INTERACTION OF β-LACTAMASE OF *STREPTOMYCES CACAOI*

II. CP-45,899, IZUMENOLIDE AND CEPHAMYCINS

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Inhibition of a β-lactamase of *Streptomyces cacaoi* by CP-45,899, izumenolide and cephamycins was investigated and compared with that of a β-lactamase of *Bacillus cereus*. *S. cacaoi* enzyme could not hydrolyze CP-45,899. Instead, hydrolysis of benzylpenicillin by the enzyme was inhibited in the presence of CP-45,899. Although inhibition increased gradually with time, the inhibition line produced by CP-45,899 with time was less curved than that produced by clavulanic acid and PS-5. Furthermore, preincubation of *S. cacaoi* β-lactamase with CP-45,899 for up to 120 seconds did not obviously affect the degree of inhibition. When the concentration was lowered, it behaved as a competitive inhibitor, a *Ki* value being 6.2×10^{-7} M. Izumenolide, on the other hand, did not inhibit the enzyme activity of *S. cacaoi* β-lactamase at 1.28×10^{-4} M, although it inhibited *B. cereus* enzyme slightly in a competitive manner. Oganomycins were inert to the both β-lactamases.

In an accompanied communication, inhibition of β-lactamases of *Streptomyces cacaoi* and *Bacillus cereus* by clavulanic acid and PS-5 was described[1]. This paper reports the effect of CP-45,899, izumenolide and oganomycins (cephamycins) on the enzymatic activity of β-lactamase of *Streptomyces cacaoi*, compared with that of *B. cereus*.

Materials and Methods

Chemicals

CP-45,899 (reference 2; a penicillanic acid sulfone) and oganomycins[3] were obtained from Taito-Pfizer Co. and Yamanouchi Pharmaceutical Co., respectively. Izumenolide[4] was a generous gift from Dr. R. B. SYKES of the Squibb Institute for Medical Research.

β-Lactamases

β-Lactamase from *S. cacaoi* was prepared as described previously[5]. *B. cereus* β-lactamase was obtained from Calbiochem and used as described[6]. Other materials and methods were described in an accompanying paper[1].

Results and Discussion

Hydrolysis of CP-45,899 was followed by change in the absorption spectrum at 229 nm, assuming $\Delta\varepsilon$ being 860. *B. cereus* β-lactamase could hydrolyze CP-45,899 at about one 180th the rate of benzylpenicillin. In contrast, *S. cacaoi* β-lactamase could not change the absorption spectrum of CP-45,899 at 0.784 mM in 0.1 M sodium phosphate buffer of pH 7.0 following 1 hour incubation.

When hydrolysis of benzylpenicillin by *S. cacaoi* enzyme was allowed to occur in the presence of CP-45,899, inhibition increased gradually with time (Fig. 1A). Similar patterns were also observed with *B. cereus* enzyme (Fig. 1B). However, compared with the case of clavulanic acid and PS-5 (reference 1), the inhibition line of CP-45,899 with time was not so significantly bent. Especially in the case of the *B.*

Fig. 1. Time course of hydrolysis of benzylpenicillin in the presence of various concentrations of CP-45,899.

(A): *S. cacaoi* β-lactamase. (B): *B. cereus* β-lactamase.

The numbers in the figure are the molar ratios of the inhibitor to benzylpenicillin. The concentration of benzylpenicillin was 0.497 mM.

