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NMR Analysis of Dog Erythrocytic Membrane Antigen

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Authors' contributions

This work was carried out in collaboration among all authors. Author GK contributed for sample collection, blood grouping, Membrane purification. Authors KGT and KV did literature collection, correction, review and editing. Author RB did sample collection, blood grouping. All authors read and approved the final manuscript.

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ABSTRACT

Aim: NMR spectroscopy analysis of dog erythrocytic membrane antigen in order to differentiate blood groups in dogs
Methodology: The purified dog erythrocytic membrane glycoprotein of DEA1.1 positive and negative blood were subjected in to NMR spectroscopy analysis. One-dimensional C13-NMR spectra were acquired at 25°C on a high resolution spectrometer Fourier 300 MHz (Bruker's, (USA) using the first increment of the pulse sequence NOESY-presat, 128 scans, sweep window 20 ppm, 32 k points and relaxation delay 5 seconds. The spectra were processed and analyzed with Bruker's. TopSpin software 300 zero-filing to 64 k points and line broadening 0.5 Hz22 and MestReNova 8.1 software (Mestrelab Research, Santiago de Compostela, Spain).
Results: The DEA 1.1 positive and negative membrane glycoprotein showed chemical shift with minimum spectral difference and functional group, CH3CO at the level of 20-30 ppm, RCH2Cl at the level of 35-45 ppm, RCH2OH, C=O (in acids and esters) at the level of 170 ppm were identified in

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both DEA 1.1 positive and negative membrane glycoprotein in NMR analysis of canine erythrocytic membrane antigen.

Conclusion: NMR studies of conformational changes of membrane proteins in response to small molecule and protein ligands and the changing lipid environment at different physiological states of cellular membranes. The exploration of the structural and mechanistic biology of membrane proteins by NMR has a bright future and bring many new exciting discoveries.

Keywords: NMR; RBC membrane; DEA1.1 positive; DEA 1.1 negative; C13-NMR spectra.

1. INTRODUCTION

"NMR spectroscopy is a very versatile, non-destructive, atomically resolved and quantifiable analytical technique, where different experiments are used depending on the desired readout. The NMR spectroscopy can yield useful information from the identification of biomarker signals up to the absolute quantification of the entire glycan moiety of a glycoprotein The NMR offers to study glycoprotein-receptor interactions under physiological conditions. The study of the structure and function of glycoproteins by NMR has difficulty due to technical barriers such as limited access to samples with the appropriate labeling or by incomplete methodology to interrogate the conformational heterogeneity and composition. The recent advancement in NMR steadily improving the toolbox for structural characterization of glycoproteins". Mateos et al.,[1].

"Nuclear magnetic resonance spectroscopy used for studying molecular interactions in biological membranes and erythrocyte membrane fragments. The ultra sonic dispersion of these fragments produces a sharp and well defined high-resolution nuclear magnetic resonance spectrum. The spectrum shows peaks which can be assigned to various groups of the lipid, sugar and protein. The choline N+ (CH), signal of the membrane lipid is prominent but the signal due to (CH), protons of the hydrocarbon chains is broadened as a result of interaction. This signal narrows at higher temperature or when exposed to increasing concentrations of sodium deoxycholate and the signals is due to amino acids of the membrane protein are unresolved in the spectrum but appear as well- defined peaks after treatment with urea or trifluoroacetic acid. Co-dispersion of the membrane fragments with lysolecithin gives a spectrum which indicates the formation of an additive complex" [2].

2. MATERIALS AND METHODS

2.1 Red Blood Cell Sample Preparation for C 13 -NMR Measurements

The RBC precipitate was washed twice with 0.9% NaCl in 5 mM phosphate buffer pH 7.2 (2,000 X g for 10 minutes) and subsequently lysed through two cycles of freezing in liquid nitrogen and thawing at 37 °C and by sonication for 30 seconds. Proteins and membranes were eliminated by ultrafiltration on membranes (cut-off 5,000 Da). The membrane glycoprotein was extracted by using glycoprotein extraction kit (Thermo Scientific, USA). The glycoprotein concentration was estimate by using phenol sulphuric acid assay. The membrane glycoprotein was freeze dried without any cryoprotectant. 1% TSP in D_2O were added to final filtrate to prepare the NMR samples [3-7].

