SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF TRIPROPEPTIN C DERIVATIVES CONTAINING THE PYRIMIDINE RING SYSTEM

Sehei Hirosawa[1], Yoshiaki Takahashi[1,*], Ayumi Murotani[1], Hideki Hashizume[2], Toshiaki Miyake,[1] and Yuzuru Akamatsu[2]

[1]*Institute of Microbial Chemistry (BIKAKEN), Hiyoshi, Japan*
[2]*Institute of Microbial Chemistry (BIKAKEN), Tokyo, Japan*

* Corresponding author: Dr Y Takahashi, Institute of Microbial Chemistry (BIKAKEN), Hiyoshi, 3-34-17 Ida, Nakahara-ku, Kawasaki, Kanagawa 211-0035, Japan.
E-mail: takashow@bikaken.or.jp

Supporting material

Index:

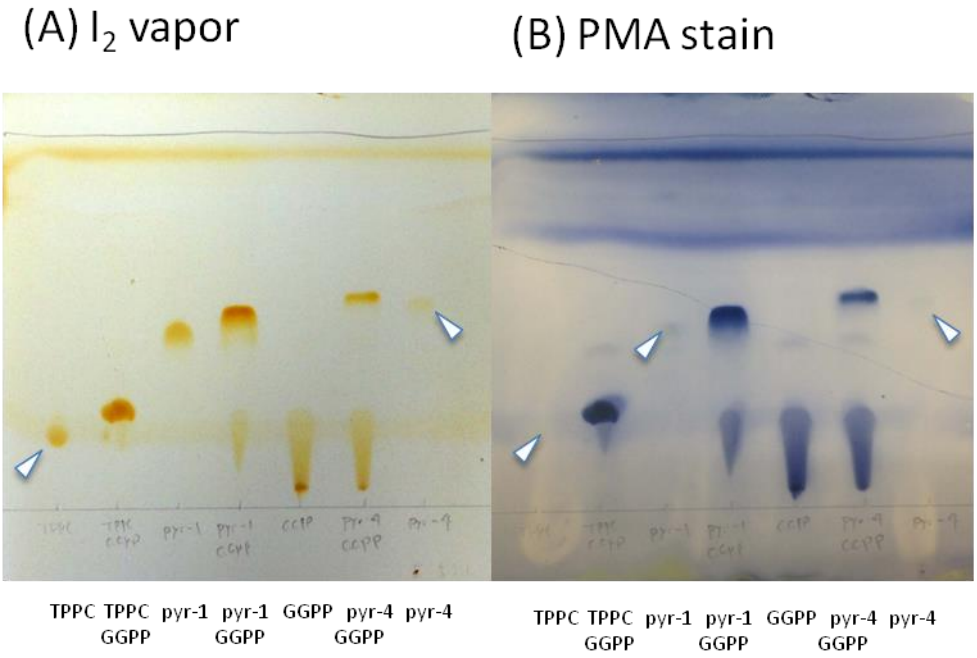
1. Thin-layer chromatography analysis of complex formation with GGPP S2
2. Mass analysis of complex formation with GGPP S3

1. Thin-layer chromatography (TLC) analysis of complex formation with GGPP

The binding ability of TPPC, and compounds **1**, and **4** to GGPP [geranylgeranylpyrophosphate (tetraprenylpyrophosphate), a model target molecule, Sigma-Aldrich, St. Louis, MO, USA] was evaluated by TLC analysis. TPPC, **1**, or **4** (each 20 μM) was mixed with GGPP (40 μM) in 80% aqueous MeOH supplemented with 20 μM calcium ions. After incubation at 37 °C for 30 minutes, the mixtures were chromatographed (chloroform/methanol/water, 8:5:1) on a silica gel 60 plate (Merck, Darmstadt, Germany). Iodide vapor and phosphomolybdic acid (PMA) color reactions were used to detect the compounds.

TPPC, **1**, **4**, and GGPP showed relative flow values of 0.20, 0.46, 0.53, and 0.06, respectively. TPPC, **1**, and **4** were hardly detectable by PMA color reactions but were detectable by iodide vapor. When combined with GGPP, the complex mixtures of TPPC, **1**, and **4** shifted to relative flow values of 0.26, 0.52, and 0.56, respectively. These results suggested that TPPC, **1**, and **4** were able to bind GGPP. However, in the case of compounds **1** and **4**, GGPP was not completely consumed and the intensity of the binding affinity was observed to be TPPC>**1**>**4**.

