PENICILLIN-BINDING PROTEINS IN *STREPTOMYCES CACAOI* THE EFFECTS ON PENICILLIN-BINDING PROTEINS AND THE ANTIBACTERIAL ACTIVITIES OF β-LACTAMS

HIROSHI OGAWARA and SUSUMU HORIKAWA

Second Department of Biochemistry, Meiji College of Pharmacy
35–23, Nozawa-1, Setagaya-ku, Tokyo 154, Japan

(Received for publication December 1, 1979)

Penicillin-binding proteins (PBPs) in membrane of *Streptomyces cacaoi* were investigated by sodium dodecylsulfate-polyacrylamide gel electrophoresis-fluorography. At the same time, eleven β-lactams were examined on the affinities for these PBPs and the antibacterial activities against *S. cacaoi*, comparing with those in *Bacillus subtilis* reported in the preceding paper. The affinity patterns of β-lactams for PBPs both in *S. cacaoi* and *B. subtilis* were similar in many points. Here again, the grouping of β-lactams based on the affinity for PBP-2 (M. W., 91,000) was in accord with that based on the antibacterial activity. These results suggest that among PBPs detected in *S. cacaoi*, PBP-2 is the most likely target of killing by these β-lactam antibiotics.

Streptomyces strains have some unique characters: a large number of strains produce β-lactamase (penicillin and cephalosporin amido-β-lactam hydrolase, E. C. 3.5.2.6)[1,2], some strains produce β-lactam compounds[3] including β-lactam antibiotics and β-lactam inhibitors. These characters provide the interest for studying the PBPs which should include the target of β-lactam antibiotics and for searching the origin of β-lactamase among the PBPs.

Previously we reported the PBPs in *S. cacaoi, S. clavuligerus* and *S. olivaceus*[4], indicating that the PBPs in *Streptomyces* were complex, for example, the differences between strains such as β-lactam producing- and non-producing strains. In above three strains, *S. cacaoi*, a β-lactam non-producing but β-lactamase producing strain, had PBPs which could bind methicillin and mecillinam like the PBPs in *Escherichia coli*[5,6].

In this paper, the affinities of β-lactams for PBPs in *S. cacaoi* and their antibacterial activities were examined and discussed, comparing with those in *B. subtilis* reported in the preceding paper[7]. In *B. subtilis*, a good correlation was observed between the antibacterial activity and the affinity for PBPs of these β-lactams. Thus, this comparison should indicate the possible lethal target of β-lactam antibiotics among PBPs in *S. cacaoi*.

Materials and Methods

Streptomyces strain

Streptomyces cacaoi subsp. *cacaoi* KCC S-0352 was a generous gift from Dr. A. SEINO of Kaken Chemicals Co.

Chemicals

All the chemicals used in this paper were the same as those in the preceding paper[7].

Antibacterial activity

The antibacterial activity of β-lactams were compared by the disc method. The mycelia were harvested from the culture[4], washed and resuspended in 0.85% sodium chloride solution. The suspen-

sion was homogenized in an ice bath by Polytron (Kinematica, Switzerland) 4 times for 2 minutes and by sonication twice for 20 seconds. The homogenized mycelial suspension whose absorbance at 600 nm was about 8, was added to the nutrient agar at 1.5% concentration. The growth inhibition was determined after incubation at 27°C for 2 days.

Other methods

Membrane fractions of *S. cacaoi* were prepared as described previously[4].

Binding of [^{14}C] benzylpenicillin to the membrane and slab gel electrophoresis-fluorography were performed as described[7].

Fig. 1. Fluorographic patterns of PBPs in *S. cacaoi*.

Slot 19 is control. In other slots, membrane samples were preincubated with β-lactams.

