論　文　の　内　容　の　要　旨

応用生命化学専攻
平成 15 年度博士課程進学
氏　名　　胡　芳宇
指導教員　　田之倉　優

論文題目

Nondestructive Observation of Bovine Milk using NMR Spectroscopy
（NMR を用いた牛乳の非破壊測定）

Introduction

Nuclear magnetic resonance spectroscopy (NMR) is a powerful tool to identify organic compounds and to analyze the structures of biopolymers in organic chemistry and biochemistry. The application of NMR spectroscopy to food science is recently being tried because it is non-destructive, selective, and capable of simultaneous detection of compounds in complex mixtures. In this study we tested the potential of NMR spectroscopy as a tool for the analysis of milk. Milk compositions were detected by NMR spectra without any pre-treatment, and their existing states were also studied in milk. Colostrum was also analyzed by NMR spectroscopy in the same way. Compounds only existing in colostrum were detected for the first time. Because the amplitude or area of NMR signals is proportional to the concentration of the corresponding molecule in the observed volume, ^{1}H, ^{31}P 1D and ^{1}H-^{13}C HSQC 2D spectra were applied to the

quantification of various milk compounds. Time course study of phosphorylated compounds in colostrum was performed by ^{31}P 1D spectra, which provided useful information on relative rates of synthesis of important milk constituents. The target of this research is to establish an effective and nondestructive NMR technique for detection and quantification of complex mixtures such as drinks and body fluids.

The Analyses of Commercial Milk by NMR Spectroscopy

One- (^{1}H, ^{13}C) and two–dimensional (^{1}H-^{13}C, ^{1}H-^{15}N, ^{1}H-^{31}P) NMR spectra of commercial milk were observed directly without any pre-treatment. The signals in each NMR spectrum were assigned, and their existing states were also analyzed. Lactose exists in a free state in milk. The signals due to the butyric acid chain can be assigned among the other fatty acid chains. Monounsaturated fatty acid (oleic acid chains) and polyunsaturated fatty acid chains (linoleic and linolenic acid) were assigned by their characteristic signals. The signals from citrate, *N*-acetylcarbohydrates, and lecithin could be observed directly in the ^{1}H-^{13}C HSQC NMR spectra; the assignment of their signals was made through the ^{1}H-^{13}C, ^{1}H-^{15}N, and ^{1}H-^{31}P HMBC spectra of extracted milk. Signals from creatine and *N*-acetylcarbohydrates were detected for the first time. The ^{1}H-^{13}C HSQC spectrum of commercial milk was shown in Figure 1.

Commercial milk has also been quantified using ^{1}H 1D and ^{1}H-^{13}C HSQC 2D NMR spectra. Fat content of commercial whole milk was detected to be 3.61 ± 0.08%, and lactose content was detected to be 47.77 ± 1.01mg/ml by ^{1}H NMR spectra. Concentrations of citrate, N-acetylcarbohydrates, butyric chain, total unsaturated fatty acid, total multi-unsaturated fatty acid and lecithin were detected to be 3.16 ± 0.22, 2.87 ± 0.11, 18.53 ± 1.44, 41.91 ± 2.76, 2.86 ± 0.33 and 4.04 ± 0.59 mM, respectively, by ^{1}H-^{13}C HSQC spectra. This is the first quantification of milk compositions using 2D

NMR spectra.

The Analyses of Colostrum by NMR Spectroscopy

Colostrum has also been analyzed by NMR spectroscopy. The one- (^{1}H, ^{13}C, ^{31}P) and two-dimensional (^{1}H-^{13}C HSQC, ^{1}H-^{31}P HMBC) NMR spectra of colostrum were successfully obtained. Fat content of colostrum in the first day postpartum was much higher than that in commercial milk as detected by ^{13}C NMR spectra. In the ^{1}H- ^{13}C HSQC spectrum of colostrum, signals due to creatine and methylene groups of lecithin could be detected directly, while these signals were not observed in that of commercial milk. Phosphorylated compounds existing in milk were thoroughly studied by ^{31}P 1D and ^{1}H-^{31}P HMBC 2D NMR spectra. UDP-glucose, UDP-galactose, phosphocreatine, glucose-2-P and galactose-2-P only exist in colostrum. Especially, glucose-2-P and galactose-2-P were detected only in colostrum in the first and second day postpartum for the first time. Contents of phosphatidyl choline, phosphatidyl ethanolamine and choline phosphate were much higher than that in commercial milk.

