PROPERTIES OF CEPHALOSPORINASE FROM *PROTEUS MORGANII*

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The cephalosporin β-lactamase (cephalosporinase) was purified from a strain of *Proteus morganii* which showed resistance to cephalosporins. The optimal pH was about 8.5, and the optimal temperature was 40°C. The isoelectric point was 8.7 and the molecular weight was estimated to be about 41,000 from sodium dodecyl sulfate-acrylamide gel electrophoresis. The enzyme activity was inhibited by cloxacillin, ampicillin, carbenicillin, cefuroxime, cefotaxime, ceftizoxime (FK749), cefmenoxime (SCE-1365), cefoxitin, cefmetazole, YM09330 and moxalactam (6059-S), but not by clavulanic acid or CP-45899. The β-lactamase also hydrolyzed cephaloridine, cefazolin, cephalothin, cephalexin, cefotiam, cefamandole and benzylpenicillin. These results suggest the possibility that the properties of β-lactamases may be characterized by measuring the kinetic parameters of the enzyme toward newly-introduced β-lactam antibiotics and β-lactamase inhibitors.

Since ABRAHAM and CHAIN[1] reported the production of β-lactamase by *Escherichia coli*, the enzyme has been considered to have a significant role in the resistance of organisms to β-lactam antibiotics.[2,3] On the one hand, β-lactamases from various bacteria have been classified with their substrate specificities, physical and biochemical properties and reactions with antisera.[3]

Recently, many β-lactam antibiotics have developed throughout the world. In our laboratory, we had a chance to investigate more broad substrate specificities by using newly-introduced drugs against 14 distinct β-lactamases. In the present work, an attempt has been made to study some of the properties of cephalosporinase produced by a strain of *Proteus morganii* GN5407. The identification of this enzyme and its distinction from other cephalosporinases were obtained by conventional methods with the addition of new substrates.

Materials and Methods

Bacterial Strains

P. morganii strains were isolated from clinical sources and β-lactam-resistant strains in which plasmids mediating penicillinase production were not detected were selected for this study.

Media

Heart infusion (HI) agar was a product of Eiken Chemical Co., Ltd. Medium-B and peptone water were used for liquid cultures. Medium-B consisted of 2 g of yeast extract, 10 g of polypeptone, 7 g of $Na_2HPO_4 \cdot 12H_2O$, 2 g of KH_2PO_4, 1.2 g of glucose and 0.4 g of $MgSO_4 \cdot 7H_2O$ in 1,000 ml of distilled water. Peptone water consisted of 10 g of polypeptone, 5 g of NaCl and 1,000 ml of distilled water.

Drug Resistance

Drug resistance was determined by using two-fold dilutions in HI agar with an inoculum of 10^4 organisms. The level of resistance was expressed as the minimum inhibitory concentration (MIC) of each drug. The MIC values were scored after 18 hours of incubation at 37°C.

Drugs

Cephalothin, cephaloridine, cefazolin, cephalexin, benzylpenicillin, ampicillin, cloxacillin and carbenicillin were commercially available materials. Cefamandole, cefuroxime, cefotaxime,[4] cefotiam,[5] cefoperazone,[6] ceftizoxime (FK749),[7] cefmenoxime (SCE-1365),[8] cefoxitin, cefmetazole,[9] YM09330,[10] moxalactam (6059-S, latamoxef),[11] clavulanic acid[12] and CP-45899[13] were used as newly-introduced drugs.

Culture and Harvesting of Organisms

Bacteria were grown overnight at 37°C in medium-B. The culture was diluted 10-fold with fresh medium-B broth and then grown at the same temperature. Ampicillin was added as an inducer for β-lactamase production to a final concentration of 100 μg/ml and the incubation was continued. The bacteria were harvested by centrifugation and washed twice with 50 mM phosphate buffer (pH 7.0). The cells obtained were then suspended with 50 mM phosphate buffer, pH 7.0 and the suspensions were disintegrated ultrasonically by treating for 5 minutes in an ultrasonic disintegrator (80W, 25 kc), and the suspensions were centrifuged at 10,000×g for 20 minutes at 4°C.

