

Mouse anti-benzylpenicilloyl IgE monoclonal antibody: preparation, characterization and cross-reactivity

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SUMMARY

Anti-benzylpenicilloyl (BPC-) monoclonal antibody of the IgE class was prepared from spleens of immune C₅₇BL/6 mice whose sera reacted with BPO-hapten, penicillin G (PCG) polymer, cephalothin (CET)-hapten and CET polymer. Affinity chromatography experiments showed that the haptenic specificity of the IgE monoclonal antibody (designated BIE-13CE) was directed mainly to phenylacetyl portion of BPO group. BIE-13CE antibody reacted on passive cutaneous anaphylaxis (PCA) assay with BPO-hapten, CET-hapten, cephaloridine-hapten and CET polymer, but did not react with PCG polymer, ampicillin-hapten, or cefazolin-hapten. These results indicated that the sera of the immune C₅₇BL/6 mice contained IgE antibodies capable of cross-reacting at the monoclonal antibody level with various forms of eliciting antigens and that the cross-reactivity of the antibody could be ascribed essentially to the structural similarity of acyl side chains of the antibiotics. The structure of the CET polymer is also discussed in terms of its PCA reactivity with the monoclonal antibody and analytical and spectral data of the polymer.

Keywords antibiotics IgE monoclonal antibody cross-allergenicity penicillin-allergy

INTRODUCTION

Clinical therapy using β -lactam antibiotics sometimes leads to allergy of the immediate type such as anaphylactic shock and acute urticaria, where IgE antibodies have been considered to be involved (Dewdney, 1977; de Weck, 1983; Sogn, 1984). In some patients, penicillins and cephalosporins cause cross-allergenicity (Levine, 1973; Petz, 1978; de Weck 1983; Sogn, 1984), and it has been regarded to be due to the structural resemblance of their acyl side chains or nuclear portions, mainly based on analyses of highly cross-reactive rabbit IgG antibodies (Shibata *et al.*, 1966; Batchlor *et al.*, 1966; Atsumi *et al.*, 1967; Nishida *et al.*, 1972; Petersen & Graham, 1974; Dewdney, 1977; Muranaka *et al.*, 1980). However, not all patients with penicillin allergy show cross-allergenicity with other antibiotics of similar structures (Abraham, Petz & Fundenberg, 1968; Girard, 1968; Assem & Vickers, 1974; Petersen & Graham, 1974; Petz, 1978; Warrington *et al.*, 1978; Solley, Gleich & Van Dellen, 1982). According to Petz (1978), cross-allergenicity of penicillin-allergic patients with cephalothin (CET), cephaloridine (CER) and cefazolin (CEZ) occurs with an incidence of 5.8%, 13.0% and 4.0%, respectively. Moreover, mouse IgE anti-benzylpenicilloyl (BPO) antibodies do not necessarily cross-react with CET-hapten (Iwata *et al.*, 1982; Harada *et al.*,

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1986a). The cross-reactivity depends on the mouse strain, the immunizing conditions and the isotypes of the antibodies examined (Harada, Watanabe & Takeuchi, 1986b). These observations made us suspect that, in the case of the IgE class, anti-BPO antibodies might not be responsible for the cross-reactivity. Antibodies other than the anti-BPO antibodies might cause the cross-reaction. More exact study is required to identify and characterize the antibodies that cause the cross-reaction, but it has been hindered by extremely low concentration of IgE in the serum. Application of monoclonal antibody techniques to this problem should provide us with a new approach for analysing the cross-allergenicity of β -lactam antibiotics.

We recently found that IgE antibodies produced against a BPO-protein conjugate under certain immunizing conditions in C α -BL/6J mice were highly cross-reactive on passive cutaneous anaphylaxis (PCA) with CET-hapten and penicillin G (PCG)- and CET polymers (Harada *et al.*, 1986a, b). In the present study, we prepared monoclonal anti-BPO antibody of the IgE class from spleens of the immune C α -BL/6J mice and examined its properties. The results revealed the presence of anti-BPO IgE antibody molecules cross-reactive with CET-hapten and CET polymer.