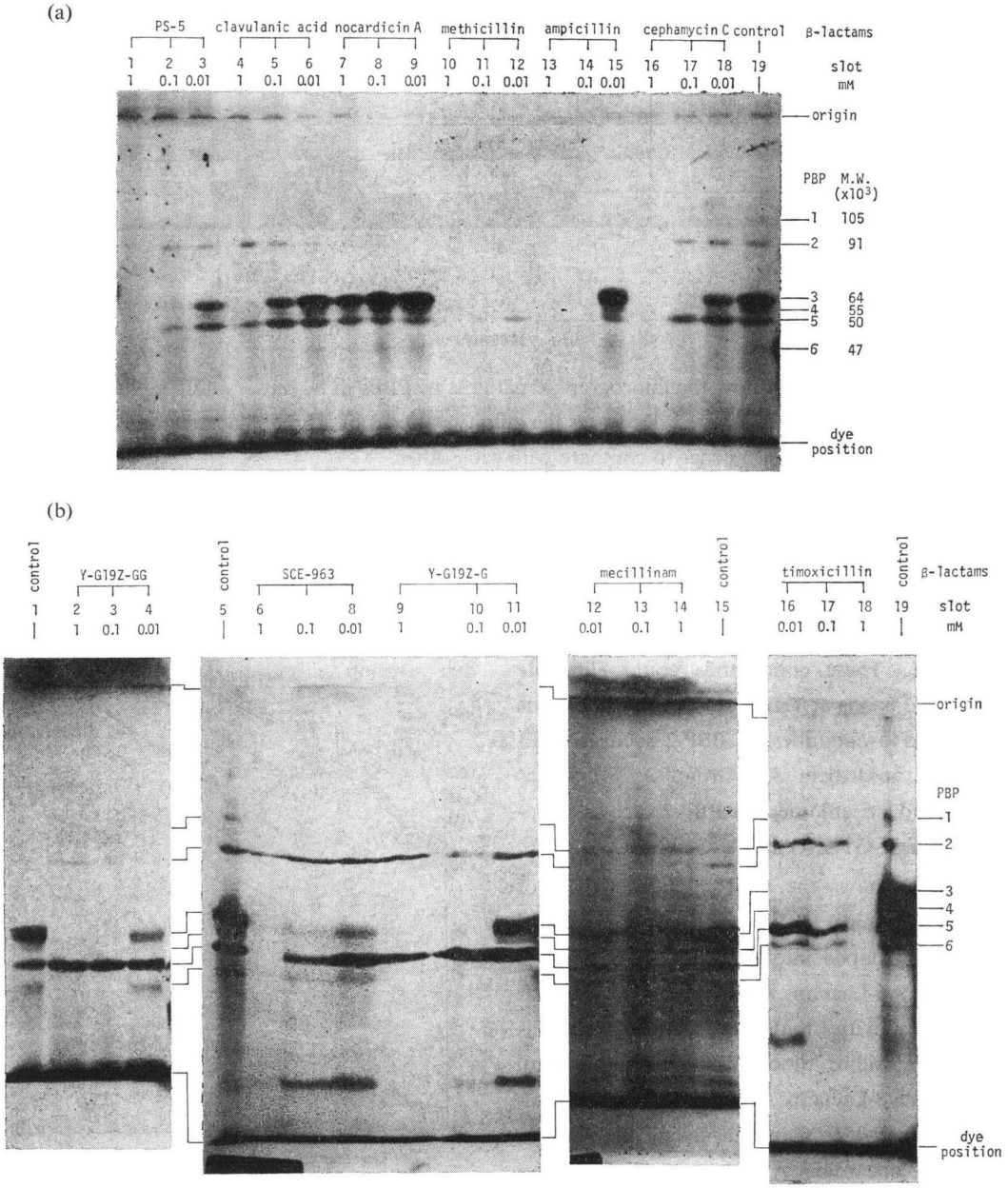
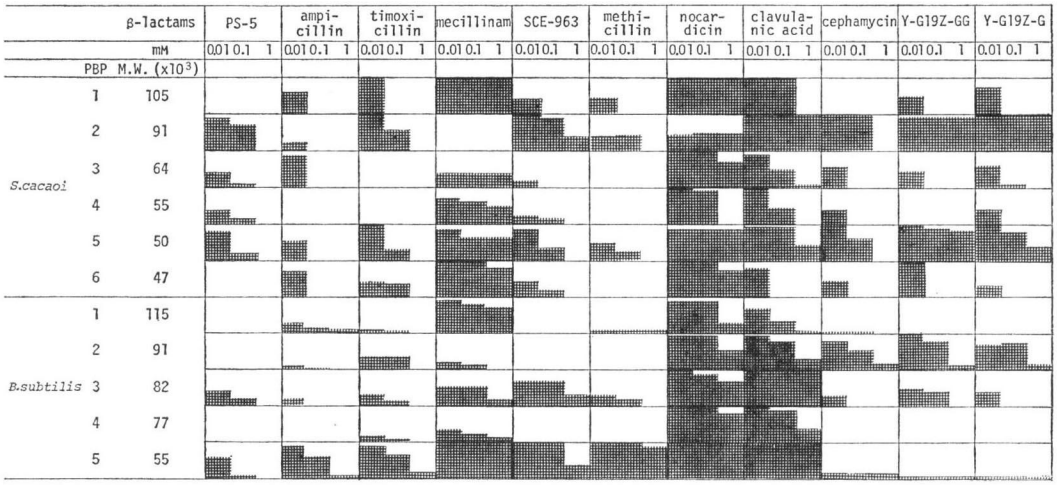