Enzyme Assay

β-Lactamase activity was determined by a spectrophotometric method[14,15] by measuring the change in absorption maximum for the β-lactam ring in a temperature-controlled spectrophotometer (Beckman model 24) at 30°C. Absorption maxima of β-lactam antibiotics were reported previously.[16] The absorption maxima of the newly-introduced drugs are moxalactam (275 nm), cefmenoxime (275 nm) and YM09330 (277 nm). One unit of enzyme activity was defined as the amount of enzyme which hydrolyzed 1 μmole of a substrate per minute at 30°C in 50 mM phosphate buffer (pH 7.0). Protein was determined by the LOWRY's method[17] with bovine serum albumin as a standard.

Determination of Molecular Weight

Molecular weight of the enzyme was determined by sodium dodecyl sulfate (SDS) polyacrylamide slab gel electrophoresis according to WEBER and OSBORN,[18] using bovine serum albumin (molecular weight, 68,000), ovalbumin (45,000), chymotripsinogen (25,000) and cytochrome C (12,500) as molecular weight standards. The gel concentration of SDS polyacrylamide gel electrophoresis was 10%.

Polyacrylamide Gel Electrophoresis

The purity of the enzyme preparation was checked by discontinuous gel electrophoresis, using 7.5% polyacrylamide gel, pH 4.0, as the separation gel.

Isoelectric Focusing

Electrofocusing was performed on a sucrose ampholyte gradient (pH 7~pH 11). The experiment was carried out at 4°C in the LKB 8100 (LKB-Produker AB, Stockholm-Bromma, Sweden) column over 48 hours with 300V. The contents of the column were collected in 3 ml fractions.

Immunological Methods

Antiserum against the purified enzyme from the strain *P. morganii* GN5407 was prepared from rabbits as described previously.[19] The effects of the antibodies on the β-lactamases were done by neutralization test of the hydrolyzing activity of the enzyme. Enzyme solution (3 units) was incubated with various amount of antibodies in 0.1 ml of 50 mM phosphate buffer, pH 7.0 at 37°C for 30 minutes and then left at 4°C for 18 hours.

Results

Resistance Levels and Substrate Specificity

All the strains of *P. morganii* were resistant to cephaloridine, cephalexin, cephalothin, cefazolin, benzylpenicillin, ampicillin and cloxacillin, and sensitive to carbenicillin and newly-introduced compounds such as cefamandole, cefotiam, cefoperazone, cefotaxime, ceftizoxime, cefmenoxime, moxalactam and YM09330 (Table 1).

Three strains of *P. morganii* (GN5307, GN5375 and GN5407) were found to synthesize an inducible

Table 1. Comparative activity of β-lactam antibiotics against *Proteus morganii*.

Drug	MIC (μg/ml)		
	P. morganii GN5307	*P. morganii* GN5375	*P. morganii* GN5407
Cephaloridine	200	200	400
Cephalexin	200	200	400
Cephalothin	>800	>800	>800
Cefazolin	100	200	200
Benzylpenicillin	400	400	800
Ampicillin	100	100	200
Carbenicillin	0.39	0.78	0.78
Cloxacillin	200	200	400
Cefotiam	0.39	0.39	0.78
Cefamandole	0.78	0.78	0.78
Cefoperazone	0.78	0.78	0.78
Cefuroxime	25	25	50
Cefotaxime	0.025	0.025	0.025
Ceftizoxime	0.05	0.0125	0.025
Cefmenoxime	0.025	0.0125	0.0125
Cefoxitin	12.5	12.5	12.5
Cefmetazole	6.25	6.25	6.25
YM09330	0.39	0.39	0.78
Moxalactam	0.20	0.20	0.10

cephalosporinase. The cephalosporinase produced by these strains hydrolyzed cephaloridine, cephalothin and benzylpenicillin more rapidly than cefazolin, cephalexin and cefotiam. However cefamandole was hydrolyzed at a low rate. *P. morganii* GN5407 was found to have the highest level of enzyme activity and was used as a source for purification of a β-lactamase.

Enzyme Purification

The purifications are summarized in Table 2. The purity of the enzyme preparation was checked by discontinuous gel electrophoresis, using a 7.5% polyacrylamide gel, pH 4.0, as a separation gel. The purified enzyme showed a single protein band in discontinuous gel electrophoresis.

Table 2. Summary of purification of β-lactamase from *Proteus morganii* GN5407.

Stage No.	Procedure*	Total protein (mg)	Specific activity (U/mg of protein)	Recovery (%)
1.	Ultrasonic disintegration	6,750	0.5	100
2.	Streptomycin treatment	6,413	0.3	56
3.	Chromatography on DEAE-Sephadex	1,698	0.9	45
4.	Chromatography on CM-Sephadex	808	13.9	35
5.	Gel filtration on Sephadex G-100	40	24.0	28

* Abbreviations. DEAE, diethylaminoethyl; CM, carboxymethyl.