MATERIALS AND METHODS

Chemicals. Bis-benzylammonium, N-(D- α -benzylpenicilloyl)- α -aminocaproate (BPO-EACA), a conjugate of PCG with α -aminocaproic acid, was prepared by the method of Levine (1962). Phenylacetyl glycine and benzylamine hydrochloride were purchased from Nakarai Chemicals Co., Osaka, Japan. Phenylacetic acid was prepared by alkaline hydrolysis of phenylacetyl chloride, and subsequent neutralization and extraction with diethylether. Phenylacetic acid was recrystallized and identified by elementary analysis. A commercial product of 2-thiopheneacetic acid was further recrystallized from petroleum ether. 2,5,5-Trimethylthiazolidine-4-carboxylic acid was prepared by the method of Nagasawa, Goon & Demaster (1978).

Preparation of antibiotic-protein conjugates. The antibiotics used were PCG (Meiji Seika Co., Tokyo, Japan), ampicillin (aminobenzylpenicillin, ABPC, Merck Banyu Co., Tokyo, Japan), CET (Shionogi Co., Osaka, Japan), CER (Shionogi Co.) and CEZ (Fujisawa Co., Osaka, Japan). Conjugates of bovine γ -globulin (BGG) with PCG (abbreviation: BPO-BGG), and conjugates of guinea pig serum albumin (GSA) with PCG, ABPC, CET, CER and CEZ (abbreviations: BPO-GSA, ABPC-GSA, CET-GSA, CER-GSA, CEZ-GSA, respectively) were prepared following the method of Levine & Gvary (1961). The total bound haptenic groups of these protein conjugates were determined by the method of Ebata, Miyake & Uchida (1976). The following conjugates were obtained: BPO₁₁-BGG, BPO₁₀-GSA, ABPC₁₃-GSA, CET₁₄-GSA, CER₁₅-GSA, CEZ₂₀-GSA.

Preparation of PCG- and CET polymers. PCG- and CET polymers were prepared as described previously (Harada *et al.*, 1986a). Thus, an aqueous solution of PCG (240 mg/ml) or CET (250 mg/ml) left at room temperature for 7 days (CET) or 15 days (PCG) was separated by Sephadex G-25 gel filtration using pure water (CET) or 0.5% NaCl solution (PCG) as the eluent. Molecular weights of fractions were estimated by relative elution volumes of actinomycin D, glucagon and polyethyleneglycol-1,000 and -4,000. In both cases, fractions containing polymers of apparent mol. wt of 1,000–3,500 daltons approximately corresponding to the trimer-decamer were pooled and lyophilized. The NaCl-containing PCG polymer was further freed of salt by the second gel filtration with pure water followed by lyophilization.

Analytical and spectral data of the CET polymer. The following data were obtained on the CET polymer dried *in vacuo* over P₂O₅ for 2 h at 25 °C. Elementary analyses: Found (%): C, 38.51; H, 3.84; N, 3.73; S, 13.68; Na, 9.70. Calculated (%) for structure III-A or V (X=OH) (Fig. 3): C, 43.37; H, 3.23; N, 5.62; S, 12.86; Na, 9.22. Calculated (%) for structure V (X, OH: SCH₂C(=CH₂)-COOONa = 2:1): C, 42.81; H, 3.03; N, 4.68; S, 14.27; Na, 10.23. UV (λ max, nm, H₂O): 193, 230, 270 (shoulder). IR (cm⁻¹, Nujol): 3380 (OH, NH), 1756 (weak, β -lactam CO), 1705 (weak, —CHO), 1624 (broad; in dimethylsulphoxide split into 1619 (COO⁻) and 1664 (CONH)). ¹H-NMR (PPM, D₂O): δ 7.38, 7.03 and 7.05 (thiophen 3 protons), 6.22 and 6.38 (weak, —SCH₂C(=CH₂)CO—), 3.92 (thiophene—CH₂CONH—), 3.41 (weak, —SCH₂C(=CH₂)CO—).

Hybridomas. The cell fusion experiment was performed essentially by the method of Galfre &

Milstein (1981). Female C₅₇BL/6JShi mice were immunized by a single intraperitoneal injection of BPO₃₁-BGG (1 mg per animal) emulsified with an equal volume of Freund's complete adjuvant (FCA) (Harada *et al.*, 1985). Splenic lymphocytes prepared from immune C₅₇BL/6JShi mice 7 days after prime immunization were fused with Sp2.0-Ag 14 myeloma cells using 50% polyethyleneglycol-4000 (Wako Pure Chemicals Co., Osaka, Japan). The fused cells were suspended in HAT selection medium containing RPMI-1640 (Nissui Seiyaku Co., Tokyo, Japan) and 20% heat-inactivated fetal calf serum, and then were mixed with a suspension of thymocytes from ⁶⁰Co-irradiated (about 1000 rad) Wistar rats as feeder cells, and finally were plated in 96-well tissue culture plates.

