Inhibition of β-Lactamase II of *Bacillus cereus* by Penamaldic Derivatives of Penicillins

Pilar Gutiérrez Navarro,* Bartolome Quintero Osso, Raquel García Ortiz, Pedro J. Martínez de las Parras, María I. Martínez Puente, and M. Carmen Cabeza González

Department of Physical Chemistry, School of Pharmacy, University of Granada, E-18071 Granada, Spain

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Bacteria have developed various strategies to deactivate β-lactam antibiotics, including the production of β-lactamase enzymes. These have been grouped in four classes (6, 10). Classes A, C, and D contain a catalytic serine residue in their active sites, and several have inhibitors utilized in therapeutics (4, 15, 16). Class B metalloenzymes require one or two zinc ions to carry out the hydrolysis of the β-lactams (3) and act on a wide range of β-lactam antibiotics, including carbapenems and inhibitors of the serine-dependent enzymes (12). Over the past 10 years, several inhibitors of these enzymes have been discovered, for instance, two esters of benzylxoycarbonyl-

The penamaldic derivatives of amoxicillin, ampicillin, and penicillins G and V, stabilized with Zn\(^{2+}\), were obtained from a methanolic medium. The enzymatic kinetic results show that the these derivatives elicit reversible inhibition of the enzyme metallo-β-lactamase from *Bacillus cereus*, with inhibition constant values determined at pH 7.0 and 25°C.

The incubation time of the inhibitor-enzyme mixture did not significantly modify the inhibition process, as demonstrated in kinetic experiments in which the enzyme and the inhibitor were incubated for different times (0, 5, and 10 min). At the end of the reaction, when the same concentration of substrate as that initially used was again added to the reaction medium, degradation of the substrate occurred at a similar rate. This finding points to the reversibility of the enzyme-inhibitor interaction. Furthermore, the value of apparent \(K_m\) is unaltered by the addition of the penamaldic derivatives—unlike the apparent \(K_m\) value—indicating that that these act as competitive inhibitors of the metallo-β-lactamase from *B. cereus*.

Since the β-lactamase II of the commercial *B. cereus* used was stabilized with bovine serum albumin, we chose to determine whether the enzymatic activity was affected by the albumin's presence. A known inhibitor of this β-lactamase, captopril, with an inhibition constant \(K_i\) of 41.6 ± 9 μM (1), was used. Under conditions described previously and using the commercial enzyme, we found the same inhibition constant (33...
indicating that the presence of albumin does not protect the enzyme from the inhibitory activity of captopril. Based on the absorbance-time kinetic curves, the values of the initial rate of absorbance change over time, $V_{ap}$, were calculated for several substrate concentrations in the absence of the inhibitor and at different concentrations of the inhibitor and then plotted according to the equation:

$$\frac{1}{V_{ap}} = \frac{1}{\Delta \varepsilon V_{max}} + \frac{K_m}{\Delta \varepsilon V_{max}} \left(1 + \frac{[I]}{K_I}\right) \frac{1}{[S]_0}$$  \hspace{1cm} (1)

where $\Delta \varepsilon$ is the change in the absorption coefficient during the enzymatic reaction, $[I]$ is the concentration of the inhibitor, and $[S]_0$ is the initial substrate concentration.

Equation 1 gives a straight line for each inhibitor concentration. When the slope of each line is plotted against the inhibitor concentration, the abscissa value where the line converges on the x axis gives the $K_I$. Values of 245 ± 11, 615 ± 10, 373 ± 12, and 522 ± 9 μM were obtained for the derivatives of amoxicillin, ampicillin, and penicillins G and V, respectively.

Of the four derivatives assayed, the best inhibitor was that of penicillin G. The inhibition constants for the derivatives of ampicillin and amoxicillin were also calculated with absorbance data from the complete kinetic curves corresponding to the degradation of the substrate, when $[S]_0 \ll K_m$ and the enzymatic reaction takes place according to a first-order process with respect to the substrate, represented by the equation:

$$\ln[A_i - A_s] = \ln[A_0 - A_s] - \frac{V_{max}}{K_m} \left(1 + \frac{[I]}{K_I}\right)$$  \hspace{1cm} (2)

The inverse values of the slopes of each straight line plotted against the inhibitor concentration (Fig. 2) give $K_I$. The values found were 406 ± 8 μM for the amoxicillin derivative and 624 ± 15 μM for the ampicillin derivative.

This method could not be used for the penamaldates of penicillins G and V since the total change in absorbance was not that expected for the degradation of the substrate in the enzymatic reaction.

The penamaldic derivatives do not exert their inhibitory activity by chelating the Zn$^{2+}$ bound to the enzyme, as the activity of the enzyme is not reestablished by the supplementary addition of external Zn ions in the form of zinc nitrate (50 μM). In view of this result, it is difficult to establish a mechanism for the interaction between the inhibitor and the $\beta$-lactamase. However, since the presence of a thiol group is considered indispensable (1) for a compound to bind with an enzyme and inhibit the metallo-$\beta$-lactamase, one possibility is that equilibrium is established between the penamaldic derivative bonded to the metal ion and that bound to the enzyme through the thiol group. This means that the enzyme and Zn compete to bind the inhibitor. Bearing in mind the large size of the molecule of the 2:1 chelate of the penamaldic derivative, this mechanism would be the most probable one.
REFERENCES