Characterization of Cephalosporinase

From the electrophoretic mobility on the SDS polyacrylamide gel, the molecular weight of the enzyme was estimated to be 41,000±1,000. The isoelectric point at the peak of protein was pH 8.7 by isoelectric focusing column chromatography. The enzyme with cephalothin as the substrate was active at

Table 3. Determination of the MICHAELIS-MENTEN kinetics of the *Proteus morganii* GN5407 cephalosporinase at pH 7.0 and 30°C.

Substrate	*Km* (μM)	*Vmax* (relative)*
Cephaloridine	500	100
Cefazolin	83	20
Cephalothin	20	46
Cephalexin	6	4
Cefotiam	19	8
Cefamandole	33	0.7
Cefoperazone	167	0.01
Benzylpenicillin	26	16

* *Vmax* values are expressed as a percentage of the rate for cephaloridine.

Table 4. Determination of the inhibition constant of the *Proteus morganii* GN5407 cephalosporinase at pH 7.0 and 30°C.

Antibiotic	*Ki* (μM)*
Cloxacillin	0.001
Ampicillin	0.99
Carbenicillin	0.009
Cefuroxime	0.05
Cefotaxime	0.07
Ceftizoxime	0.24
Cefmenoxime	0.08
Cefoxitin	0.22
Cefmetazole	0.40
Moxalactam	0.16
YM09330	0.11

* The *Ki* values were determined with cephalothin as a substrate.

pHs 6.0 to 9.0. However, the enzyme was most active at pH 8.5 and the temperature optimum was 40°C.

The kinetic parameters (*Km* and *Vmax*) of the enzyme activity are given in Table 3. The *Km* values and *Vmax* values that were calculated relative to cephaloridine (taken as 100) were obtained from LINEWEAVER-BURK plots. Cephaloridine, cefazolin, cephalothin and benzylpenicillin showed higher relative *Vmax* than with cephalexin and cefotiam. On the other hand, cefamandole and cefoperazone showed very low *Vmax* values. Cloxacillin, ampicillin, carbenicillin, cefuroxime, cefotaxime, ceftizoxime, cefmenoxime, cefoxitin, cefmetazole, moxalactam and YM09330 were resistant to hydrolysis by the cephalosporinase from *P. morganii* GN5407, and they inhibited the enzyme activity competitively. The dissociation constants of the enzyme-inhibitor complex (*Ki*) are shown in Table 4. The *Ki* values for various compounds were determined with cephalothin as a substrate. These compounds, especially cloxacillin and carbenicillin, had very low *Ki* values, indicating their high affinity to the enzyme.

A number of inhibitors of the enzyme were tested for their inhibitory effects on the activity of the purified enzyme. After preincubation in distilled water with each of the inhibitors or ions for 10 minutes at 30°C, the enzyme activity was assayed using 100 μM of cephalothin as a substrate.

The enzyme activity was almost completely inhibited by 0.01 mM iodine and 1.0 mM mercury ions. In contrast, 0.5 mM *p*-chloromercuribenzoate, 0.1 mM clavulanic acid and 0.1 mM CP-45899 had no effect on the enzyme activity.

Immunological Properties of the Purified Enzyme

The antibody inhibited the β-lactamase activity of the purified enzyme preparation of *P. morganii* GN5407 completely. The neutralizing capacity of the antibody was checked on various β-lactamases obtained from resistant strains including *P. morganii* GN5307 and GN5375, *E. coli* GN5482,[20] *Enterobacter cloacae* GN7471,[21] *Citrobacter freundii* GN7391,[22] *Serratia marcescens* GN10857, *Pseudomonas aeruginosa* GN10362, *Proteus rettgeri* GN4430,[23] *Proteus vulgaris* GN7919,[24] *Pseudomonas cepacia* GN11164,[16] *Bacteroides fragilis* GN11477,[25] the transconjugants of Rms212, Rms213 and Rte16 in *E. coli* W3630 and the transconjugant of Rms139 in *P. aeruginosa* M1. The enzyme obtained from the homologous strains, *i.e.*, GN5307 and GN5375 were neutralized by the anti-GN5407. However, there was no reaction with the other β-lactamases.