PCA assay. The PCA assay was performed by the method of Mota & Wong (1969) and Ovary, Caizza & Kojima (1975). Each female Wistar Shi rat was injected intracutaneously with 50 μ l of serially diluted culture supernatants of hybridoma-growing wells or ascites of hybridoma-bearing mice. After 18–24 h, each sensitized rat was injected intravenously with 0.5 ml of PBS (phosphate-buffered 0.15 M saline) solution containing eliciting antigen (1 mg unless otherwise noted) and Evans Blue dye (5 mg). In the case of PCA in mice, each female DS/Shi mouse was injected intracutaneously with 50 μ l of antibody sample, then after 1 h or on the next day, was injected intravenously with 0.2 ml of PBS solution of eliciting antigen (1 mg unless otherwise noted) and Evans Blue dye (0.5 mg). Both rats and mice were killed 1 h after the antigen challenge, and the PCA titre was recorded as the reciprocal of the highest dilution that gave a 5 mm blue spot inside the skin.

Production of monoclonal antibody. BIE-13CE hybridomas producing IgE anti-BPO monoclonal antibody were cloned twice successively by limiting dilution in a single cell culture. Large amounts of monoclonal antibodies were produced in the ascites of (BALB/c $\times$ C₅₇BL/6)F₁ (CBF₁) or (C₅₇BL/6 $\times$ DBA/2)F₁ (BDF₁) mice which had been injected intraperitoneally with 0.3 ml of 2,4,6,10-tetramethyldecapentane (pristane), then intraperitoneally with BIE-13CE hybridomas after 1 week or more.

Purification of IgE monoclonal antibody by affinity chromatography. IgE monoclonal antibodies were isolated from the ascites of hybridoma-bearing CBF₁ mice by ammonium sulphate precipitation (Liu *et al.*, 1980). Proteins soluble at 30% saturation, but precipitable at 60% were isolated, redissolved in PBS and dialyzed exhaustively against PBS. This sample was then applied to BPO-GSA-Sepharose 4B gel column prepared by coupling BPO-GSA and BrCN-Sepharose 4B (Pharmacia Japan Co.). Unbound materials were washed out from the immunoadsorbent column with PBS. The bound IgE monoclonal antibodies were eluted with 0.02 M BPO-EACA in PBS solution. Elution fractions or their dialyzates were tested for the IgE antibody by the rat PCA assay. In order to compare the ability of various haptens to elute the IgE monoclonal antibody, we prepared seven sets of the gel column. The partially-purified CBF₁ ascites (total PCA titre: 2^{9.8}) was applied to each gel column (4.2 ml, bed volume). Elution buffers were prepared by dissolving phenylacetyl glycine, phenylacetic acid, 2-thiophenacetic acid, benzylamine hydrochloride, 2,5,5-trimethylthiazolidine-4-carboxylic acid, BPO-EACA or PCG in PBS, if necessary, with the aid of minimum amounts of sodium hydroxide. The final concentration of the haptens was adjusted to 20 mM except for 66.7 mM of 2,5,5-trimethylthiazolidine-4-carboxylic acid. This is because NMR analysis of the compound showed it to be a stereoisomeric mixture of 2S,4S (70%) and 2R,4S (30%), the latter isomer corresponding to the configurations of the PCG and the BPO group (Levine, 1962; Nagasawa, Goon & Demaster, 1978). The eluate was fractionated into 2 ml portions and the second to the fifth fractions were assayed for the IgE antibody by rat PCA.

Chemical analysis of monoclonal antibody. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli (1970). Marker proteins for mol. wt estimation were from the Biorad standard kit 161-0304 (Biorad Chemical Division, USA). Antibodies used for Ouchterlony's double diffusion test were goat anti-mouse IgE antiserum (Fc γ -specific) (Nordic Co., Netherlands), goat anti-mouse IgG, IgM, IgA antibodies (Fc γ - μ , α -specific) (Cappel Co., USA), and rabbit anti-mouse κ , λ antibodies (Miles Lab., USA).

