

NMR spectroscopy was first introduced to the world with a measurement performed on a mobile organic sample. The observed nuclear induction of protons in paraffin was used to calculate the gyro magnetic ratio of the proton. The discovery of the chemical shift propelled NMR into chemistry and analysis and identification of small molecules. An early application (reported about a decade later in 1957) to biological molecules reported four resonances for the protein ribonuclease, which at the time was subject to intense investigation by all available techniques.

Intense interest in membranes later catalyzed applications to multi-component biological systems containing both mobile and immobile phases. A  $^1\text{H}$  NMR study of a rabbit sciatic nerve reported in Science in 1972 [1] attracted much attention by its conclusion that the phospholipids (PL) of the myelin sheath were mobile enough to be seen with the standard “solution state or high-resolution” method of NMR. Other studies of membranes isolated from cells showed observable proton peaks. These findings seemed to contradict measurements by other techniques that implied very high rigidity in biomembranes. The NMR spectra of membranes revealed peaks for protons in the acyl chain of esterified lipids. Missing were peaks for cholesterol and even the phospholipid choline headgroup, which should be mobile because of internal rotations.

The sciatic nerve was re-investigated by the newer method of  $^{13}\text{C}$  NMR spectroscopy [2], which with its much better resolution showed many acyl chain peaks, and only a very weak choline methyl peak. Remarkably, after rinsing the nerve preparation in ethanol, the intact nerve revealed no NMR signals. The ethanol rinse contained the species contributing to the NMR spectrum: triglyceride. Most triglyceride in biological systems is in a liquid-isotropic state, which explains its high resolution spectrum. This study first illustrated that a mobile species can dominate the NMR spectrum of a multi-component system, even if it is a minor species, in this case contaminating fat tissue adhering to the dissected nerve.

Detection of triglycerides (TG) can however be important because of their involvement in diseases, including some cancers [3], obesity and diabetes. In these cases, application of high resolution NMR spectroscopy takes advantage of the high mobility of the TG and the low mobility of the many other cell components, which include membrane lipids. The enrichment of TG in fatty liver can be detected *in vivo* by voxel-guided  $^1\text{H}$  NMR spectroscopy [4]. This is an outstanding illustration of the differential mobilities of cell components, and the basis for detection of the excess fat in liver derives from the observation of fat adherent to the sciatic nerve.

At the same time as most studies focused on membranes,  $^{13}\text{C}$  NMR spectroscopy was applied to plasma lipoproteins [5], which circulate in the plasma as spherical microemulsions and deliver fatty acids and cholesterol to cells as well as remove excess cellular cholesterol.  $^1\text{H}$  spectra had shown about a half dozen signals, mostly from acyl chains [6]. These could not be distinguished for the three types of acylated lipids present in lipoproteins: PL, TG, and cholesteryl esters (CE).

In NMR studies of membranes, one key lipid constituent was consistently missing: cholesterol. The first observation of NMR signals from the rigid cholesterol ring came in the  $^{13}\text{C}$  natural abundance spectra of lipoproteins, which contained numerous well-resolved

peaks, including those from the acyl chains, and the steroid ring and its side chain. The most abundant form of cholesterol in the lipoproteins is the esterified form, and model systems studies [7] later demonstrated that the steroid rings peaks originated predominantly from CE in a liquid state. Moreover, a small proportion of the unesterified cholesterol dissolved in the liquid core of the lipoprotein and gave rise to observable signals whereas the majority of cholesterol in a less mobile environment did not give any observable signal [8]. Thus, the plasma lipoproteins represent a valuable case study of differential mobilities of lipids.

The most complex multi-component, multi phase lipid system that has been studied by NMR is probably the atherosclerotic plaque [9]. At body temperature, the plaque lipids may be present in a liquid state (CE, TG and some cholesterol), liquid crystalline state (phospholipid/ cholesterol bilayers) and the crystalline state (cholesterol) [10]. Plaque tissue, which contains many other components in addition to lipids, give rise to a remarkably simple high resolution spectrum that originates from the liquid lipid phase, which is mainly comprised of CE. As expected, the crystalline state of cholesterol is not observed with solution-state NMR. However, with solid-state magic angle spinning (MAS) NMR, crystalline cholesterol is detected and the liquid CE are now absent [11, 12]. Conditions of the MAS experiment can be modified to reveal the liquid-crystalline phase of cholesterol without interference from the crystalline cholesterol.

Recent studies of atherosclerotic plaque have achieved the convergence of NMR spectroscopy and MRI with the issue of lipid phases and mobilities. With the goal of simplifying the images of plaques to achieve enhanced detection of individual plaque components, an application of diffusion-weighted imaging suppressed the signals of the mobile water protons to enhance the signals from protons attached to the more viscous/less mobile lipids [13]. Pulse parameters to provide the high contrast between lipids and water were derived from model lipid systems. What was the chemical identity of the lipid seen by MRI? This question was answered definitively by voxel-guided  $^1\text{H}$  NMR spectroscopy [14]: CE, the only abundant liquid lipid in plaques, the result predicted from the first  $^{13}\text{C}$  NMR study of atherosclerotic plaques.

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