POLYMERIZATION OF CEPHALOSPORINS IN AQUEOUS SOLUTION

Q Zhang, MB Yamamoto*, HM Aki*, KH Kubo* and GT Liao

(*School of Pharmacy, West China University of Medical Sciences, Chengdu 610041;*
** Faculty of Pharmacy, Fukuoka University, Fukuoka, Japan 814-01*)

ABSTRACT The polymerization of 7 cephalosporins, including ceftizoxime Na (CZX), cefotaxime Na (CTX), ceftriaxone Na (CTRX), cefotiam 2 HCl (CTM), cefamandole Na (CMD), cefazolin Na (CZL) and cefalotin Na (CLT), in aqueous solution has been investigated in relation to their chemical structures. The aqueous solution of cephalosporins was eluted on DEAE-Sephadex A-25 column by a linear gradient of 0.2～3.0 mol·L^{-1} NaCl (pH 7.4～9.4) at ambient temperature. The fractions were characterized by UV, CD, FAB-MS, and NMR spectrometries. No polymers were observed from CTM, CTRX, CMD, CZL, and CLT solutions, while CZX and CTX were found to form dimers within a few days. The dimers were evidently confirmed to form through the intermolecular aminolysis of the β-lactam carbonyl moiety by the NH_2 group in the second molecule. However, the steric and ionic charge hindrance may have a direct effect on the polymerization.

Key words Cephalosporins; Polymer; Ion exchange chromatography

The polymers of β-lactam antibiotics, mostly the penicillins, have been the subject of many studies[1～5], because these polymers have been found to possess strong antigenic properties in animal experiments[6,7] and, therefore, may play a role in some clinical allergic reactions caused by using such antibiotics. Many questions in this field still remain to be answered, and one of them is the relationship between chemical structure and the polymer formation. In the present paper, the polymerization of 7 cephalosporins, ceftizoxime Na (CZX), cefotaxime Na (CTX), ceftriaxone Na (CTRX), cefotiam 2HCl (CTM), cefamandole Na (CMD), cefalotin Na (CLT) and cefazolin Na (CZL) in aqueous solution has been investigated in order to understand the properties of their polymerization and the relationship with their chemical structures. Chemical structures of the 7 cephalosporins are shown in Tab 1.

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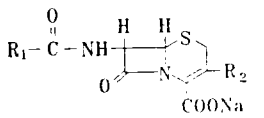
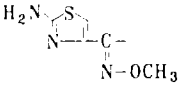
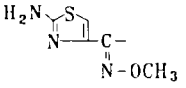
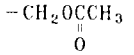
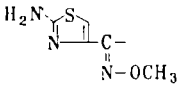
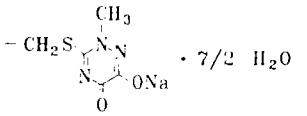
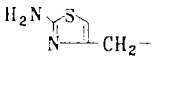
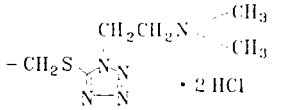
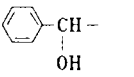
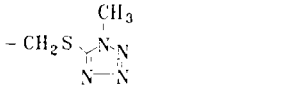
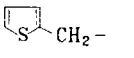
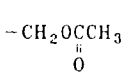
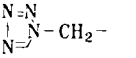
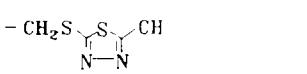
Tab 1 Chemical structures of 7 tested cephalosporins

Compound	 (R_1)	(R_2)	Molecular formula (m. w.)
Ceftizoxime Na (CZX)		-H	$C_{13}H_{12}N_5O_5S_2Na$ (405.39)
Cefotaxime Na (CTX)			$C_{16}H_{16}N_5O_7S_2Na$ (477.44)
Ceftriaxone Na (CTRX)			$C_{18}H_{16}N_2O_7S_3Na_2 \cdot 7/2H_2O$ (661.59)
Cefotiam 2HCl (CTM)			$C_{18}H_{23}N_9O_4S_3 \cdot 2HCl$ (598.54)
Cefamandole Na (CMD)			$C_{18}H_{17}N_6O_5S_2Na$ (484.48)
Cefalotin Na (CLT)			$C_{16}H_{15}N_2O_6S_2Na$ (418.41)
Cefazolin Na (CZL)			$C_{14}H_{13}N_8O_4S_3Na$ (476.48)