RESULTS

Production of IgE monoclonal antibody. Splenic lymphocytes of C₅₇BL/6 mice primed with a

BPO-BGG conjugate were fused with Sp 2, non-immunoglobulin-producing myeloma cells. Culture supernatants of hybridoma-growing wells were screened in the rat PCA assay for the detection of IgE antibodies reactive with BPO-hapten, PCG polymers or BGG. Of 743 supernatants, four reacted with BPO-GSA, two reacted with BGG, but none with PCG polymer. Among these IgE antibody-producing hybridomas only one clone (BIE-13CE) showed stable production of IgE antibody and could be propagated. We first confirmed the isotype of the BIE-13CE antibody, then examined the specificity of antigen binding and PCA reactivity of the antibody.

Isotype analysis of monoclonal antibody. The isotype of the monoclonal antibody, BIE-13CE, was determined by the PCA reaction, SDS-PAGE analysis and double diffusion test. (I) PCA reaction: BIE-13CE antibody could sensitize rat skin. This activity was substantially abolished by heat treatment of the sample at 56 °C, for 30 min. The PCA titres in rats for both long (72 h) and short (4–24 h) sensitization periods were the same, showing persistent skin-sensitization capacity. These results were consistent with known characteristics of mouse IgE antibodies (Mota & Perini, 1983; Tung, 1983). BIE-13CE antibody also could sensitize DS/Shi mice for 4 days or more, though even a short sensitization period (1 h) was sufficient to give the maximum antibody titre. Again, the skin-sensitizing activity of this antibody was heat-labile. (II) SDS-PAGE analysis and double diffusion test: the monoclonal antibody was purified by antigen column. Affinity-bound IgE antibody was eluted with BPO-EACA (Fig. 1A) and detected by the rat PCA reaction. SDS-PAGE analysis of the purified antibody run under reducing conditions (Fig. 1B) showed two bands with apparent mol.wt of 76×10^3 and 24×10^3 , corresponding to heavy and light chains. The mol.wt of the heavy chain was distinct from that of the γ chain (mol.wt 50×10^3) like other known mouse antibodies of the IgE class (Eshar Ofarim & Waks, 1980; Liu *et al.*, 1980). In Ouchterlony's double diffusion test, the affinity-purified antibody reacted with anti-mouse IgE (F ϵ -specific) or anti-mouse κ antibody but not with antibodies to mouse IgG, IgM, IgA and λ .

Antigen binding of BIE-13CE IgE monoclonal antibody. The binding of BIE-13CE antibody with various chemically synthesized haptens was estimated by means of affinity chromatography. The amounts of the antibody eluted under similar conditions were compared by the rat PCA assay (Fig.