Fig. 2. The illustration of the affinity patterns of all the β-lactams for PBPs in *S. cacaoi* and *B. subtilis*.
The rods in each columns indicate the percentage of residual binding capacity of [^{14}C] benzylpenicillin to PBPs after the treatment with β-lactam compounds.



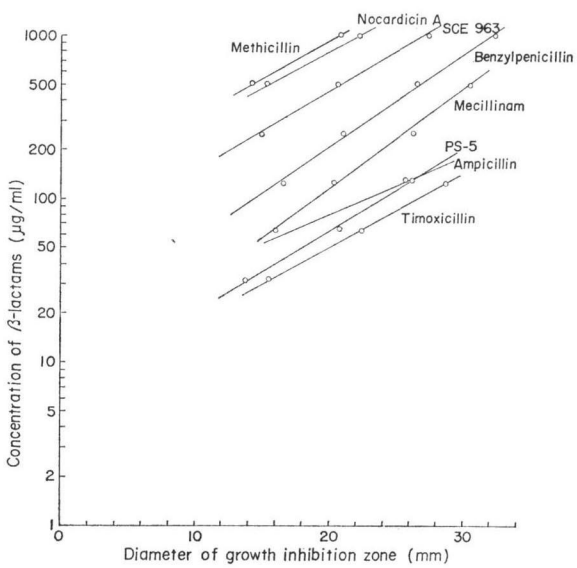
Results

Figs. 1(a) and 1(b) show the fluorographic patterns of PBPs in *S. cacaoi* and the effects of several β-lactams on these PBPs. Fig. 2 illustrates the affinity patterns of all the β-lactams tested for PBPs in *S. cacaoi* and *B. subtilis* in order to compare with each other.

In *S. cacaoi*, β-lactams could be classified into three groups on the basis of their affinities for PBP-2. Group 1: β-Lactams which had high affinities for PBP-2 such as PS-5, ampicillin, timoxicillin, mecillinam, methicillin and cephamycin C. These compounds could eliminate the PBP-2 bands at 1 mM. Group 2: β-Lactams which had low affinities for PBP-2 such as SCE-963 and nocardicin A. Group 3: β-Lactams which had no affinities for PBP-2 such as clavulanic acid, Y-G19Z-G and Y-G19Z-GG.

