ob mouse as far as the response of BAT 5'D to cold exposure and to noradrenaline is concerned.

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Letters to the Editor

The application of circular dichroism (CD) to a binding study of latamoxef and β-lactamase

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β-lactam antibiotics (BLA) exert their action on two main groups of bacterial enzymes. One group, the cell wall synthesizing enzymes, penicillin binding proteins (PBPs), are inactivated by BLA. The second group, the β-lactamases, inactivate BLA and thereby protect the cell against BLA attack.

The difference in binding ability of BLA to these two groups of enzymes is closely correlated with BLA action.

In the present study, the antibiotic latamoxef has been subjected to a binding study with β-lactamase because latamoxef undergoes little hydrolysis by β-lactamase but has a high affinity for the enzyme. A significant spectral change in circular dichroism (CD) was observed after mixing it with β-lactamase. Our findings are given herein.

Method

Latamoxef was kindly donated from Shionogi Ph. Co. Ltd., Osaka, Japan. β-lactamase was prepared according to the method of Minami et al (1980). Briefly, *Enterobacter cloacae* NUH10 isolated clinically from Nagasaki University Hospital were harvested by centrifugation and washed twice with 0·05 M phosphate buffer (pH 7·0). The cells, suspended in 200 mL of the same buffer, were disrupted by an ultrasonic oscillator (Branson sonifier cell disrupter 185) in an ice bath. The sonicate was centrifuged at 1500 *g* for 30 min at 4°C, to give the crude β-lactamase in the supernatant fraction. The crude β-lactamase was purified with column chromatography using Carboxymethyl-Sephadex C-50 (Pharmacia Fine Chemicals Inc., Uppsala, Sweden).The β-lactamase used in CD measurement was the enzyme showing an absorbance 2·98 at 278 nm. CD measurements were using a JASCO Model J-500A spectropolarimeter in cells of pathlength 10 mm. The dynode voltage was kept below 600 V and measurement was made at room temperature (22°C). CD studies of latamoxef-β-lactamase interaction were carried out between 280 and 350 nm and the following conditions: sensitivity; 1 milli degree cm^{-1}, time constant; 8 s, wavelength expansion; 10 nm cm^{-1}. The observed ellipticities were the actual CD of the latamoxef-β-lactamase complex, while the

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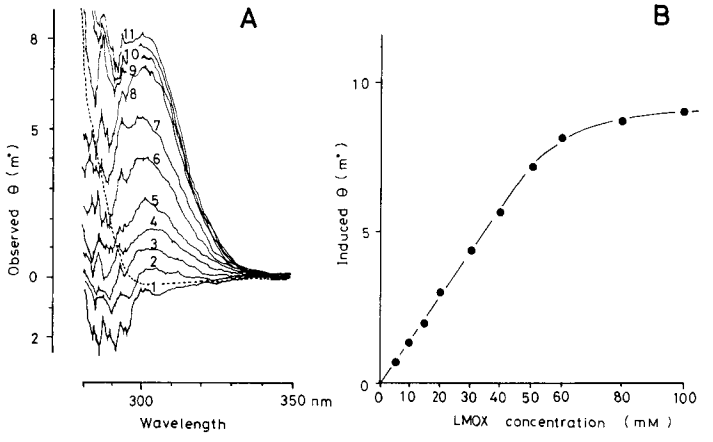

FIG. 1. Observed ellipticities (A) and induced ellipticities (B) of latamoxef(LMOX)-β-lactamase complexes at various concentration of antibiotic at pH 7·0 in 10 mm cells. β-lactamase with an absorbance of 2·98 at 278 nm was used in the CD experiment. The CD of LMOX (200 mM) alone is shown with dotted line. LMOX concentrations (mM) are: 1 = 0, 2 = 5, 3 = 10, 4 = 15, 5 = 20, 6 = 30, 7 = 40, 8 = 50, 9 = 60, 10 = 80, 11 = 100.

induced ellipticity was derived from the observed ellipticity of the complex minus the ellipticity of β-lactamase alone at 300 nm.

Results

The observed CD spectra of the latamoxef-β-lactamase complex are shown in Fig. 1A. The extrinsic Cotton effects generated by the binding of latamoxef to β-lactamase arise only from the bound fraction of antibiotic at 300 nm at pH 7·0. It follows from Fig. 1B that the induced ellipticity increases proportionally with the increase in the antibiotic concentration below 50 mM and is rapidly saturated thereafter. The non-sigmoidal character of the titration curves suggest latamoxef binding to one site on the β-lactamase. Therefore, the method of drawing a tangent to a plot of induced ellipticity against antibiotic concentration, which allows an estimation of free and bound fractions of the drug (Rosen 1970; Matsuyama et al 1987), can be used for the determination of the binding constant after confirmation of the molar extinction coefficient of β-lactamase at 278 nm.

BLA are effective antibacterial agents only if they can reach penicillin-binding proteins. However, β-lactamase inactivates BLA before they gain access to those proteins. The evidence of the hyperproduction of β-lactamase found in a resistant strain, i.e. *Enterobacter cloacae* against BLA (Bruno et al 1987; Lindberg & Normark 1987), suggests that kinetic factors for the binding of β-lactamase and BLA are the most important determinants in the fate of BLA. From that point of view, the binding of β-lactamase and BLA has been widely studied (Fisher et al 1980; Bush et al 1982; Seeberg et al 1983). However, those authors expressed potency as indirect parameters, i.e. K_m or K_i values. The present study has shown the possibility for the binding constant of β-lactamase and BLA to be determined using the CD method without consumption of much β-lactamase.

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