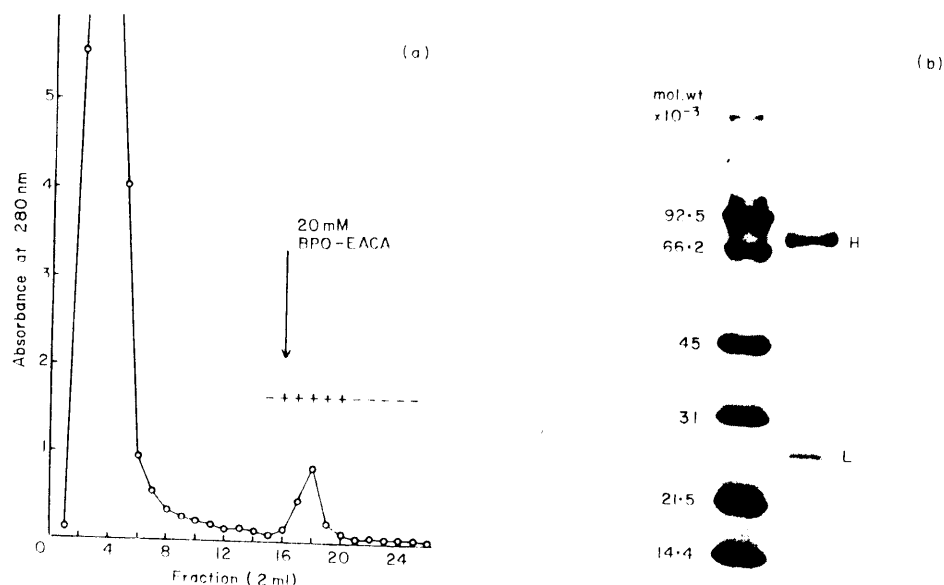


Fig. 1. (a) Affinity chromatography of IgE monoclonal antibody (BIE-13CE). The sample partially purified from CBF $_1$ ascites was loaded on a BPO-GSA-Sepharose gel column. Fractions eluted with BPO-EACA were tested by rat PCA assay. +, PCA titre ≥ 10 ; —, PCA titre < 10 . (b) SDS-PAGE analysis of affinity-purified IgE monoclonal antibody (BIE-13CE). Left, Marker proteins. Right, Affinity-purified antibody treated with SDS and 2-mercaptoethanol. Acrylamide gel (10%) was used. H, heavy chain. L, light chain.

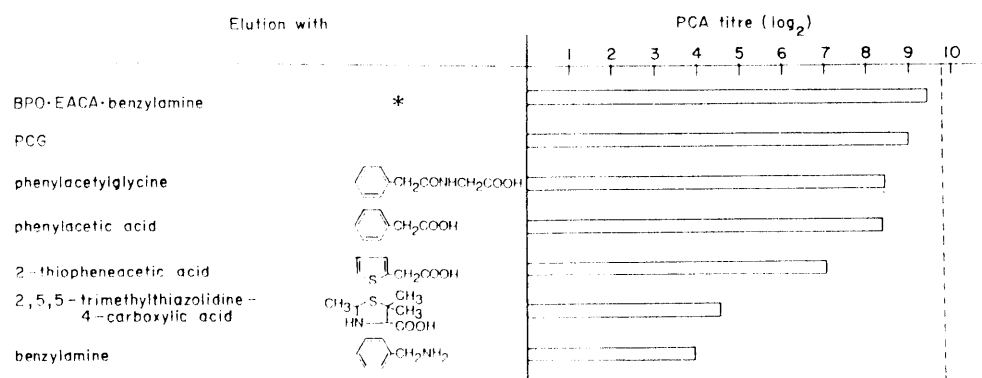


Fig. 2. Comparative effectiveness of haptens to elute the affinity bound IgE monoclonal antibody (BIE-13CE). Antibodies eluted under similar conditions from BPO-GSA-Sepharose column were assayed by rat PCA. Broken lines indicate the total amount of antibody loaded. * The structure is given in Fig. 3 (structure I).

2). Although the PCA assay is semi-quantitative, it can be used to estimate the relative affinities of the antibody for various haptens. BPO-EACA and PCG were the most effective eluents among the haptens examined. Even phenylacetyl glycine and phenylacetic acid, both corresponding to the acyl side chain of the BPO group, could elute the antibody effectively. In addition, 2-thiopheneacetic acid, corresponding to the acyl side chain of CET, could elute the antibody, having a little lower PCA titre than phenylacetic acid. The amount of antibody eluted with 2,5,5-trimethylthiazolidine-4-carboxylic acid, corresponding to the thiazolidine ring portion of the BPO group, was about one-fifteenth of that with phenylacetic acid. The results shown in Fig. 2 indicate that the haptenic specificity of the antibody was directed mainly to the phenylacetyl portion and, to some extent, to the thiazolidine ring portion of the BPO group.

PCA reactivity of BIE-13CE IgE monoclonal antibody. The antigen specificity of the BIE-13CE antibody was examined by PCA assay using various eliciting antigens. It is noteworthy that BIE-13CE IgE antibody in both ascites and affinity-purified preparations highly cross-reacted with CET-hapten in the PCA assay in rats (overnight sensitization) and mice (1-h sensitization). As shown in Table 1, almost the same PCA titre was observed with BPO₁₀-GSA and CET₁₄-GSA. This

Table 1. PCA reactions of BIE-13CE IgE monoclonal antibodies with BPO₁₀-GSA and CET₁₄-GSA: cross-reactivity with BPO- and CET-haptens

Antibody preparation	Eliciting antigen*			
	Rat PCA [†]		Mouse PCA [‡]	
	BPO ₁₀ -GSA	CET ₁₄ -GSA	BPO ₁₀ -GSA	CET ₁₄ -GSA
CBF ₁ ascites	2 ^{12.5} §	2 ¹²	2 ^{11.5}	2 ^{11.5}
BDF ₁ ascites	2 ^{12.5}	2 ^{12.5}	2 ¹²	2 ¹²
Affinity-purified	2 ^{11.5}	2 ^{10.5}	2 ¹¹	2 ^{9.5}

* 1 mg animal.