Discussion

Previous studies in our laboratory[26] have characterized a cephalosporinase found in *P. morganii*. By combining several newly described methods, including isoelectric focusing and the kinetic constants of cephalosporinase, it was possible to characterize a new property of β-lactamase in *P. morganii* using newly-introduced compounds as substrates.

The enzyme from *P. morganii* GN5407 has a molecular weight of about 41,000. The isoelectric point (8.7), optimal temperature (40°C) and optimal pH (pH 8.0) are close to those of cephalosprinases from *E. coli* GN5482,[20] *E. cloacae* GN7471,[21] *C. freundii* GN7391,[22] *S. marcescens* GN10857, *P. aeruginosa* GN10362 and *P. rettgeri* GN4430.[23]

The isoelectric points of cephalosporinases from 50 *P. morganii* strains were reported by KAZMIERCZAK *et al.*[27] (pI 5.9~8.98). LABIA and co-workers[28] have reported that the isoelectric points of cephalosporinases from *P. morganii* RL 19 and F 20 B were 8.33 and 8.29, respectively. Furthermore, *P. morganii* 1510 enzyme (pI 7.2) and *P. morganii* NCTC235 enzyme (pI 8.3) were reported by FUJIKURIYAMA *et al.*[29] and BARTHELEMY *et al.*,[30] respectively. However, the isoelectric point of *P. morganii* GN 5407 enzyme was more basic than that of *P. morganii* 1510 enzyme, and our results fully agree with previous reports[31] that suggest a basic (7.5~9.5) pI for cephalosporinases.

Semisynthetic penicillins, cloxacillin, ampicillin and carbenicillin, were found to be not only resistant to hydrolysis by cephalosporinase from *P. morganii* GN5407 but had a strong inhibitory activity against the enzyme. The same situation was observed with 7α-methoxylated derivatives of cephalosporin such as cefoxitin, cefmetazole and YM09330 and 1-oxa cephalosporin such as moxalactam. *Ki* values of these compounds were 100 times lower than the *Km* values of cephalosporins, such as cefazolin, cephalothin, cefotiam and cefamandole. Furthermore, cefuroxime-type cephalosporins including cefuroxime, cefotaxime, ceftizoxime and cefmenoxime showed low *Ki* values. On the other hand, new β-lactamase inhibitors such as clavulanic acid and CP-45899 had no affinity to the enzyme from *P. morganii* GN5407. These substrate specificities of *P. morganii* GN5407 cephalosporinase prove to be close to those reported for cephalosporinases from *E. coli* GN5482,[20] *E. cloacae* GN7471,[21] *C. freundii* GN7391,[22] *S. marcescens* GN10857, *P. aeruginosa* GN10362 and *P. rettgeri* GN4430.[23]

DARLAND *et al.*[32] have shown that cefoxitin was an effective inhibitor against *B. fragilis* β-lactamase, but cefuroxime was hydrolyzed by the β-lactamase from *B. fragilis*. Studies in our laboratory also proved that cefuroxime-type cephalosporins were well hydrolyzed by the β-lactamase from *B. fragilis* GN-11477[25] and moreover, those from *P. vulgaris* GN7919[24] and *P. cepacia* GN11164.[16] In addition the hydrolysis of cephalothin by β-lactamases from *B. fragilis*, *P. vulgaris* and *P. cepacia* was competitively inhibited by both clavulanic acid and CP-45899. The hydrolysis of cephalothin by cephalosporinases from *B. fragilis* and *P. vulgaris* was also inhibited by cephamycin antibiotics, but not by β-lactamase from *P. cepacia*. These substrate specificities indicated that cephalosporinase of *P. morganii* was apparently distinct from those of *B. fragilis, P. vulgaris* and *P. cepacia*. The antibody against the purified cephalosporinase produced by *P. morganii* GN5407 did not cross-react with the cephalosporinase of other *Proteus* species, *E. coli, E. cloacae, C. freundii, S. marcescens, P. aeruginosa, P. cepacia, B. fragilis* and penicillinase types I, II, III and IV. This result indicates that the enzyme from *P. morganii* GN5407 is the "species-specific" cephalosporinase.

In the course of this experiment, we found that the "species-specific" cephalosporinase from *P. morganii* was close to those from *E. coli, E. cloacae, C. freundii, S. marcescens, P. aeruginosa* and *P. rettgeri* in view of substrate specificities with the addition of new substrates. However, we found that the "species-specific" cephalosporinases from *B. fragilis, P. vulgaris* and *P. cepacia* were apparently different from *P. morganii* cephalosporinase. Therefore, the addition of newly-introduced β-lactam antibiotics, such as β-lactamase inhibitors, 7α-methoxylated cephalosporins and cefuroxime-type cephalosporins to substrate-specificities studies might contribute to the characterizations of the β-lactamases from various bacteria.

