IMMUNOLOGICAL PROPERTIES OF A NOVEL β-LACTAMASE PRODUCED BY *BACTEROIDES FRAGILIS*

AKIRA YOTSUJI, SHINZABUROU MINAMI, SEIKI OKAMOTO, TAKASHI YASUDA, AKIRA TAKAI, ISAMU SAIKAWA, MATSUHISA INOUE† and SUSUMU MITSUHASHI†

Research Laboratory, Toyama Chemical Co. Ltd., Toyama
†School of Medicine, Gunma University, Maebashi, Japan

(Received for publication June 23, 1984)

In the previous paper[1], we reported the comparative study of the enzymatic and physicochemical properties of a novel β-lactamase produced by *Bacteroides fragilis* G-237 with those of a typical *B. fragilis* β-lactamase produced by *B. fragilis* G-242. The enzyme produced by *B. fragilis* G-237 showed a unique substrate profile in hydrolyzing cephalosporins including 7α-methoxycephalosporins, penicillins and carbapenems. The enzyme activity was inhibited by *p*-chloromercuribenzoate and iodine, but not by clavulanic acid and sulbactam. The isoelectric point was 4.8, and molecular weight was estimated to be 26,000. This paper deals with the immunological properties of purified β-lactamase from *B. fragilis* G-237.

Bacterial strains used in this study were: *B. fragilis* G-237 and G-242, *Bacteroides vulgatus* G-101, *Bacteroides thetaiotaomicron* G-282, *Bacteroides ovatus* G-53, *Bacteroides distasonis* G-50, *Bacteroides uniformis* G-294, *Proteus vulgaris* GN7971[2], *Pseudomonas cepacia* GN11164[3], Rtk3$^+$/*Escherichia coli* TK-3 (penicillinase type I) (Richmond class III)[4,5,6], Rms213$^+$/*E. coli* W3630 (PCase type II) (Richmond class V type a)[7], Rte16$^+$/*E. coli* W3630 (PCase type III) (Richmond class V type b)[8], Rms219$^+$/*Pseudomonas aeruginosa* M1 (PCase type IV) (Richmond class V type d), *Staphylococcus aureus* F-186 (PCase type V), *E. coli* GN5482[9] and *Enterobacter cloacae* H-27.

For the preparation of crude enzymes, we followed the method described previously[10]. For the production of specific anti-enzyme serum, the enzymes from *B. fragilis* G-237 and G-242 were purified in a previously-described manner[1]. Other enzymes were purified by absorption and elution on a DEAE-Sephadex A-50 column or CM-Sephadex C-50 column[11]. Antisera against purified *B. fragilis* G-237 and G-242 enzymes were obtained from rabbits. One-tenth milligram of enzyme protein was dissolved in 0.5 ml of saline, emulsified with 0.5 ml of Freund complete adjuvant (Difco), and used for the first injection. An injection of 0.5 ml was given under the skin of the footpad, and the remaining dose was given in the thigh. Three weeks after the first injection, a booster injection containing 0.1 mg of enzyme protein in 0.5 ml of saline was administered intravenously. Antisera were collected 2 weeks after the last injection. The immunological identification of a β-lactamase was demonstrated by a neutralization test using spectrophotometric method[10]. The enzyme solution was mixed with the antiserum at 37°C for 1 hour and then kept at 0°C for 18 hours. The mixture was centrifuged for 10 minutes at 3,000 rpm, and the remaining enzyme activity in the supernatant was assayed[12].

The β-lactamase activity was inhibited by specific anti-enzyme serum. The standard neutralization curve was obtained by adding increasing quantities of antiserum to a fixed quantity of antigen (Fig. 1). The enzyme activities were al-

Fig. 1. Neutralization of the activities of β-lactamase from *B. fragilis* G-237 (○) or G-242 (△) by each homologous antiserum.

β-Lactamase activity was assayed by spectrophotometry. Cephaloridine (100 μM) was used as a substrate.

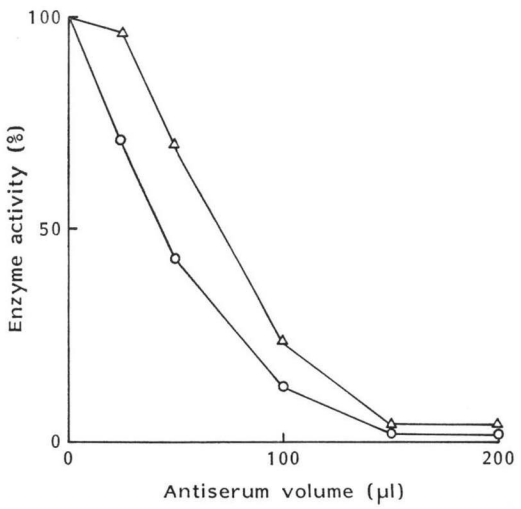

Table 1. Neutralization of β-lactamase activities with anti-β-lactamase sera.

