Water Interaction with Membrane Phospholipid and Protein by Proton HR-MAS NMR

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Introduction: Understanding water distribution and dynamics in intact cell and tissue systems is important given its central role in biological processes. Although water relaxation times have been used extensively to guide parameter selection in MRI imaging, the relative contributions of phospholipids and protein to water relaxation has not been determined. In this hr-MAS NMR study of intact cultured cells and tumor tissue, we first evaluate the mechanism of interaction of protein with water and then analyze the relative contribution of protein and membrane phospholipid to water relaxation. Finally, the mechanism of magnetization transfer under static conditions was evaluated.

Method : HCT-116 cell, 3T3 cell lines and GIST tumors were studied on a Bruker 600 MHz at 20 ^{o}C using a MAS rate of 5000 Hz. Magnetization transfer from water to protein amide protons and ring protons and to the phospholipid protons was measured using a water selective excitation-NOE experiment. The chemical exchange rate between amide proton and water, the NOE rates between water and protein ring protons were linearly fitted from the initial rate of the selective-NOE experiment. The auto-relaxation rate was fitted from the build curves as shown in Fig. 1d and 1f.

Results: Water transfers magnetization to the amide protons of observed overall protein through chemical exchange with an average time of 0.74 s. Water transfers magnetization to the surface protons of phosphatidylcholine through NOE with the NOE rate of 1.23 s^{-1}. The longitudinal relaxation time of water exchangeable with amide protons is 3 s and demonstrates free motion. The water that interacts with the phosphatidylcholine surface protons has a T1 of 0.8 s and illustrates restricted motion. The magnetization transfer rate of protein protons and membrane protons with water are comparable. From a comprehensive relaxation analysis the distance between water and phosphatidylcholine surface protons was estimated to be 2.4 Å.

Conclusion: HR-MAS NMR provides a unique approach to study the dynamics, structure and interaction of water with other cellular metabolites. In cultured cells and tumor tissue, both phospholipid and protein influence the longitudinal water relaxation to the same degree. However, water motion is much more restrictive around phosphatidylcholine surface protons than amide protons.

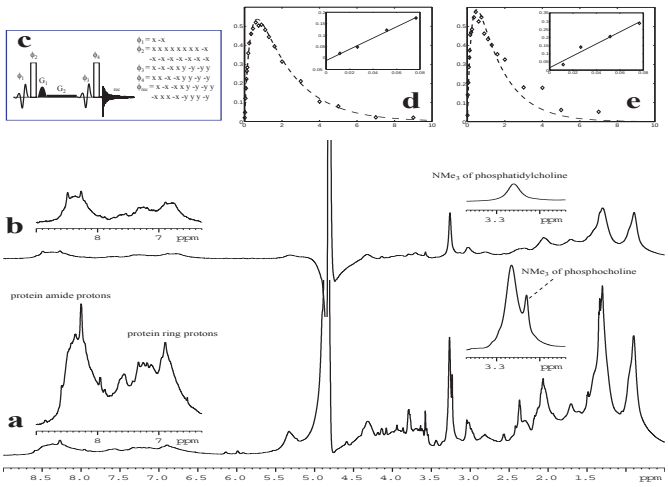

Fig. 1. **a**). 3T3 cells acquired using SEEN; **b**). acquired using selective-NOE sequence of (**c); d** and **e** are build up curves of the NMe_3 through NOE and protein amide protons through chemical exchange. Inserts in **d** and **e** showed the initial rate of build curves, respectively.

Reference:

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