References

1) ABRAHAM, E. P. & E. B. CHAIN: An enzyme from bacteria able to destroy penicillin. Nature (London) 146: 837, 1940
2) MITSUHASHI, S.; S. YAMAGISHI, T. SAWAI & H. KAWABE: Biochemical mechanisms of plasmid-mediated resistance. *In* "R Factor-Drug Resistance Plasmid" (*ed.* by S. MITSUHASHI). pp. 195~254, University of Tokyo Press, Tokyo, 1977
3) RICHMOND, M. H. & R. B. SYKES: The β-lactamases of Gram-negative bacteria and their possible physiological role. Adv. Microb. Physiol. 9: 31~88, 1973
4) HEYMES, R.; A. LUTZ & E. SCHRINNER: Experimental evaluation of HR 756, a new cephalosporin derivative. Current Chemotheraphy, Proc. 10th Internatl. Congr. Chemoth. II: 823~824, 1978
5) TSUCHIYA, K.; M. KIDA, M. KONDO, H. ONO, M. TAKEUCHI & T. NISHI: SCE-963, a new broad-spectrum cephalosporin: *In vitro* and *in vivo* antibacterial activities. Antimicr. Agents & Chemoth. 14: 551~568, 1978
6) MATSUBARA, N.; S. MINAMI, T. MURAOKA, I. SAIKAWA & S. MITSUHASHI: *In vitro* antibacterial activity of cefoperazone (T-1551), a new semisynthetic cephalosporin. Antimicr. Agents & Chemoth. 16: 731~735, 1979
7) TAKAYA, T.; T. KAMIMURA, H. KOJO, Y. MINE, M. NISHIDA, S. GOTO & S. KUWAHARA: Ceftizoxime (FK749), a new parenteral cephalosporin: *In vitro* and *in vivo* antibacterial activities. Current Chemotherapy and Infectious Disease, Proc. 11th Internatl. Congr. Chemoth. & 19th Intersci. Conf. Antimicr. Agents Chemoth. I: 255~257, 1979
8) GOTO, S.; M. OGAWA, A. TSUJI, S. KUWAHARA, K. TSUCHIYA, M. KONDO & M. KIDA: SCE-1365, a new cephalosporin: *In vitro* antibacterial activities. Current Chemotherapy and Infectious Disease, Proc. 11th Internatl. Congr. Chemoth. & 19th Intersci. Conf. Antimicr. Agents Chemoth. I: 264~266, 1979
9) SUGAWARA, S.; M. TAJIMA, I. IGARASHI, S. OHYA & Y. UTSUI: CS-1170, a new cephamycin derivative. II. *In vitro* and *in vivo* antibacterial activities. Abstracts, 16th Intersci. Conf. Antimicr. Agents & Chemoth. No. 231, 1976
10) TODA, M.; T. SAITO, K. YANO, K. SUZAKI, M. SAITO & S. MITSUHASHI: *In vitro* and *in vivo* antibacterial activities of YM09330, a new cephamycin derivative. Current Chemotherapy and Infectious Disease, Proc. 11th Internatl. Congr. Chemoth. & 19th Intersci. Conf. Antimicr. Agents Chemoth. I: 280~281, 1980
11) NEU, H. C.; N. ASWAPOKEE, K. P. FU & P. ASWAPOKEE: Antibacterial activity of a new 1-oxa cephalosporin compared with that of other β-lactam compounds. Antimicr. Agents & Chemoth. 16: 141~149, 1979
12) READING, C. & M. COLE: Clavulanic acid: a beta-lactamase-inhibiting beta-lactam from *Streptomyces clavuligerus*. Antimicr. Agents & Chemoth. 11: 852~857, 1977
13) ENGLISH, A. R.; J. A. RETSEMA, A. E. GRIRARD, J. E. LYNCH & W. B. BARTH: CP-45899, a beta-lactamase inhibitor that extends the antibacterial spectrum of beta-lactams: Initial bacteriological characterization. Antimicr. Agents & Chemoth. 14: 414~419, 1978
14) ROSS, G. W.; K. V. CHANTER, A. M. HARRIS, S. M. KIRBY, M. J. MARSHALL & C. H. O'CHALLAGHAN: Comparison of assay technique for beta-lactamase activity. Anal. Biochem. 54: 9~16, 1973
15) WALEY, S. G.: A spectrophotometric assay of β-lactamase action on penicillins. Biochem. J. 139: 780~789, 1974
16) HIRAI, K.; S. IYOBE, M. INOUE & S. MITSUHASHI: Purification and properties of a new β-lactamase from *Pseudomonas cepacia*. Antimicr. Agents & Chemoth. 17: 355~358, 1979
17) LOWRY, O. H.; N. J. ROSEBROUGH, A. L. FARR & R. J. RANDALL: Protein measurement with the FOLIN phenol reagent. J. Biol. Chem. 193: 265~275, 1951
18) WEBER, K. & M. OSBON: The reliability of molecular weight determinations by dodecyl sulfate polyacrylamide gel electrophoresis. J. Biol. Chem. 244: 4406~4412, 1969
19) KURIYAMA, Y.: Studies on microsomal nucleoside diphosphatase of rat hepatocytes. J. Biol. Chem. 247: 2979~2988, 1972
20) MINAMI, S.; M. INOUE & S. MITSUHASHI: Purification and properties of cephalosporinase in *Escherichia coli*. Antimicr. Agents & Chemoth. 18: 77~80, 1980
21) MINAMI, S.; M. INOUE & S. MITSUHASHI: Purifications and properties of a cephalosporinase from *Enterobacter cloacae*. Antimicr. Agents & Chemoth. 18: 853~857, 1980
22) TAJIMA, M.; Y. TAKENOUCHI, S. SUGAWARA, M. INOUE & S. MITSUHASHI: Purification and properties of β-lactamase from *Citrobacter freundii* GN7391. J. Gen. Microbiol. 121: 449~456, 1980
23) MATSUURA, M.; H. NAKAZAWA, M. INOUE & S. MITSUHASHI: Purification and biochemical properties of β-lactamase produced by *Proteus rettgeri*. Antimicr. Agents & Chemoth. 18: 687~690, 1980

