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研究課題名（和文）Development of a novel gel-display drug screening technique

研究課題名（英文）Development of a novel gel-display drug screening technique

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研究成果の概要（和文）：本研究の目的は、薬剤耐性菌感染に対する治療のための新しいペプチド医薬の開発である。我々は、耐性菌由来の薬剤耐性酵素の阻害剤として16種類のペプチドの候補を取得することに成功し、そのうちの4種類が非常に有力な候補であることを見出した。さらに複数の薬剤耐性菌の酵素に対して活性を示した。これらの化合物は、抗生物質耐性細菌感染の治療のための薬物を開発するために使用され得る。

研究成果の概要（英文）：The goal of this work was to identify new antibiotics for the treatment of drug resistant bacterial infections. We identified 16 candidate inhibitors of bacterial antibiotic-resistance enzymes. Four of which are highly potent and exhibited activity against enzymes from multiple different drug-resistant strains of bacteria. These compounds may be used to develop drugs for the treatment of antibiotic resistant bacterial infections.

研究分野：　Chemical Biology

キーワード：　Chemical Biology　Peptide　Antibiotic

１．研究開始当初の背景

(1) Bacterial antibiotic resistance is an increasing problem in healthcare settings, and new antibiotics are required for the treatment of relatively common infectious diseases. Macrocyclic peptides are a validated chemical class for the development of antibiotics, and we, and others have shown that display screening techniques can be used to identify macrocyclic peptides with high affinities to targets of interest.

２．研究の目的

(1) Macrocyclic peptides are promising candidates for revolutionary drug discovery, with the capability to disrupt protein-protein interactions. mRNA display-based techniques allow for rapid screening of cyclic peptides for affinity to a disease-related target, however, they do not select for bioactivity.

(2) The present research had two main goals. Overall the aim was to identify antibacterial macrocyclic peptides with a novel mechanism of action. This was proposed to be achieved through the development of a novel display screening technique (gel display screening), which would screen for bioactivity. The development of this screening methodology was the second primary purpose of the research.

３．研究の方法

(1) The proposed gel display screening method (Figure 1) was as follows: DNA template, gelling agent and RNA polymerase enzyme will be combined at 4°C. Warming to 37°C will simultaneously set the gel and initiate RNA synthesis, forming “colonies” of clonal RNA molecules. Genetically reprogrammed translation on the gel surface will then be used to synthesize clonal pools of cyclic peptides. The gel will then be overlaid with target cells leading to the formation of growth inhibition plaques around bioactive peptides, which can be recovered by PCR followed by re-translation.

４．研究成果

(1) The original proposal for this project outlined the optimization of the screening system over the first year of studies (FY2015) with screening for novel

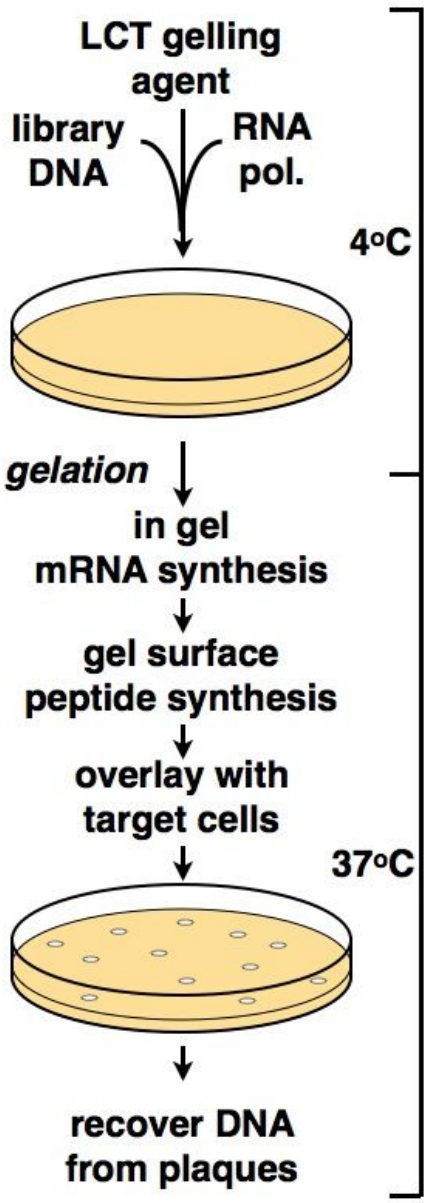

Figure 1 - Workflow of proposed gel display screening system.

antibiotics occupying the second year (FY2016). This initial year of optimization was further planned to involve i) optimization of gel casting, ii) optimization of in gel enzymatic reactions, iii) optimization of local DNA concentration and iv) optimization of cell growth conditions. Of these, i) and ii) were optimized relatively easily, with methyl-cellulose concentrations around 3% found to be optimal for both casing and in gel enzymatic reactions. Using a fluorescent reporter protein it was also found that peptides could be synthesized in under gelation conditions (data not shown).

