Int. J. Pure Appl. Sci. 4(1): 47-50 (2018)

IJPAS
ijpas@munzur.edu.tr
ISSN: 2149-0910

Research article/Araştırma makalesi

DOI: 10.29132/ijpas.359756

Determination of Cell Membrane Signal by using NMR Spectroscopy

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Received date: 30.11.2017, Accepted date: 25.04.2018

Abstract

The cell membrane forms the boundaries of the cell and regulates the passage of matter between the inside and the outside of the cell. Cell membranes play a major role in the process of taking the necessary nutrients from the outside and exerting the harmful metabolites resulting from the metabolism. In this study, 400 MHz ^{1}H-NMR spectrum of a membrane sample in an aqueous solution was taken using single pulse and presaturation method and it has been tried to determine the location of the membrane signal by comparing these two spectrums.

Keywords: Cell membrane, nuclear magnetic resonance, presaturation method

Hücre Zarı Sinyalinin NMR Spektroskopisi ile Belirlenmesi

Öz

Hücre zarı, hücrenin sınırlarını oluşturur ve hücre içi ile dışı arasındaki madde geçişini düzenler. Hücrenin kendisi için gerekli besin maddelerini dış ortamdan alıp metabolizma sonucu oluşan zararlı metabolitleri dış ortama verme olayında hücre zarlarının büyük bir rolü bulunmaktadır. Bu çalışmada sulu çözeltideki bir hücre zarı örneğinin 400 MHz ^{1}H-NMR spektrumu, tek puls ve presaturasyon metodu kullanılarak alınmış ve bu iki spektrumun karşılaştırmasıyla hücre zarı sinyalinin yeri saptanmaya çalışılmıştır.

Anahtar kelimeler: Hücre zarı, nükleer manyetik rezonans, presaturasyon metodu

INTRODUCTION

The nuclear magnetic resonance (NMR) phenomenon has been known for decades and NMR spectroscopy has been used as a powerful tool in physicochemical analyzes for almost fifty years (Marion, 2013). With the progress of the technology, the free induction decay (FID) signals can be transformed into shapes by using programs similar to those used in the analogue scaling programs used in the scantography (Grover et al., 2015). At the basis of nuclear magnetic resonance studies lies the magnetic nature of the core (Webb, 2007). The electric current creates a magnetic field around it. An atomic nucleus that rotates around the axis forms a magnetic field around it, as it is charged due to the electrons in it. Technically, magnetic resonance studies the behavior of the nuclei of atoms with magnetization properties.

Biological membranes are dynamic systems containing lipids and proteins. Membranes act as selective permeable barrier and contain transport and displacement (translocation) systems that regulate the structure of the intracellular environment, as well as specific receptors for cell signaling studies (Schnell and Hebert, 2003).

Homology calculations of the human genome sequence reveal that 30% of the proteins are membrane-associated (Babcock and Li, 2014). Since a large of majority of best-selling drugs target membrane receptors, membrane-related proteins are of interest to the pharmaceutical and biotechnology industry (Bull and Doig, 2015).

Investigation of the interaction of drugs with biological membranes and membrane-associated proteins using NMR spectroscopy and other spectroscopic methods has been the focus of recent years (Opella et al., 2001; Xu et al., 2002; Nielsen et al., 2004; Middleton et al., 2004; Pignatello et al., 2011).

Liquid NMR has been recognized as an important method in the determination of structures of readily soluble protein and protein complexes in aqueous solutions. (Thennarasu et al., 2005; Tamm and Liang, 2006). It is also possible to obtain information about the molecular dynamics of the system by calculating T_1 and T_2 relaxation times in

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aqueous solutions with Liquid NMR. (Wüthrich, 1986; Yilmaz and Zengin, 2013; Yilmaz and et al., 2014).

When membrane proteins are embedded in lipid layers, using liquid NMR techniques is not appropriate. Because the rotation of these membrane proteins around their surroundings is slow and largely anisotropic. This leads to improper relaxation and very large resonance signals that cannot be detected. The solid state NMR is a successful method for finding high-resolution spectra of membranes attached to peptides and proteins in lipid layers (Abu-Baker and Lorigan, 2012).