EXPERIMENTAL

Materials

Seven cephalosporins for injection were the preparations commercially available in Japan. DEAE-Sephadex A-25 (40～120 μm) was purchased from Pharmacia, Sweden. HP-20 was supplied by Diaion Company, Japan. All chemicals and solvents were of analytical reagent grade.

Instrumentation

The chromatographic system consisted of a 45 cm × 2.6 cm column (Pharmacia SD450 Excel Column, Sweden), a peristaltic pump (Mitsumi Scientific Industry, Japan), a spectrophotometer (Shimadzu UV-120-02, Japan), a potentiometric recorder (Yoko Gawa LER-12A, Japan) and a fraction collector (Gilson FC-220, France). The detection wavelength was 260 nm; and the whole system was kept at ambient temperature. A lyophilizer (VirTis, Bench Top 3, USA) was used to isolate the polymer, while a spectropolarimeter (Spectroscopia JASCO J500S, Japan), a 400 MHz NMR analyzer (Jeol JNM-GX400, Japan) and a mass spectrometer (Jeol JMS-HX, Japan) were

applied to characterize the polymer. The scanning speed was 100 nm·min^{-1}, and the wavelength ranged from 195 to 350 nm in the circular dichroism (CD) analysis. The solvents were DMSO-d_6 and *m*-NBA/methanol for NMR and FAB-MS measurement, respectively.

Sample preparation

The dry powder of each cephalosporin was dissolved in distilled water to a concentration of 20%～50%, then the pH value of each solution was adjusted to 8.5±0.1 with 2 mol·L^{-1} HCl. The solution of each cephalosporin was aged at room temperature for 3 to 8 days. Details are shown in Tab 2.

Tab 2 The experimental conditions of 7 cephalosporins

Experimental condition		CZX	CTX	CTRX	CTM	CMD	CLT	CZL
Concentration		50	50	50	20	25	25	50
Aged time (d)		3	3	3	4	6	8	5
Charged volume (ml)		0.1	0.1	0.1	0.05	0.2	0.2	0.1
LG*	pH	7.4～9.4	7.4～9.4	7.4～9.4	7.4～7.4	7.4～7.4	7.4～7.4	7.4～7.4
	NaCl conc. (mol·L^{-1})	0.2～3.0	0.2～3.0	0.2～3.0	0.2～2.5	0.2～1.8	0.2～2.5	0.2～1.8

*LG: linear gradient

Elution operation

The aqueous solution of each cephalosporin was eluted on the column packed with DEAE-Sephadex A-25 gel which was previouly equilibrated on a steam bath for 3 h with 0.05 mol·L^{-1} phosphate buffer containing NaCl 0.2 mol·L^{-1} at pH 7.4. Separation was carried out with a linear NaCl gradient (0.2 to 3 mol·L^{-1}) at a constant flow rate of 70 ml·h^{-1}, by UV monitoring, the effluent was collected in fractions of 10 ml and estimated by CD spectrometry. Experimental details are summarized in Tab 2.

Isolation and structrure analysis

These effluents related to the peaks in the UV monitored elution pattern were freeze-dried first, and the freeze-dried sample was desalted by ion exchange resin HP-20, collected by methanol, dried in vacuum at room temperature and finally characterized by FAB-MS and NMR spectrometries.

RESULTS AND DISCUSSION

Fig 1 shows the UV monitored elution patterns by anion exchange chromatography. The five cephalosporins, CZX, CTX, CTRX, CTM and CLT, gave two major peaks (peak A and B) in aqueous solution after aging at room temperature for several days, while CMD and CZL showed only one peak each.