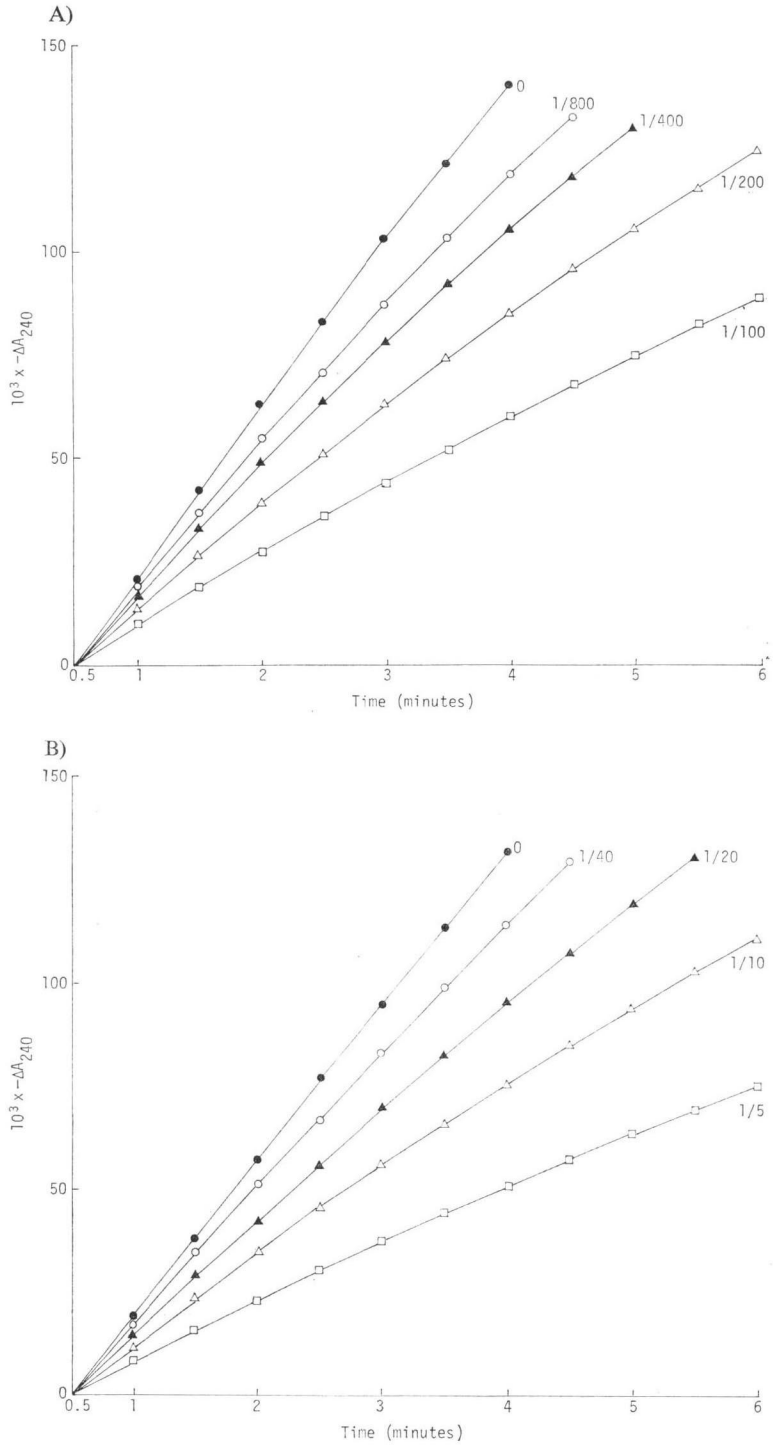
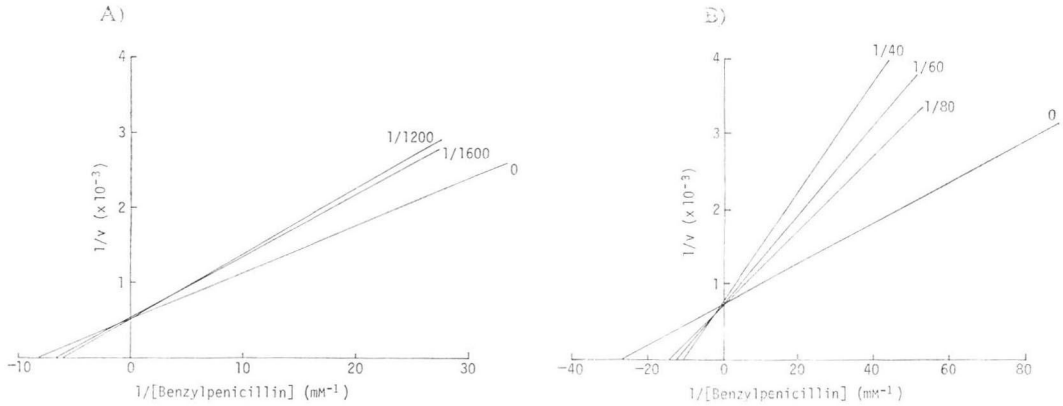


Fig. 2. The double reciprocal plots of the inhibition by CP-45,899.
(A): *S. cacaoi* β-lactamase. (B): *B. cereus* β-lactamase.
The numbers in the figure are the molar ratios of the inhibitor to benzylpenicillin. The concentration of benzylpenicillin was 0.333 mM.



cereus enzyme, an almost straight line was observed (Fig. 1B). Furthermore, when the enzymes were preincubated with CP-45,899 for 0 to 120 seconds and then the remaining enzyme activity was determined no apparent difference in the degree of inhibition was observed among them. Therefore, it is concluded that the mechanism of inhibition by CP-45,899 and/or the rate constants are different from that by clavulanic acid and PS-5. The fact that ratio of the concentrations showing the same degree of inhibition by clavulanic acid and CP-45,899 was different between *S. cacaoi* enzyme and *B. cereus* enzyme supports the above notion.

In spite of these facts, when the concentration of CP-45,899 was lowered, it behaved as a competitive inhibitor for both the enzymes from *S. cacaoi* (Fig. 2A) and *B. cereus* (Fig. 2B). *Ki* values calculated from the integrated form of LINEWEAVER-BURK type were 6.2×10^{-7} M and 5.5×10^{-6} M for *S. cacaoi* and *B. cereus* enzymes, respectively.

Turning to izumenolide, this did not show the progressive inhibition of the enzymatic activity of either enzyme, in contrast to other inhibitors described previously[1] and above, and other enzymes[4,7]. In the case of *B. cereus* β-lactamase, izumenolide even at high concentrations of 0.64 and 1.28×10^{-4} M inhibited the enzymatic activity competitively. *Ki* value calculated by the integrated equation of the LINEWEAVER-BURK type was found to be 1.5×10^{-4} M. On the contrary, the β-lactamase from *S. cacaoi* could not be inhibited even after incubation for 10 minutes with 1.28×10^{-4} M izumenolide at 30°C. This is in accord with the fact that izumenolide inhibits β-lactamases from Gram-negative bacteria strongly but those from Gram-positive organisms less strongly. These facts suggest that although the β-lactamase from *S. cacaoi* is similar to that from Gram-negative bacteria in that both enzymes can catalyze the hydrolysis of cloxacillin and methicillin[5], it also possesses properties of the β-lactamases from Gram-positive bacteria.

As for cephamycin group antibiotics, oganomycin G and oganomycin GG[3] could not be hydrolyzed by the above two enzymes, since no changes occurred in their absorption spectra during incubation at 30°C for over 1 hour. In addition, both compounds failed to inhibit the hydrolysis of benzylpenicillin by either the *B. cereus* or *S. cacaoi* enzyme. This indicates that methoxy group of cephamycin has a significant role in the binding of β-lactams to the above enzymes.

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