[†] Wistar rats sensitized overnight.

[‡] DS mice sensitized for 1 h.

§ PCA titre.

Index numbers are mean values from duplicate assays.

Affinity-purified by antigen column using BPO-EACA.

Table 2. PCA reactions of BIE-13CE IgE monoclonal antibodies with aged CET solution and CET polymer

	Eliciting antigen*			
	Rat PCA†		Mouse PCA‡	
Antibody preparation	CET aged§	CET polymer	CET aged	CET polymer
CBF ₁ ascites	2 ^{7.5} (5 mg)**	2 ⁷ (0.5 mg, 2 mg) 2 ⁵ (0.2 mg)	2 ^{5.5} (5 mg)	2 ^{5.5} (0.4 mg)
Affinity purified††	2 ⁵	2 ⁸	2 ⁶	2 ⁶

* 1 mg animal, unless otherwise noted.

† Wistar rats sensitized overnight.

‡ DS mice sensitized overnight.

§ CET aqueous solution left standing for 1 week.

¶ Polymers of CET separated from aged CET solution.

• PCA titre from duplicate assays.

** Eliciting antigen dose animal.

†† Affinity-purified using BPO-EACA.

indicates a high degree of cross-reactivity between BPO- and CET-haptens, since BIE-13CE antibody does not react with GSA (data not shown). BIE-13CE antibody cross-reacted also with CER-hapten, the PCA titre being nearly the same as that with CET-hapten. Cross-reactivity was also found with aged CET solution and its fractionated materials, CET polymer (Table 2). The degree of cross-reactivity of BIE-13CE antibody with CET-derived elicitors is comparable to that found in conventional antibodies of immune sera of C₅₇BL/6 mice from which spleen cells were obtained for the hybridoma formation (Harada *et al.*, 1986a, b). On the other hand, BIE-13CE antibody caused no cross-reaction with PCG polymer unlike the conventional antibodies mentioned above (Harada *et al.*, 1986a). CET-hapten, aminobenzylpenicilloyl (ABPO)-hapten, BGG (carrier portion of the immunogen), BPO-EACA and a fresh solution of PCG and CET also could not elicit rat PCA with BIE-13CE antibody, the PCA titre being less than 2¹.

DISCUSSION

The cross-reaction among β -lactam antibiotics may occur with antibodies specific to some structure common to various antibiotic-protein conjugates, e.g. a linkage portion between the antibiotic-hapten and the protein carrier as suggested by Haan *et al.* (1985). The cross-reaction is also possible with antibodies produced against some immunogenic impurities common to various antibiotic preparations. Minute traces of protein impurities, which are penicilloylated with penicillin, are contained in penicillin preparations (Kristofferson *et al.*, 1977; Ahlstedt, Kristofferson & Petersson, 1979; Bundgaard, 1983). Carrier portions of penicilloyl-protein impurities may induce the formation of cross-reactive antibodies. Considering these possibilities, we examined the specificity of binding and PCA reactions of BIE-13CE monoclonal antibody.

BIE-13CE antibody reacted in the rat PCA assay with BPO-, CET- and CER-haptens. Aged CET solutions and CET polymer also reacted with this antibody. Evidently, BIE-13CE antibody is directed to the BPO group rather than to the carrier portions of penicilloyl-protein impurities within the preparation, since it binds to recrystallized pure BPO-EACA. Furthermore, BIE-13CE antibody is not an antibody specific to the linkage portion of BPO-protein conjugates, because of its effective binding to phenylacetic acid.