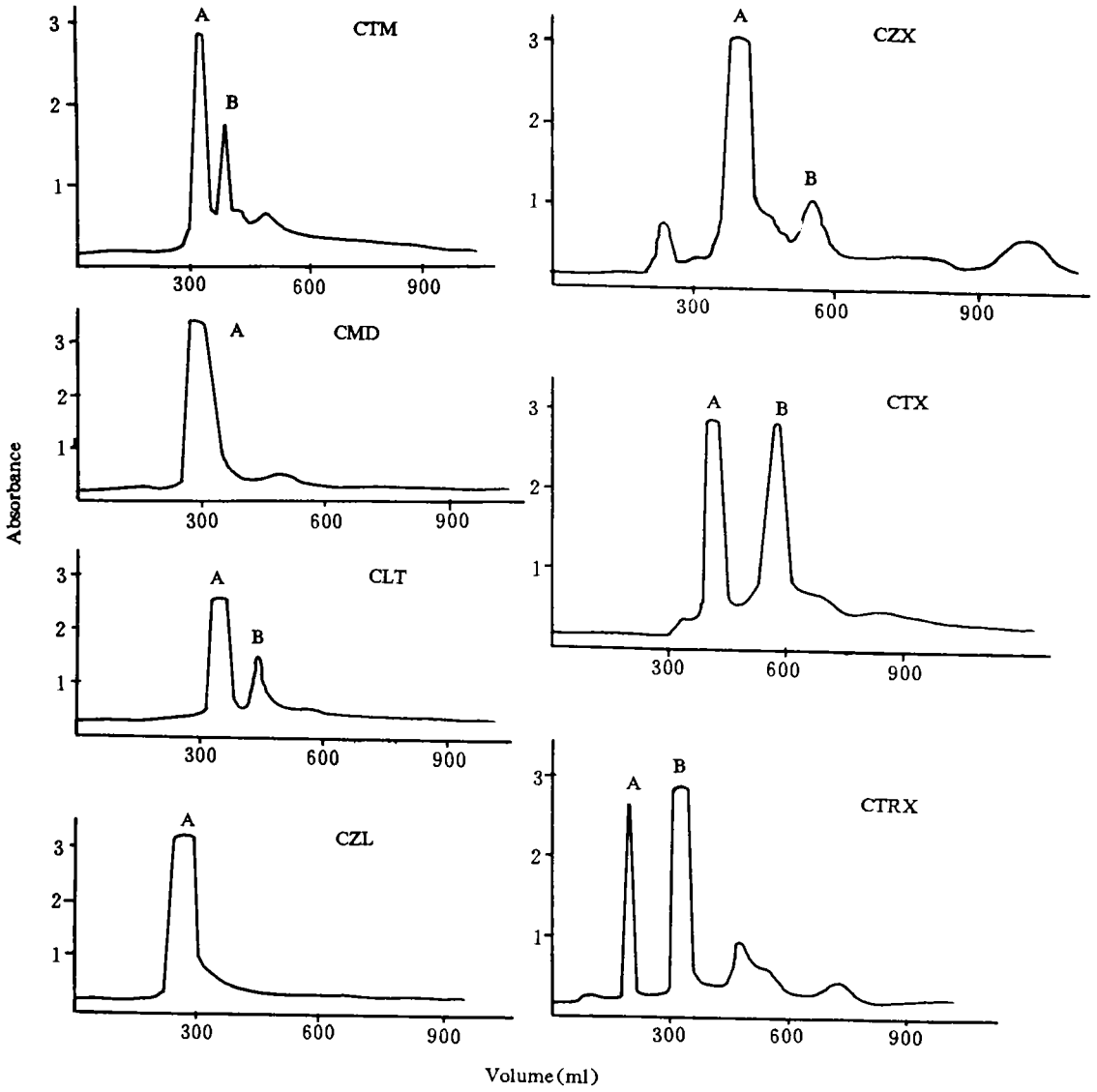


Fig 1 The UV monitored elution patterns of 7 cephalosporins by anion exchange chromatography. A,B: Codes of major peaks in UV monitored elution pattern.

