NOTE

Components of Phosphomonoesters Observed by ^{31}P NMR of the Spleen: Results from Normal Rats and Patients with Liver Cirrhosis

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Composition of the PME resonance was determined in rat spleens, and in surgical specimens of human spleens enlarged due to liver cirrhosis. Specimens were excised rapidly in one group of rats (n=5), and in the other group (n=5) after clamping of splenic artery and vein for 1 hour. Phosphoethanolamine (PE) was the major component of PME resonance in both groups, and PE/PC was 3.51±0.18 in the former group and 3.45±0.17 in the latter, suggesting anaerobic condition hardly affected PE/PC ratio. PE was also dominant in human spleen with the PE/PC ratio of 5.0. Intensity of 2,3–DPG resonance was not greater than that of PC in rapidly excised spleens of rat.

INTRODUCTION

MR spectroscopy (MRS) can detect some important metabolites in tissues *in situ*, and this ability of MRS makes it a useful diagnostic probe. Extensive studies of various tissues using ^{31}P MRS have shown that among several resonances detected on *in vivo* spectra, the intensity of the PME resonance is frequently elevated in many malignant lesions compared to that in control tissues[1].

Some of malignant neoplasmas, such as lymphoma and lymphatic leukemia, have tendency to infiltrate diffusely into the spleen. In cases with possible infiltration to the spleen, MRS may be a useful modality because the infiltration can hardly be detected by imaging techniques. To the contrary, MRS can essentially detect the alternations of metabolic conditions in infiltrated spleen. A couple of spectroscopic studies of the infiltrated spleen have shown a prominently elevated PME in malignant disorders compared to controls[2),3)], and a shift of the chemical shift of PME resonance to downfield[4)]. By spectroscopic follow-up of therapy, a significant reduction in the PME peak was observed in the spleen of patients who had chron-

Keywords ^{31}P MRS, NMR, spleen, phosphomonoesters

ic lymphatic leukemia and showed clinical improvement after therapy[3]. As the PME resonance increases also in splenomegaly due to benign causes[2], the components of the PME resonance in the spleen should be clarified for understanding the nature of the PME peak elevated due to various causes.

In the extract of a lymphomatous lymph node, most of the PME was identified as PE[5]. The elevation of PME in the hepatic lymphoma might presumably be due to the increase in PE content in the liver[5]. As PME resonance cannot be resolved into the components without proton-decoupling technique, we proposed a method to estimate the major components of PME on *in vivo* MR spectra[6]. In that method, the PE/PC ratio is estimated from the chemical shift of PME and Pi peak, assuming that the chemical shift of PME might depend on the relative contents of PE and PC. This method was applied to the spectra of spleens enlarged due to hematologic disorders[4].

Components of the PME resonance of the spleen has not been analyzed and is still uncertain as far as we know. The purpose of this study is to investigate the components of the PME peak of the normal spleen as the basis for the understanding of *in vivo* MR spectra. We need to consider a possible anaerobic effect on the components of the PME resonance because specimens excised at surgery for further *in vitro* NMR analysis are usually under anaerobic conditions of some duration before excision.

SUBJECTS AND METHODS

Adult male rats of Wistar strain weighing about 200 g were used for this study. Rats were anesthetized under intraperitoneal injection with sodium pentobarbitone (20 mg/kg). Rapidly after abdominal incision, spleens were resected from five rats in order to avoid the ischemic effect on metabolite concentrations. Other five rats had splenectomy after clamping of splenic artery and vein for 1 hour in order to investigate the anaerobic effect on the metabolite ratios. Each specimen weighed from 0.8 up to 1.4 g. Specimens of human spleen were obtained intraoperatively in two patients with enlarged spleens due to liver cirrhosis. Specimen were immersed in liquid nitrogen immediately after excision, and stored in a −80℃ freezer till chemical procedures.

The specimen was pulverized in a ceramic mortar immersed in liquid nitrogen and then co-pulverized with 10% perchloric acid of the same volume with specimen. They were homogenized in an ice-cooled mortar. After centrifugation, EDTA-2Na solution was added into the supernatant to the concentration of 15 mM EDTA. Acidity of the supernatant was adjusted to pH 8.4–8.8 with 20% potassium bicarbonate solution, and resulting precipitate was removed by centrifugation.