Figure S1. TLC analysis of the interaction of TPPC, **1**, or **4** with GGPP



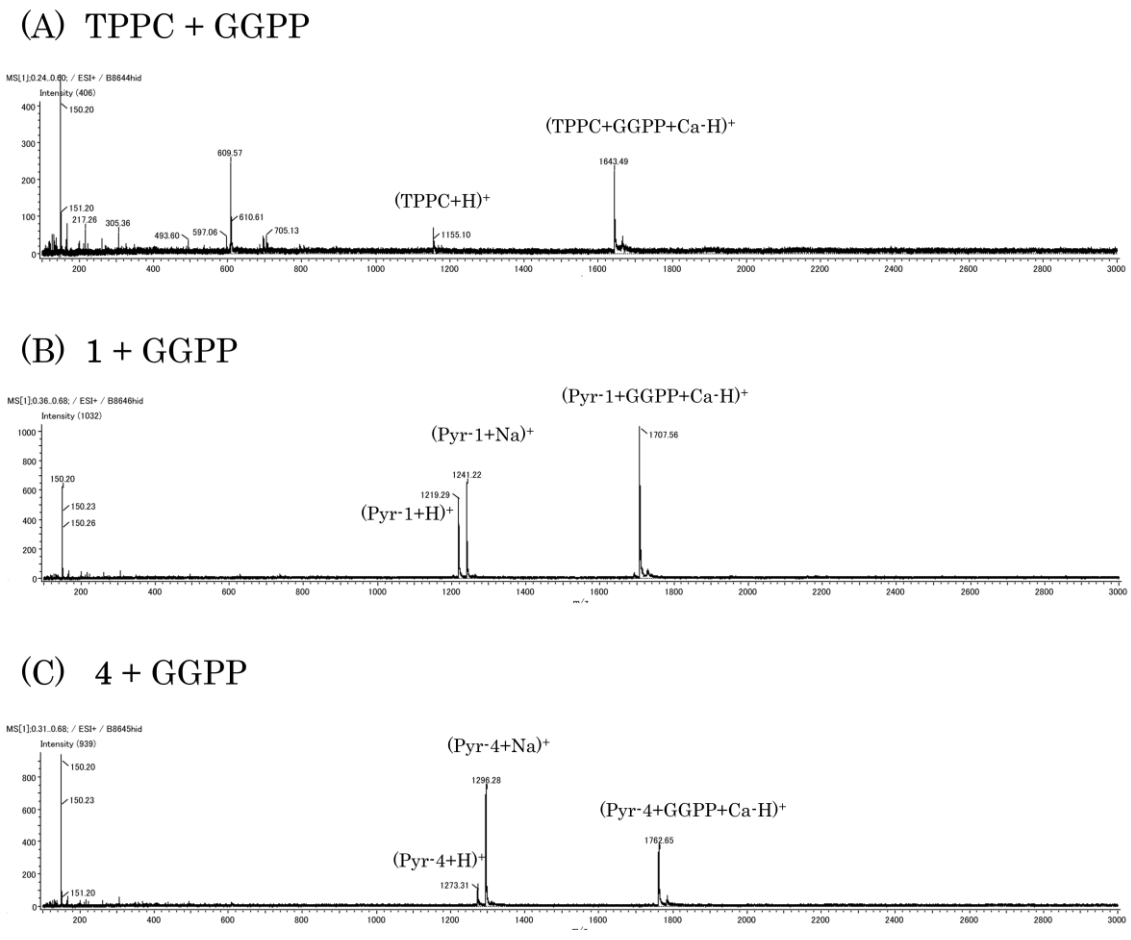
Abbreviations: pyr-1, compound **1**; pyr-4, compound **4**.

2. Mass analysis of the complexes formed by TPPC or its analogs (1 or 4) with GGPP

Each complex mixture, prepared as for TLC analysis, was subjected to liquid chromatography-mass spectrometry (JMC-T100LC mass spectrometer; JEOL, Tokyo, Japan).

In all cases, the complex of TPPC ($C_{51}H_{83}N_{11}O_{19}$) or its analog (**1** or **4**) / GGPP ($C_{20}H_{36}O_7P_2$) / Ca^{2+} was observed at a molar ratio of 1:1:1 by time-of-flight mass spectrometry. However, the ratios of GGPP-adduct form / free form of TPPC or its analog (**1** or **4**) were different (TPPC>**1**>**4**).

Figure S2. MS analysis of the interaction of TPPC, **1**, or **4** with GGPP



Abbreviations: GGPP, geranylgeranylpyrophosphate (tetraprenylpyrophosphate).