β-Lactamase prepared from	Neutralization (%) by antisera against the enzyme of[a]:	
	B. fragilis G-237	*B. fragilis* G-242
B. fragilis G-237	98	30
B. fragilis G-242	60	96
B. vulgatus G-101	96	45
B. uniformis G-294	50	30
B. thetaiotaomicron G-282	0	0
B. ovatus G-53	0	0
B. distasonis G-50	0	0
P. vulgaris GN7971	0	0
P. cepacia GN11164	0	0
Rtk3$^+$/*E. coli* TK-3	0	0
Rms213$^+$/*E. coli* W3630	0	0
Rte16$^+$/*E. coli* W3630	0	0
Rms219$^+$/*P. aeruginosa* M1	0	0
S. aureus F-186	0	0
E. coli GN5482	0	0
E. cloacae H-27	0	0

[a] Neutralization of β-lactamase activity was expressed by the following equation: (a−b)/a (%), where a is enzyme activity without antiserum, and b is enzyme activity with antiserum.

most completely neutralized after 60 minutes of incubation at 37°C with 150 μl of homologous antiserum.

The neutralizing activities of antisera to *B. fragilis* G-237 and G-242 were examined by using various β-lactamases obtained from 16 strains of clinical isolates including the above two strains.

As shown in Table 1, antiserum to *B. fragilis* G-237 β-lactamase cross-reacted with enzyme from *B. vulgatus* G-101, and showed weak cross-reaction with enzymes from *B. fragilis* G-242 and *B. uniformis* G-294. There was no cross-reaction with enzymes from other strains. Antiserum to *B. fragilis* G-242 β-lactamase was cross-reactive with enzymes from *B. fragilis* G-237, *B. vulgatus* G-101 and *B. uniformis* G-294, but not cross-reactive with enzymes from other strains.

The novel β-lactamase produced by *B. fragilis* G-237 is unique in terms of it's broad substrate profile, inhibitory profile and physico-chemical properties[1]. Antisera to *B. fragilis* G-237 β-lactamase and G-242 β-lactamase cross-reacted with enzymes from *B. fragilis* groups, *i.e.*, *B. fragilis*, *B. vulgatus* and *B. uniformis*, but did not cross-react with enzymes from *B. thetaiotaomicron*, *B. ovatus* and *B. distasonis*. With regard to immunological properties of β-lactamases from *B. fragilis*, *B. vulgatus* and *B. thetaiotaomicron*, each of the mouse antisera to those three enzymes completely neutralized not only the activities of their own enzymes, but also those of the other enzymes from the *B. fragilis* groups[13]. However, in our observations, antiserum to *B. fragilis* G-242 showed no cross-reaction with the enzyme from *B. thetaiotaomicron*. This might be due to immunological differences between the two enzymes or related to the species of animal used for the production of anti-enzyme sera.

No cross-reaction was observed between antiserum to *B. fragilis* and the enzymes produced by *P. vulgaris* and *P. cepacia*. Although the β-lactamase produced by *B. fragilis* G-237 is very similar to oxacillin-hydrolyzing β-lactamases (PCase type II, III) (Richmond class V type a, b) and carbenicillin-hydrolyzing β-lactamases (PCase type V) (Richmond class V type d) mediated by a transferable R plasmid among members of the Enterobacteriaceae[4,5,6,14,15] in substrate profiles and physico-chemical properties, no immunological cross-reaction were exhibited between antiserum to *B. fragilis* G-237 and these enzymes.

Cross-reaction between antisera prepared to specific β-lactamases and β-lactamase produced by different species of bacteria are rare[4,6,12,16,17]. Immunological properties as well as enzymatic and physico-chemical properties of the enzyme from *B. fragilis* G-237 are different from those of the enzyme from *B. fragilis* G-242, and appear different from those of β-lactamases of any class proposed by MITSUHASHI and INOUE[4], RICHMOND and SYKES[5], and SYKES and MATTHEW[6].

Acknowledgment

We thank Dr. K. UENO for kindly supplying the clinical isolates of anaerobic bacteria.

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