The spectra were acquired on a Varian 7 T spectroscopy system (Unity 300) at a ^{31}P NMR frequency of 121 MHz. The spectra were the sum of 400 to 900 transients, collected with 45 degree pulse, a 5-sec interpulse delay, and a spectral window of 6000 Hz. Proton decoupling was applied during the acquisition period and

Received Oct. 17, 1996; revised Dec. 26, 1996
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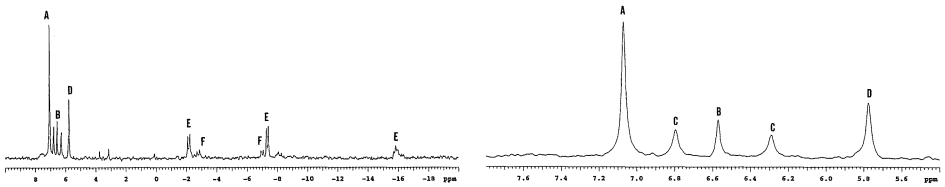
the sample was placed in room temperature during measurement. Acquisition with 30-sec delays was performed to correct the saturation effect at 5-sec interpulse delay, and a spectrum of 5-sec interpulse delay was acquired without proton decoupling to evaluate nuclear Overhauser effect (NOE). Chemical shift was referenced to external phosphoric acid of pH 7.3 at 5.12 ppm. Peak assignments were confirmed by spiking the extracts with authentic PC and PE solutions, separately. 2,3–DPG was assigned based on the titration data of 2,3–DPG, PE and PC[7),8)]. The PE/PC ratios were estimated from peak integrals.

RESULTS AND DISCUSSION

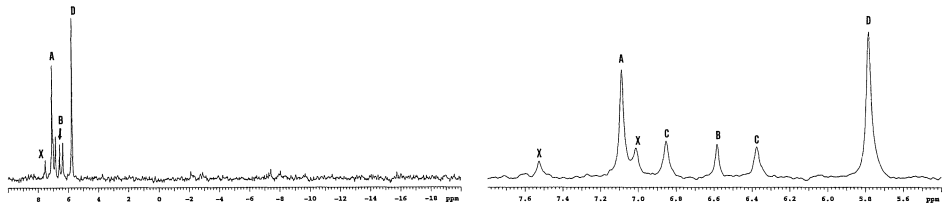
PE was the dominant component of PME with smaller amounts of PC in rapidly excised rat spleen (Fig. 1). ATP, and 2,3–DPG which is a major component of blood PME[9),10)] were observed in rapidly excised spleen showing similar peak height. Except for PE, PC and 2,3–DPG, no other resonances were identified in the PME region of rapidly excised spleen. In an anaerobic rat spleen (Fig. 2), the PE/PC ratio was similar to those of rapidly excised spleens. ATP was slightly discernible and 2,3–DPG did not decrease so much as ATP, suggesting that decomposition or chemical change of 2,3–DPG is slower than ATP.

As shown in Table, PE/PC was averaged as 3.51 ± 0.18 (mean$\pm$SD) in rapidly excised spleens ($n=5$), and 3.45 ± 0.17 in spleens experienced anaerobic conditions for 1 hour ($n=5$), respectively. As no significant differ-



(a) (b)

Fig. 1. NMR spectra of the perchloric acid extract of the spleen rapidly excised from a normal rat. Whole spectrum (a) and its PME-to-Pi region (b). PE is a dominant component of the phosphomonoester resonance. Peak assignments : A : PE, B : PC, C : 2,3–DPG, D : Pi, E : ATP, F : ADP, X : unknowns.



(a) (b)

Fig. 2. NMR spectra of perchloric acid extract of rat spleen experienced anaerobic condition of 1 hr. Whole spectrum (a) and its PME-to-Pi region (b). The PE/PC ratio changed little compared to that of the rapidly excised spleen. Comparing to the spectrum in Fig. 1, additional peaks were detected. They were not assigned and indicated with "X". For peak assignments, see the legend of Fig. 1.

ence was found statistically between the two groups, clamping of splenic artery and vein for 1 hour had little effect on the PE/PC ratio of the spleen.