In this study, a 400 MHz ^{1}H-NMR spectrum of a normal (healthy) membrane sample in an aqueous solution was taken by two techniques (single puls and presaturation) and the position of membrane signal in this spectrum was tried to be determined by the comparison method of two spectrum.

MATERIAL AND METHOD

In this work, D_2O used as solvent was obtained from Sigma-Aldrich catalog. Membrane samples were obtained from the Department of Biophysics, Faculty of Medicine, Ankara University. Normal (healthy) membrane samples were heart homogenates obtained from 4-month-old healthy rabbits. The normal membrane sample was reacted with D_2O and 400 MHz ^{1}H-NMR spectra of these samples were investigated.

In order to determine the membrane signal, 400 MHz ^{1}H-NMR spectrum of D_2O and the buffer solution mixture in which the membrane samples were homogenized was taken using single pulse and presaturation (suppression) method. Then 400 MHz ^{1}H-NMR spectrum of the D_2O and the membrane sample mixture was taken using single pulse and presaturation method. As a result of these two spectrum comparisons, we have an idea of the location of the membrane sample in the spectrum.

An NMR spectrum example with and without solvent presaturation method was shown in Figure 1 (Jao, 2005). In Figure 1(a), the solvent peak is very intense, so the intensity of the other peaks is rather small. In Figure 1(b), the solvent pike was suppressed by solvent presaturation method, so that the intensity of the other peaks was increased.

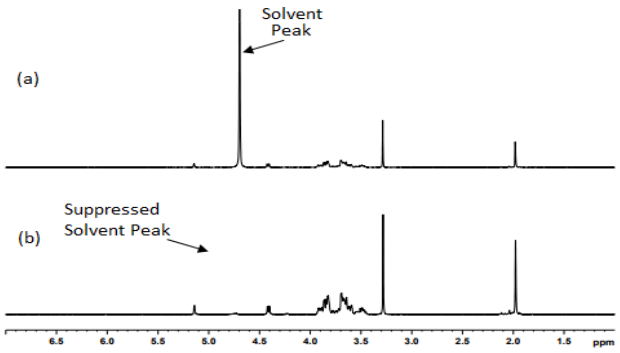

Figure 1. (a) Spectrum without solvent suppression method (b) Spectrum with solvent suppression method

When the H_2O + D_2O mixture was used as the solvent, the water peak is quite large. In order to be able to see the other signs in the spectrum, the water peak has to be suppressed effectively. In this study, in order to effectively suppress the water peak and to see the other peaks better, the ZGPR pulse step was selected in the BRUKER NMR AVANCE 400 Running TOPSPIN program. In order to effectively suppress the water peak with the ZGPR pulse program, a single pulse experiment was performed first. Then, the O1 resonance frequency, which will suppress the water peak, was detected from the spectrum and this frequency value was written in the pulse program. The water peak suppression experiment was completed by selecting the other parameters in the pulse program appropriately.

RESULTS AND DISCUSSION

The 400 MHz ^{1}H-NMR spectrum of the membrane sample with the buffer solution in which the membrane samples were homogenized. These spectrums were given in Figure 2. Figure 2a shows the ^{1}H-NMR spectrum of a 900 µL D_2O mixture with 100 µL of the buffer solution and Figure 2b gives the ^{1}H-NMR spectrum of the 980 µL D_2O mixtures with the 20 µL membrane sample on the same vertical scale. When these two spectra were compared, it was seen that the only difference was the signal observed at the chemical shift value of -0.0046 ppm in the ^{1}H-NMR spectrum of the D_2O mixture with the membrane sample. As a result, this signal was taken into account in the membrane samples.