Phosphorylated compounds (UDP-glucose, UDP-lactose, phosphatidyl choline, phosphatidyl ethanolamine, phosphocreatine and choline phosphate) occurring as lactation progressed from day 1 to 30 have been quantified easily by ^{31}P NMR spectra.

Conclusion

Commercial milk and colostrum have been successfully studied using NMR spectroscopy. General compounds such as fatty acid and lactose were easily detected in ^{1}H and ^{13}C 1D NMR spectra. Minor compounds such as phosphorylated sugars, creatine and phospholipid were also detected by the combination with various NMR measuring methods. Most of them were detected in milk for the first time. Compounds existed in

commercial milk were quantified by ^{1}H 1D and ^{1}H-^{13}C HSQC 2D spectra. Time course study of phosphorylated compounds in colostrum was performed by ^{31}P 1D spectra. This research showed the convenience, sensitivity and efficiency of NMR spectroscopy. NMR spectroscopy is considered to be applicable to quality control or specification of various types of mixtures.

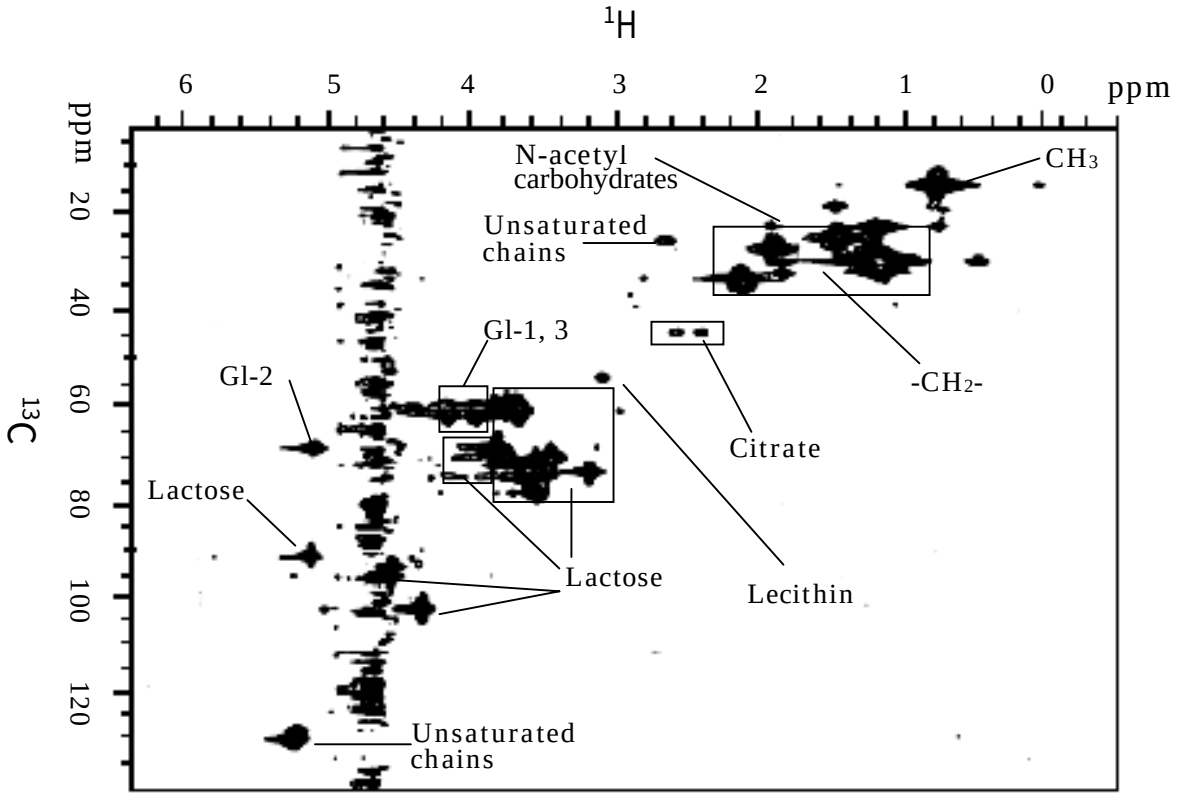

Figure 1. ^{1}H-^{13}C HSQC spectrum of commercial milk

Reference

Hu. F., Furihata, K., Ito-Ishida, M., Kaminogawa, S., Tanokura, M. (2004). Nondestructive observation of bovine milk by NMR spectroscopy: analysis of existing states of compounds and detection of new compounds. *J. Agric. Food Chem*, 52, 4969-4974.