Fig. 3 shows the antibacterial activities of β-lactams against *S. cacaoi*. These β-lactams could be grouped into A, B and C as follows: Group A: β-Lactams which had stronger antibacterial activities than benzylpenicillin such as PS-5, ampicillin, timoxicillin and mecillinam. Group B: β-Lactams which had weaker antibacterial activities than benzylpenicillin such as SCE-963, nocardicin A and methicillin. Group

Fig. 3. Antibacterial activities against *S. cacaoi*, determined by the disc method.
Absolute values of diameter of growth inhibition zone were not the same in another experiment, but the order of antibacterial activity was the same.



C: β-Lactams which had no antibacterial activities against *S. cacaoi* such as clavulanic acid, cephamycin C, Y-G19Z-G and Y-G19Z-GG.

The groups of β-lactams determined by their affinities for PBP-2 had a good correlation with those defined by their antibacterial activities with a few exceptions, especially in the case of cephamycin C.

Discussion

The penicillin-binding proteins (PBPs) have been studied in many bacteria. In some bacteria, the lethal target of β-lactam antibiotics and the functions of PBPs in cell division and cell elongation have been suggested[6,8,9]. In *Streptomyces* strains that produce β-lactam compounds, however, examination of the PBPs may throw light on the elucidation of the self resistance mechanism. Previously we reported that the PBPs detected in *S. clavuligerus* which produced clavulanic acid and cephamycin C and those in *S. olivaceus* which produced MC-696-SY2-A could bind neither clavulanic acid and cephamycin C (natural products) nor mecillinam and methicillin (semi-synthetic compounds). These characters were markedly different from those in other bacteria[5,6]. We show in this paper, that the PBPs in *S. cacaoi* which does not produce a β-lactam compound have a character similar to those observed in *B. subtilis*[7], and that the PBP-2 may be the lethal target of β-lactam antibiotics in *S. cacaoi* as follows.

First, the classification into the groups 1, 2 and 3 of the β-lactams determined by their affinities for PBP-2 had a good correlation with those classified by their antibacterial activities.

Second, as observed in Fig. 2, the binding patterns of β-lactams for PBPs in *S. cacaoi* and *B. subtilis* had similarities in many points. Nocardicin A and clavulanic acid had low affinities for PBPs in both strains. The affinity patterns of cephamycin C, Y-G19Z-G and Y-G19Z-GG for PBPs in both strains resembled each other and these β-lactams had weak antibacterial activities against both strains. Thus, although a discrepancy from the correspondence was observed in the cases of PS-5, ampicillin, SCE-963 and methicillin, these similarities indicate the possibility that the PBP-1 to PBP-5 in *S. cacaoi* would correspond to PBP-1 to PBP-5 in *B. subtilis*, respectively. In addition, the difference of their affinities for PBPs, especially PBP-2, reflected upon their antibacterial activities. Consequently, PS-5 and ampicillin had strong antibacterial activities against both strains and showed strong affinities for PBP-2 in both strains. SCE-963 and methicillin had strong antibacterial activities against *B. subtilis* but weak activities against *S. cacaoi*. In accord with these facts, these compounds had strong affinities for PBP-2 in *B. subtilis* but fair affinities for PBP-2 in *S. cacaoi*. The small difference in the antibacterial activities between SCE-963 and methicillin (Fig. 3) may be explained by the fact that methicillin is hydrolyzed more easily than SCE-963 by the β-lactamase. *S. cacaoi* produces a β-lactamase hydrolyzing methicillin as well as benzylpenicillin[10]. Although this activity could not be detected in the membrane fraction, it could have an influence upon the result of the antibacterial activity.

Third, the binding patterns of many β-lactams for PBP-5 were similar to those for PBP-2. However, the possibility that PBP-5 was the target was eliminated by the facts that mecillinam had low affinity for PBP-5 but strong antibacterial activity and that SCE-963 had a fair affinity for PBP-5 but did not show strong antibacterial activity. PBP-5 may be DD-carboxypeptidase as the molecular weight is similar to that in other *Streptomyces* strains[11].