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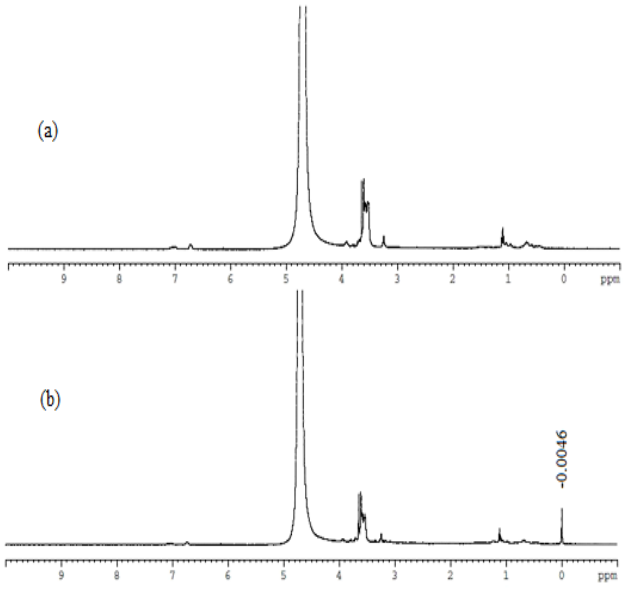

Figure 2. (a) NMR spectrum (with single puls techniques) of Buffer solution+D_2O mixture (b) NMR spectrum (with single puls techniques) of membrane+D_2O mixture

Figure 3 shows the result of suppression of the HDO signal using the presaturation method in the 400 MHz ^{1}H-NMR spectrum of the membrane sample with the buffer solution in which the membrane samples were homogenized.

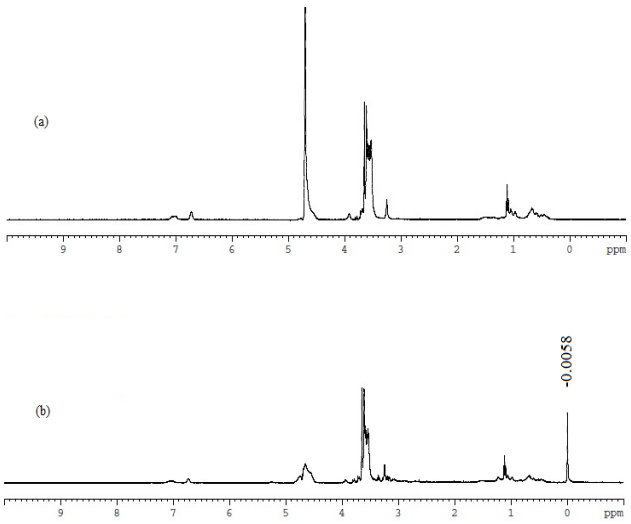

Figure 3. (a) NMR spectrum with presaturation method of Buffer solution+D_2O mixture (b) NMR spectrum with presaturation method of membrane+D_2O mixture

Figure 3a gives the ^{1}H-NMR spectrum using the presaturation method of the D_2O mixture with the buffer solution and Figure 3b shows the same vertical scale of the ^{1} H-NMR spectrum of the D_2O mixture with the membrane sample. When these two spectra were compared, it was seen that the signal observed at the chemical shift value of -0.0058 ppm in the ^{1}H-NMR spectrum of the D_2O mixture with the membrane sample was observed. This signal observed at -0.0046 ppm in Figure 2b shifted to -0.0058 ppm in the case of presaturation.

CONCLUSION

As a result of comparison of the 400 MHz ^{1}H-NMR spectrum of the membrane sample with the buffer solution in which the membrane samples were homogenized, the signal to be considered was taken into account in the membrane samples. The chemical shift values and the intensity of this signal were investigated in single pulse and presaturation experiments. It is possible to measure T_1 and T_2 relaxation times at different concentrations and temperatures by adding various proteins and drugs after the normal membrane signal is located in the spectrum.

ACKNOWLEDGEMENTS

First, I thank my doctoral advisor Prof. Dr. Ali YILMAZ. This work includes a part of the doctoral dissertation that supported Dicle University Scientific Research Project (DÜBAP 08-FF-10).

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