in the opposite direction. The sequence in front of cba contains the SOS box (27) which can serve as a binding site for the LexA repressor protein. Apparently, expression of cma is regulated at the same SOS box upstream of cba. This conclusion was supported by the strongly reduced expression of cma displayed by transposon insertion mutations in cba (20).

LITERATURE CITED