It is known that rabbit IgG anti-BPO antibodies comprise antibodies raised to the whole BPO group or its partial structures such as the acyl side chain and the nuclear portion (Atsumi *et al.*, 1967, 1968). Antibodies mainly directed to the nuclear portion have been considered to be

responsible for the cross-reactivity with several penicillins and those to the acyl side chain for the cross-reactivity with cephalosporins (Atsumi *et al.*, 1967; Nishida *et al.*, 1972). In the present study, affinity chromatography experiments (Fig. 2) showed that B1E-13CE antibody had much higher affinity for the phenylacetyl portion than for the thiazolidine ring portion of the BPO group. The binding reactivity of B1E-13CE antibody to 2-thiopheneacetic acid suggests that the acyl side chain is responsible for the cross-reactivity with CET-hapten. This view is consistent with the PCA reactivity of the antibody with other antibiotic haptens such as ABPC, CER and CEZ. B1E-13CE antibody distinguished BPO-hapten from the 6-amino-substituted derivative (ABPO-hapten), both of which have common thiazolidine ring portions. Success of the antibody to produce PCA with CER-hapten but not with CEZ-hapten is easily explainable because both CER and CET but not CEZ have the common structure of a thiophene ring as an acyl side chain.

The present study showed that mouse anti-BPO IgE antibody reacted with CET-hapten at the monoclonal antibody level without participation of other antibodies. Thus, it might be possible that the cross-reactivity and its heterogeneity observed with the immune sera of BPO-sensitized mice are ascribed only to anti-BPO antibodies. The heterogeneity might be due to differences in the affinities of anti-BPO antibodies (Eisen & Siskind, 1964; Siskind & Benaceraf, 1969), which are known, in rabbit IgG anti-BPO antibodies, to be accompanied by differences in binding specificities of the antibodies (Atsumi *et al.*, 1968). Characterization at the monoclonal antibody level of many IgE antibodies, including non-cross-reactive IgE anti-BPO antibodies, is needed for complete understanding of the cross-reactivity and its heterogeneity.

The antibody titre in PCA elicited with the aged CET solution was high and comparable to that of the CET polymer as shown in Table 2. PCA-elicitable dimer and polymer are contained in amounts of about 10% in the aged materials and their amounts are sufficient to elicit the high-titre PCA. The contaminating monomers which might inhibit the PCA by competing with polymeric materials would have been diffused out rapidly by 1 h after the elicitor challenge from the sensitized site of the skin because of lower avidity of the monomer than the dimer and polymer for IgE antibodies bound to the mast cells. Our preliminary experiment shows that inhibition of PCA by CET monomers mixed with eliciting polymers did not last for half an hour after the elicitor challenge (data not shown).

In the PCA assay, B1E-13CE antibody cross reacted with the CET polymer but not with the PCG polymer. One possibility is that the CET polymer having much higher degree of polymerization is more antigenic than the PCG polymer, as has been demonstrated in polymers of penicillins (Munro *et al.*, 1976). This possibility can be excluded since the difference in mol.wt and hence the degree of polymerization between these polymers is not so prominent, and the doses of these polymers are high enough for PCA elicitation. It is probable that CET is polymerized in a different manner from PCG, so that B1E-13CE antibody can distinguish the structural differences of these polymers. In contrast to many reports on the structures of polymers of penicillins (Smith & Marshall, 1971; Bundgaard & Larsen, 1977; Bundgaard, 1983), the structure of the CET polymer has not been studied. The CET polymer is probably dissimilar in structure to the PCG polymer (II in Fig. 3) formed by acylation with penicillenic acid, since formation of the penicillenic acid equivalents from cephalosporins appears difficult (Dewdney, 1977; Bundgaard, 1983; de Weck, 1983). The acyl side chain may be postulated as the repeating structure since the monoclonal antibody specific mainly for the acyl side chain can elicit PCA reaction with the CET polymer. The presence of the thiopheneacetamido group in the polymer as an essential component is supported by IR, NMR and UV spectral data (see Materials and Methods section). Elementary analysis of the CET polymer (see Materials and Methods) roughly accords with structure III-A (Fig. 3) which has been considered to be a hydrolysis product of cephalosporins (Newton, Abraham & Kuwabara, 1968; Schwartz, 1969; Hamilton-Miller, Newton & Abraham, 1970). Thus the repeating unit of the polymer would be structure III-A. The polymerization may occur by aldol condensation of III-A to yield a polymer of structure V (X = OH) shown in Fig. 3. Then the hydroxy group in V may condense partly with compound IV, another possible hydrolysis product of cephalosporins (Abraham & Newton, 1961; Newton, Abraham & Kuwabara, 1968; and unpublished data of Dr M. Yoshioka in our laboratories) to give structure V (X = OH; $\text{SCH}_2\text{C}(\text{=CH}_2)\text{COCOONa} = 2:1$). The partial presence of the $\text{SCH}_2\text{C}(\text{=CH}_2)\text{COCOONa}$ grouping is supported by the NMR data of the