The λ_{max}(nm) values of CD spectra are given in Tab 3. All cephalosporins have a positive band at 245～312 nm and a negative band at 217～220 nm. The positive CD band is due to the Cotton effect of the β-lactam ring of cephalosporin, suggesting the intact β-lactam ring of cephalosporins. For CZX, CTX, CTRX and CTM, the λ_{max} values of peak A and peak B are almost the same, indicating that there existed the same β-lactam ring in the substances corresponding to peak A and B. But for CLT, the CD spectrum of peak B is not detectable.

Tab 3 The λ_{max} values of CD spectra of the substances corresponding to peaks A and B of 7 cephalosporins in their chromatograms

	CZX	CTX	CTRX	CTM	CMD	CLT	CZL
λ_{max} of peak A (nm)	245(+)	253(+)	312(+)	255(+)	255(+)	250(+)	253(+)
	217(−)	219(−)	220(−)	220(−)	220(−)	217(−)	218(−)
λ_{max} of peak B (nm)	248(+)	253(+)	312(+)	250(+)		ND*	
	218(−)	219(−)	220(−)	220(−)			

*ND: not detectable

The FAB-MS spectra of substances isolated from the fractions of peaks A and B of cephalosporins in their chromatograms were measured and the values of $(M+H)^+$(m/z) are summarized in Tab 4. The values of peak A for CZX(384), CTX(456), CTM(526), CMD(463), CLT(397), CZL (455) and peak B for CTRX(555) are assignable to their monomers, while the values of peak B for CTX(910) and CZX(766) are obviously due to their dimers. The $(M+H)^+$ at m/z 485 for peak A of CTRX and the $(M+H)^+$ at m/z 900 and 493 for the peak B of CTM and CLT do not correspond to their molecular dimers, but these ions may come from the fragments of their dimers or the dimers of their fragments. Therefore, further studies on their polymerization are needed.

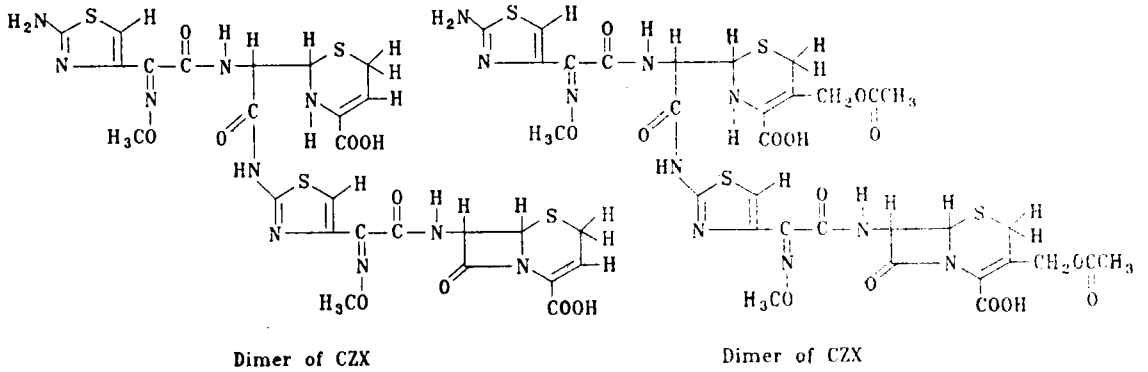
Tab 4 The $(M+H)^+$(m/z) values of FAB-MS of substances corresponding to peaks A and B of 7 cephalosporins in their chromatograms

	CZX	CTX	CTRX	CTM	CMD	CLT	CZL
$(M+H)^+$ of peak A (m/z)	384	456	485	526	463	397	455
	Monomer	Monomer		Monomer	Monomer	Monomer	Monomer
$(M+H)^+$ of peak B (m/z)	767	911	555	900		493	
	Dimer	Dimer	Monomer				

Proton NMR spectra were analyzed to clarify the chemical structures of the dimers, and the chemical shifts (ppm) of CTX are summarized in Tab 5. The signals at 9. 32 and 2. 68 ppm correspond to the 8-CONH-proton on the thiazole ring and the 5-NH-proton on the β-lactam ring in the dimer of CTX, reflecting that the β-lactam ring was cleaved by the amino group of a second molecule of cephalosporins in forming the dimer. The proposed structures of such dimers are depicted in Fig 2.

