Structural Characterization of Cytolysin from the Pathogenic *Enterococcus faecalis*

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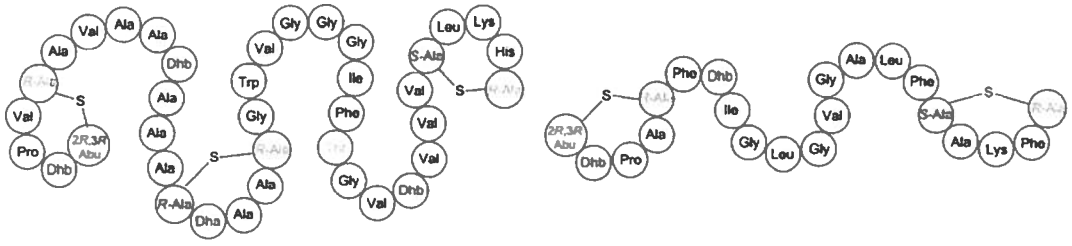
The enterococcal cytolysin is a unique two-component lantibiotic with hemolytic activity against various types of eukaryotic cells. Its production in *Enterococcus faecalis*, which is a major cause of surgery infection, has been found to be related to the enhanced virulence of the bacteria and associated with acute patient mortality. Despite the intensive research on its biological functions, the enterococcal cytolysin's structure remains unknown. In previous studies, the structural characterization of cytolysin is very challenging, most probably because the purification of large amount of cytolysin from the producing bacteria is difficult. To overcome this challenge, we have successfully expressed cytolysin in *E. coli* and obtained sufficient purified cytolysin for structure studies. By tandem MS and chiral GC-MS techniques, the structure of cytolysin was determined as shown in the figure.



Systematic Evaluation of the Dependence of Deoxyribozyme Catalysis on Random Region Length

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Functional nucleic acid DNA/RNA sequences can be identified from random-sequence pools by in vitro selection, which requires choosing the length of the random region. Shorter random regions allow more complete coverage of sequence space but may not permit the structural complexity necessary for binding or catalysis. In contrast, longer random regions are sampled incompletely but may allow adoption of more complicated structures that enable function. We systematically examined random region length (N_{20} through N_{60}) for two particular deoxyribozyme catalytic activities, DNA cleavage and tyrosine-RNA nucleopeptide linkage formation. In the case of DNA cleavage, we found that shorter N_{20}, N_{30}, and N_{40} regions allowed robust catalytic function, either by DNA hydrolysis or by DNA deglycosylation and strand scission via β-elimination, with an interesting interplay of metal ion cofactors. In contrast, longer N_{50} and N_{60} regions did not lead to catalytically active DNA sequences. Separately, for Tyr-RNA linkage formation, N_{30} and N_{60} regions provided catalytically active sequences, whereas N_{20} was unsuccessful, and the N_{40} deoxyribozymes were functionally superior (in terms of rate and yield) to N_{30} and N_{60}. Collectively, the results indicate that with future in vitro selection experiments for DNA and RNA catalysts, and by extension for aptamers, random region length should be an important experimental variable.