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Analysis of RF heating and sample stability in aligned static solid-state NMR spectroscopy

Conggang Li [a,b], Yiming Mo [a,b], Jun Hu [a,b], Eduard Chekmenev [b], Changlin Tian [a,c], Fei Philip Gao [a,b], Riqiang Fu [b], Peter Gor'kov [b], William Brey [b], Timothy A. Cross [a,b,c,*]

[a] *Department of Chemistry and Biochemistry, Florida State University, Tallahassee, FL 32310, USA*
[b] *National High Magnetic Field Laboratory, Florida State University, Tallahassee, FL 32310, USA*
[c] *Institute of Molecular Biophysics, Florida State University, Tallahassee, FL 32310, USA*

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Abstract

Sample instability during solid-state NMR experiments frequently arises due to RF heating in aligned samples of hydrated lipid bilayers. A new, simple approach for estimating sample temperature is used to show that, at 9.4 T, sample heating depends mostly on ^{1}H decoupling power rather than on ^{15}N irradiation in PISEMA experiments. Such heating for different sample preparations, including lipid composition, salt concentration and hydration level was assessed and the hydration level was found to be the primary parameter correlated with sample heating. The contribution to RF heating from the dielectric loss appears to be dominant under our experimental conditions. The heat generated by a single scan was approximately calculated from the Q values of the probe, to be a 1.7 °C elevation per single pulse sequence iteration under typical sample conditions. The steady-state sample temperature during PISEMA experiments can be estimated based on the method presented here, which correlates the loss factor with the temperature rise induced by the RF heating of the sample.

Keywords: RF heating; Sample stability; Temperature measurement; Non-spinning sample; PISEMA; Solid-state NMR

1. Introduction

Radio frequency heating of biological solid-state NMR samples is a well known problem. In particular, meaningful studies of membrane proteins require the observation of well hydrated samples and often require the use of negatively charged lipids and hence counter ions. Furthermore, due to the dramatic influence of the gel to liquid crystalline phase transition, membrane protein samples are often characterized above this phase transition reflecting the native membrane protein environment and hence the need to work at temperatures above room temperature for many lipids. For solid-state NMR of aligned samples using glass slides, even with the 30 μm thick glass now available, the filling factor for the RF coil is small and therefore, relatively large samples are required and hence very high RF power levels. All of these factors have the potential to exacerbate RF heating that leads to a dangerously high temperature for these temperature sensitive samples. In addition thermal gradients across the samples lead to additional problems. Here, we demonstrate an approach for measuring the internal temperature of aligned membrane protein samples for solid-state NMR and we characterize how the samples are being heated by radio frequency irradiation. Such characterizations suggest approaches for minimizing the heating effect.

The heating of aligned membrane protein samples can have several consequences. Visually, the sample may become cloudy in the center with droplets of water forming on the interior of the glass tube containing the stack of

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* Corresponding author. Fax: +1 850 644 1366.
E-mail address: cross@magnet.fsu.edu (T.A. Cross).

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glass plates. In other words, the central portion of the sample has been significantly dehydrated; so that there is a hydration gradient across the sample. Via thermal or hydration gradients, the mosaic spread in bilayer alignment may significantly increase. Not only is the protein likely to be functionally and structurally sensitive to an increase in temperature, but the protein–lipid complex may also undergo a phase separation either as a result of the temperature or hydration gradient [1]. Consequently, it is of great importance to minimize the radio frequency heating of membrane protein samples.

RF heating of biological samples has been discussed by various authors [2–5] in the early literature and RF heating by solid-state NMR has been studied by many others in the more recent literature [6–19]. The sample can induce a loss in RF efficiency for generating B_1 fields by two mechanisms. Inductive losses are proportional to ion conductivity and dominate many solution NMR situations [5]. Ion conductivity can also be an important part of the dielectric loss. However, dielectric losses also derive from oscillating dipoles interacting with the oscillating electric field and therefore has a maximum effect at $\omega\tau = 1$, where ω and τ denote the RF frequency and the correlation time of the sample's dipole. Bulk water has such a critical frequency in the low to mid-GHz range (~20 GHz). For constrained water molecules interacting with a surface such as the waters in the lipid bilayer interfacial region the correlation times are reduced by 1–2 orders of magnitude and hence the critical frequency is much lower [20,21,33,34].