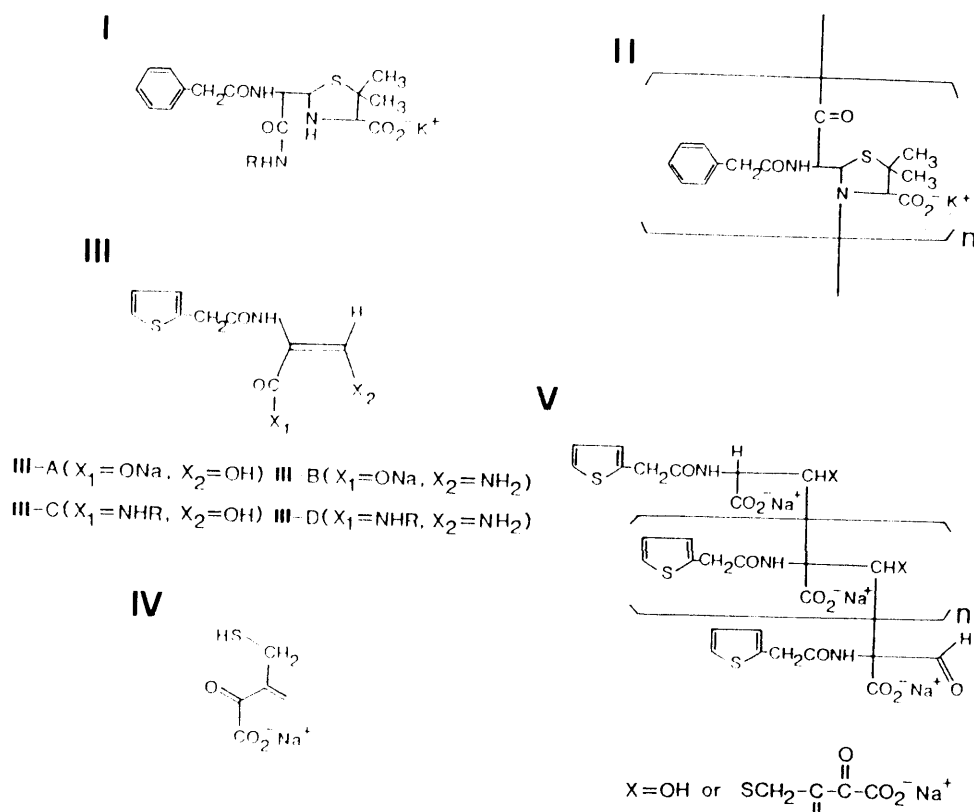


Fig. 3. Chemical structures of antibiotic-haptens, antibiotic hydrolysis products and antibiotic polymers. I, BPO-hapten. BPO-EACA [$R = (\text{CH}_2)_6\text{COOH}$]; II, PCG polymer; III-A, III-B, IV, hydrolysis products of CET; III-C, III-D, CET-hapten. V, an estimated structure of CET polymer.

CET polymer as well as a better accordance with the elemental analysis data. The actual structure, however, would be more complicated since the estimated structure cannot completely explain all spectral data, especially many unidentified complicated $^1\text{H-NMR}$ signals appearing at 2-4 ppm (data not shown). Although the actual structure is still unknown, the presence of monoclonal antibody which can distinguish the PCG polymer from the CET polymer in spite of binding specificity for the acyl side chain strongly suggests difference in steric structures between these polymers. As far as structures II and V are compared, PCG polymer retaining the thiazolidine appears more rigid than the CET polymer. The less-flexible structure of PCG polymer may hinder the binding with the monoclonal antibody or the effective cross-linking of IgE antibodies bound to Fc ϵ receptors enough to trigger the PCA reaction (Levine, 1965a, b; Ishizaka & Ishizaka, 1978).

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