In vitro spectra of the specimen obtained from human spleen enlarged due to liver cirrhosis showed that PE was the dominant component of the PME region (Fig. 3). The PE/PC ratios of the two human spleens were 4.2 and 5.8. The average was 5.0, which was higher than that of the spleen of normal rats.

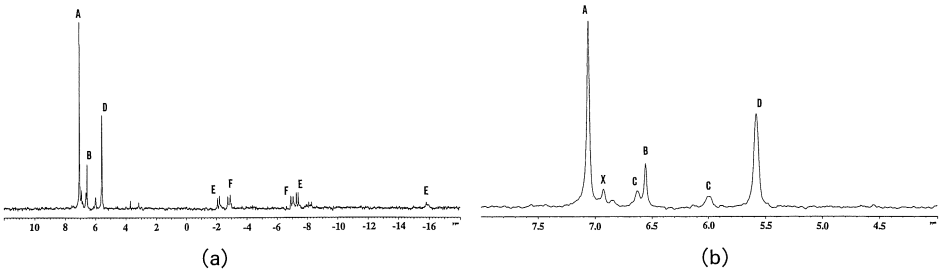
Spectrum with 30-sec interpulse delay was acquired from the extract of a rat (No. 2). It was showen that the PE and PC resonances were fully relaxed during the 5-sec interpulse delay because the PE/PC ratio on 30-sec spectrum relative to that on 5-sec spectrum was 1.01. The PE/PC ratio measured without proton decoupling was 0.99 of that with proton decoupling showed that NOE unaffected on the PE/PC ratio.

Table　PE/PC Ratios in the Perchloric Extracts from Normal Rat Spleens

Rapidly excised		Anaerobic condition	
Rat No.	PE/PC	Rat No.	PE/PC
1	3.09	6	3.35
2	3.63	7	3.37
3	3.69	8	3.57
4	3.46	9	3.75
5	3.66	10	3.21
Av	3.51		3.45
SD	0.18		0.17

In anaerobic spleens of rats, two peaks which were not observed in the spectra of rapidly excised spleens were additionally detected . The chemical shifts were 7.5 and 7.0 ppm, respectively, suggesting sugar phosphates. The intensity of these resonances was smaller than PE or PC (see Fig. 2, indicated with "X"). Perry et al.[11] determined contents of 35 amino acids and related compounds in whole rat brain, and in biopsied and autopsied human brain in order to study postmortem changes of the contents. It was observed that PE began to decrease after 24 hour in rat brain, but was stable in human brain[9]. PC was not measured in their study.

In a conclusion, the dominant component of the PME peak was identified as PE both in the spleen of normal rats and also in enlarged human spleens due to liver cirrhosis; the PE/PC ratio was averaged as 3.51 ± 0.18 in the former and 5.0 in the latter, respectively. The content of 2,3–DPG was found to be lower than PE in



(a)　(b)

Fig. 3. NMR spectra of perchloric acid extracts from the human spleen enlarged due to liver cirrhosis. PE was also dominant in phosphomonoesters of the human spleen. The PE/PC ratio was somewhat higher than those of rats. Whole spectrum (a) and its PME-to-Pi region (b). For peak assignments, see the legend of Fig. 1.

the rapidly excised spleen. The PE/PC ratio in rat spleen changed little after anaerobic condition of 1 hour. Therefore, it appears that anoxic condition, which likely occurs during intervals between the vascular clamping and excision of human organ specimen at surgery, does not essentially affect the tissue PE/PC ratio. This is the first report of the NMR study of the components of the spleen PME.

ACKNOWLEDGMENT

The authors thank James C. Ehrhardt, PhD, of The University of Iowa for his advice in the preparation of this manuscript, Dr. Y. Seo of National Institute of Physiological Science for his encouragement of this work, and also Dr. Y. Hoshikawa and Dr. M. Ishikawa for their assistance in sample preparation.

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Abbreviations :

ATP (adenosine triphosphate)
2,3–DPG (2,3–diphosphoglycerate)
NOE (nuclear Overhauser effect)
PC (phosphocholine)
PE (phosphoethanolamine)
Pi (inorganic phosphate)