24) MATSUBARA, N.; A. YOTSUJI, K. KUMANO, M. INOUE & S. MITSUHASHI: Purification and some properties of a cephalosporinase from *Proteus vulgaris*. Antimicr. Agents & Chemoth. 19: 185~187, 1981
25) SATO, K.; M. INOUE & S. MITSUHASHI: Activity of β-lactamase produced by *Bacteroides fragilis* against newly introduced cephalosporins. Antimicr. Agents & Chemoth. 17: 736~737, 1980
26) YAGINUMA, S.; T. SAWAI, S. YAMAGISHI & S. MITSUHASHI: β-Lactamase formation and resistance of *Proteus morganii* to various penicillins. Jpn. J. Microbiol. 18: 113~118, 1974
27) KAZMIERCZAK, A.; M. BOLLE, E. SIEBOR, P. POTHIER & R. LABIA: Characterization of *Proteus morganii* cephalosporinases and evaluation of their role in LY127935 (6059-S) and HR-756 (cefotaxime) antimicrobial activities. Current Chemotherapy and Infectious Disease, Proc. 11th Internatl. Congr. Chemoth. & 19th Intersci. Conf. Antimicr. Agents Chemoth. I: 71~72, 1980
28) LABIA, R.; M. GUIONIE & C. FABRE: Céphalosporinases inductibles de *Proteus morganii*. Biochimie 58: 1083~1087, 1976
29) FUJII-KURIYAMA, Y.; M. YAMAMOTO & S. SUGAWARA: Purification and properties of beta-lactamase from *Proteus morganii*. J. Bacteriol. 131: 726~734, 1977
30) BARTHELEMY, M.; M. GUIONIE & R. LABIA: Beta-lactamases: Determination of their isoelectric points. Antimicr. Agents & Chemoth. 13: 695~698, 1978
31) MATTHEW, M.; A. M. HARRIS, M. J. MARSHALL & G. W. ROSS: The use of analytical isoelectric focusing for detection and identification of β-lactamase. J. Gen. Microbiol. 88: 169~178, 1975
32) DARLAND, G. & J. BIRNBAUM: Cefoxitin resistance to beta-lactamase: A major factor for susceptibility of *Bacteroides fragilis* to the antibiotic. Antimicr. Agents & Chemoth. 11: 725~734, 1977