(2) However, optimization of DNA concentration and cell growth conditions was more problematic. The original proposal was to use a known translatable antibiotic peptide as a tool for the final optimization of in gel peptide synthesis and cell growth conditions. However, even the most potent such peptide know, Cecropin P1, turned out to be insufficiently active for this purpose, with concentrations in excess of 10 uM required for bacterial inhibition. This was a major obstacle since it made further optimization of the screening system impossible.

(3) At this point, the focus of the project changed to the identification of translatable peptides with sufficient antibiotic activity that they could be used to finalize the optimization of the screening system. We reasoned that by finding such a peptide targeting an antibiotic resistance protein, we could a) identify an potential treatment for antibiotic resistance thereby achieving the overall goal of the project and b) find a molecule that could be used to optimize the screening system.

(4) To this end, we set up a collaboration with the laboratory of Christopher Schofield at the University of Oxford; world leaders in the study of metallo-beta-lactamases, which are a class of clinically significant antibiotic resistance genes for which there are no known potent inhibitors. Screening to identify inhibitors of this enzyme class was conducted using RaPID technology developed at the University of Tokyo, and targeting three different enzyme homologues: IMP-1, NDM-1 and VIM-2.

(5) This screening identified 16 candidate macrocyclic peptide metallo-beta-lactamase inhibitors, exhibiting different binding kinetics with respect to each of the three related enzymes (Table 1). Of these, MBL-L-06, MBL-L-07, MBL-D-01 and MBL-D-02 appear the most promising, with low nanomolar dissociation constants observed against all three targets.

Table 1 – Dissociation constants (nM) for macrocyclic peptide inhibitors of metallo-beta-lactamases.

	IMP-1	NDM-1	VIM-2
MBL-D-01	136	1.2	44
MBL-D-02	275	44	79
MBL-D-03	0.9	262	ND
MBL-D-04	1.2	>1000	>1000
MBL-D-05	261	109	>1000
MBL-D-06	804	806	20
MBL-D-07	ND	ND	ND
MBL-D-08	>1000	134	951
MBL-D-09	391	502	392
MBL-L-01	3.3	520	>1000
MBL-L-02	45	8	118
MBL-L-03	3.6	355	309
MBL-L-04	6.4	144	ND
MBL-L-05	50	801	11
MBL-L-06	36	2	50
MBL-L-07	132	36	98

(6) Preliminary assays for enzyme inhibition have also been conducted, and appear to demonstrate inhibitory potencies in line with the dissociation constants, particularly for the more potent/higher affinity molecules. For example, IC_{50} values for MBL-D-01, MBL-D-02, MBL-L-06 and MBL-L-07 against VIM-2 are of the order of 33.1 nM, 33.9 nM, 33.8 and 111.0 nM respectively. Further activity assays as well as x-ray crystallography studies are under way in order develop these molecules into clinically useful therapeutics for multi-drug resistant bacterial infections.

(7) Thus, while we have not been able to fully develop the proposed gel display screening system due to unforeseen difficulties, we have achieved the overall goal of the project, which was to identify

compounds with the potential to treat drug-resistant bacterial infections. Although most of the work supported by this grant has not yet been published, we anticipate at least one very significant paper will arise from the new molecular entities identified as a result of these studies, once further characterization of the compounds of interest is completed, and the necessary intellectual property protections are in place. Additionally, a number of incidental aspects of this work have made it into the scientific literature, as detailed in the following sections.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　1　件）

A RaPID way to discover nonstandard macrocyclic peptide modulators of drug targets. Passioura T, Suga H.
Chem Commun (Camb). 2017 53(12):1931-1940. doi: 10.1039/c6cc06951g. (Peer reviewed)

〔学会発表〕（計　3　件）

(1) Drug-like macrocyclic peptides comprised of diverse non-canonical amino acids assembled through genetic code reprogramming. Passioura T. The 42nd Lorne Conference on Protein Structure and Function. 7th Feb. 2017. Lorne (Australia)
(2) Ribosomal synthesis of highly *N*- and *O*-methylated cyclic peptide libraries for selection of protein-protein interaction inhibitors with “drug-like” characteristics. Passioura T., Liu W. and Suga H. 11th Australian Peptide Association Meeting. 28th Oct. 2015. Kingscliff (Australia)
(3) Drug-like non-canonical cyclic peptides selected from diverse libraries.
Passioura T. and Suga H. 3rd International Conference on Cyclic Proteins. 2nd Nov. 2015. Morton Island (Australia)

〔図書〕（計　0　件）

〔産業財産権〕

○出願状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計　0　件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

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