These results suggest that the affinity for PBP-2 in *S. cacaoi* is correlated with the antibacterial activity and, in addition, the PBP-2 is the lethal target of these β-lactams in *S. cacaoi* as in the case of *B. subtilis*.

Among the β-lactams that had stronger antibacterial activities than benzylpenicillin against *S. cacaoi*, mecillinam had a higher affinity for PBP-2 but weaker antibacterial activity than PS-5, ampicillin and timoxicillin. One possible explanation for this is the different stabilities of complex of PBP-2 and mecillinam, or the differences in their permeability, although the exact reason is not clear at the present time.

Acknowledgments

We are grateful to Prof. H. UMEZAWA for his hearty encouragement throughout the present work. We also thank Dr. A. SEINO for his kind gift of *Streptomyces* strain, and Drs. T. ISHIKURA, K. NARA, S. WATANABE, T. HIRAOKA and H. IMANAKA for their kind supplies of β-lactams. We thank the Institute of Microbial Chemistry and the Ministry of Education, Science and Culture in Japan for the kind support of this work.

References

1) OGAWARA, H.: Production and property of β-lactamase in *Streptomyces*. Antimicr. Agents & Chemoth. 8: 402~408, 1975
2) OGAWARA, H.; S. HORIKAWA, S. SHIMADA-MIYOSHI & K. YASUZAWA: Production and property of β-lactamase in *Streptomyces*: Comparison of the strains isolated newly and thirty years ago. Antimicr. Agents & Chemoth. 13: 865~870, 1978
3) NAGARAJAN, R.: β-Lactam antibiotics from *Streptomyces*. *In* E. H. FLYNN (*ed.*), Cephalosporins and Penicillins. pp. 636~661, Academic Press Inc., New York, 1972
4) OGAWARA, H. & S. HORIKAWA: Penicillin-binding proteins of *Streptomyces cacaoi*, *Streptomyces olivaceus*, and *Streptomyces clavuligerus*. Antimicr. Agents & Chemoth. 17: 1~7, 1980
5) HORIKAWA, S. & H. OGAWARA: Membrane-bound penicillin-binding proteins of *Escherichia coli*: Comparison of a strain carrying an R-factor and the parent strain. J. Antibiotics 31: 1283~1291, 1978
6) SPRATT, B. G.: Distinct penicillin-binding proteins involved in the division, elongation and shape of *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S.A. 72: 2999~3003, 1975
7) HORIKAWA, S. & H. OGAWARA: Penicillin-binding proteins in *Bacillus subtilis*. The effects on penicillin-binding proteins and the antibacterial activities of β-lactams. J. Antibiotics 33: 614~619, 1980
8) MATSUHASHI, M.; Y. TAKAGAKI, I. MARUYAMA, S. TAMAKI, U. NISHIMURA, H. SUZUKI, U. OGINO & Y. HIROTA: Mutants of *Escherichia coli* lacking in highly penicillin-sensitive D-alanine carboxypeptidase activity. Proc. Natl. Acad. Sci. U.S.A. 74: 2476~2479, 1977
9) IWAYA, M. & J. L. STROMINGER: Simultaneous deletion of D-alanine carboxypeptidase 1B-C and penicillin-binding component IV in a mutant of *Escherichia coli* K12. Proc. Natl. Acad. Sci. U.S.A. 74: 2980~2984, 1977
10) OGAWARA, H.: Actinomycete Biology. *In* K. P. SCHAAL (*ed.*), Gustav Fischer Verlag, Stuttgart (in press)
11) GHUYSEN, J. M.: The Bacterial DD-Carboxypeptidase-Transpeptidase Enzyme System. University of Tokyo Press, Tokyo, 1977