Recently, the importance of both ion and dielectric conductivity for solution NMR samples has been demonstrated in the reduced quality factor of cryogenic probes leading to a loss in NMR sensitivity at high fields [6,7]. Indeed, quality factors are an important characterization of all NMR probes. Here, for aligned membrane protein samples we show a quantitative correlation between the quality factor and observed RF heating.

Measurement of sample temperature for solid-state NMR has been the object of considerable literature pointing to the many reasons why it is important to know the temperature and that it is not enough to know the temperature of the gas flowing over the sample [8–13,15–17]. Thermal calibration is usually achieved by the chemical shift temperature dependence of reference compounds, such as lead nitrate, added to the sample. However, reference compounds can interact specifically with the sample. Therefore, adding a ^{207}Pb RF channel or making a channel broadband does not fulfill the need. Furthermore, since our samples are static the observation of spectral discontinuities as opposed to isotropic resonances reduces the precision of the measurements [11,16]. Recently, a fiber optic infrared temperature measurement system has been designed for MAS spectroscopy, but it is not very effective for the temperature range we are interested in [17]. Here, we will use chemical indicators within the sample tube to report on the maximum temperature achieved during the experiment.

2. Experimental

2.1. Temperature measurements

The temperature measurement method is illustrated in Fig. 1. The irreversible temperature indicator (Omega ML4C non-reversible temperature monitor, Omega Engineering (Stamford, CT)) is attached to the surface of the stack of glass slides sealed in a glass tube. The white dot above the indicated temperature value changes to black indicating the temperature has been exceeded. Two indicator strips were used in this work, one strip contains the values 40, 43, 46, and 49 °C, and the other contains 54, 60, 65, and 71 °C indicators, so the lowest and highest temperature we can measure are 40 and 71 °C, respectively. Control experiments of several types were used to show that these indicators were appropriate. First, a sample tube with only an indicator strip was tested. Under the application of an RF field, the indicator did not change color indicating that it does not directly absorb the RF energy. Second, measurements at different air flow temperatures from 40 to 72 °C were carried out in sample tubes containing only temperature indicator strips. In each case, the indicator dye turned black within 1 °C of the air flow temperature value as measured by a thermocouple. Finally, 0.5, 1, 2, 4 and 8 h PISEMA experiments were carried out on our test sample. After 1 h, no further changes in temperature were observed, so we conclude that the sample reached its steady-state temperature within this time frame under the probe conditions declared below. All further experiments were conducted for 1 h.

2.2. NMR experimental conditions

All experiments were carried out on a Bruker DRX 400 spectrometer at ^{15}N and ^{1}H Larmor frequencies of 40.596 and 400.533 MHz, respectively. A NHMFL built double resonance probe was used. PISEMA [25–27] experiments

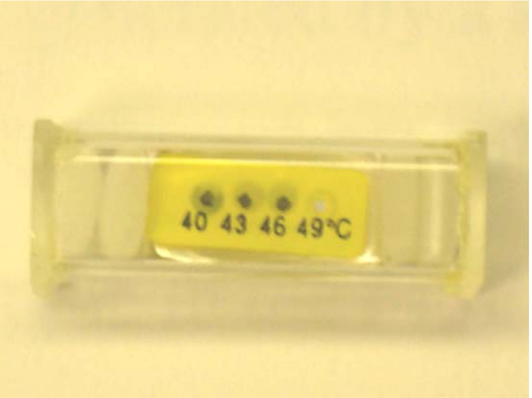

Fig. 1. Temperature measurement is based on temperature indicator strips. The strip is attached to the surface of the glass slides and sealed in the glass tube. The accuracy of the indicator is ±1 °C. Here, the strip indicated that the maximum temperature of the sample reached between 46 and 49 °C. This is reported in Table 1 as 47.5 ± 2.5 °C.