Tab 5 The chemical shifts (ppm) of CTX monomer and dimer by ^{1}H-NMR

Assignment	Chemical shifts (ppm)	
	Monomer	Dimer
$2H_2$	3.47, 3.62(2H,ABq)	3.47, 3.31(2H,ABq)
$3\text{-}CH_2$	4.71, 4.99(2H,ABq)	4.71, 4.99(2H,ABq)
$\text{-}OCOCH_3$	2.03(3H,s)	2.03(3H,s)
6H	5.15(1H,d)	5.14(1H,d); 5.36(1H,d)
7H	5.77(1H,q)	5.70(1H,q); 5.77(1H,q)
9H	9.49(1H,d)	9.41(1H,d); 9.47(1H,d)
13H	3.85(3H,s)	3.84(3H,s)
5'H	6.76(1H,s)	6.74(1H,s)
$2'NH_2$	2.50(2H,s)	2.49(2H,s)
5NH		2.68(1H,s)
8CONH-		9.32(1H,s)



Dimer of CZX Dimer of CZX

Fig 2 The proposed structures of CTX and CZX dimer.

Accordingly, it can be pointed out that the free NH_2 group is essential for polymer formation, since all the tested cephalosporins which do not contain any NH_2 group, such as CMD, CZL and CLT, were not found to form polymers, while those cephalosporins which contain thiazole NH_2 group, for instance, the CTX and CZX, were observed to form their dimers after standing for several days in aqueous solution. However, CTRX and CTM are exceptions which led to such assumption that besides the NH_2 group, other elements may affect the formation of polymer. By careful comparison, it seems logical to suppose that the behavior of CTRX and CTM is due to their steric hindrance and/or ionic charge hindrance, since both CTRX and CTM have ionic charge and possess much larger side chain than CTX and CZX.

CONCLUSIONS

CTX and CZX were shown to form dimers in aqueous solution, and no polymers were observed for other tested cephalosporins. It seems that polymer is only found in those cephalosporins which possess free NH_2 group and have no relatively large side chain or ionically charged group. The dimers

of CTX and CZX were formed through intramolecular aminolysis at the β-lactam carbonyl moiety by the free NH_2 group of a second molecule.

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头孢类抗生素在水溶液中的聚合

张 强 MB Yamamoto*, HM Aki*, KH Kubo*, 廖工铁

(华西医科大学药学院,成都 610041; *日本福冈大学药学部,福冈 814-01)

摘要 对7种头孢类抗生素:头孢唑肟钠(CZX),头孢噻肟钠(CTX),头孢曲松钠(CTRX),头孢替安(CTM),头孢孟多钠(CMD),头孢唑啉钠(CZL)和头孢噻吩钠(CLT)在水溶液中的聚合行为进行了研究。将头孢类抗生素的水溶液(10%～50%)在 DEAE-Sephadex A-25离子交换色谱柱上,用含 NaCl 0.2～3.0 mol·L^{-1}的磷酸盐缓冲液(pH 7.4～9.4),在室温下进行线性梯度洗脱,在260 nm 监测,收集各流分。用 CD,FAB-MS 和 NMR 等确证洗脱图谱中峰值对应的化合物,发现 CMD,CZL,CLT,CTRX 和 CTM 在实验条件下没有形成聚合物,CTX 和 CZX 的水溶液在室温下放置几天可形成二聚物,表明分子中不含自由氨基或含有氨基,但空间或电荷位阻大时,没有聚合物形成。形成的二聚物是由于自由氨基向核进攻另一分子的 β-内酰胺环,引起分子间氨解的结果。

关键词 头孢类抗生素;聚合物;离子交换色谱