2.2 NMR - C13 Experimental Analysis of Canine Erythrocytic Membrane Glycoprotein

One-dimensional C13-NMR spectra were acquired at 25°C on a high resolution spectrometer Fourier 300 MHz (Bruker's, (USA) using the first increment of the pulse sequence NOESY-presat, 128 scans, sweep window 20 ppm, 32 k points and relaxation delay 5 seconds. The spectra were processed and analyzed with Bruker's TopSpin software 300 zero-filing to 64 k points and line broadening 0.5 Hz22 and MestReNova 8.1 software (Mestrelab Research, Santiago de Compostela, Spain).

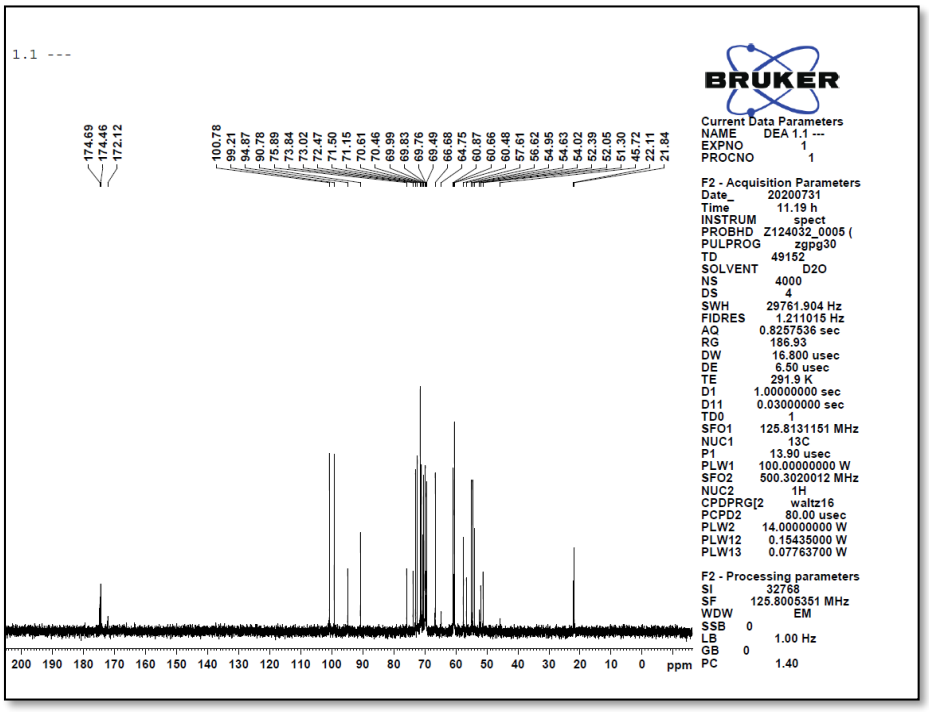
3. RESULTS AND DISCUSSION

The DEA 1.1 positive and negative membrane glycoprotein showed chemical shift with minimum spectral difference and functional group, CH3CO at the level of 20-30 ppm, RCH2Cl at the level of 35-45 ppm, RCH2OH, C=O (in acids and esters) at the level of 170 ppm were identified in both

DEA 1.1 positive and negative membrane glycoprotein in NMR analysis of canine erythrocytic membrane antigen. One-dimensional C13-NMR spectra were acquired at 25°C on a high resolution spectrometer Fourier 300 MHz (Bruker's, (USA) using the first increment of the pulse sequence NOESY-presat, 128 scans, sweep window 20 ppm, 32 k points and relaxation delay 5 seconds. The spectra were processed and analyzed with Bruker's [8,9]. TopSpin software 300 zero-filing to 64 k points and line broadening 0.5 Hz22 and MestReNova 8.1 software (Mestrelab Research, Santiago de Compostela, Spain). The DEA 1.1 positive and negative membrane glycoprotein showed chemical shift with minimum spectral difference and functional group, CH3CO at the level of 20-30 ppm, RCH2Cl at the level of 35-45 ppm, RCH2OH, C=O (in acids and esters) at the level of 170 ppm were identified in both DEA 1.1 positive and negative membrane glycoprotein [10].

Smith et al., (1995) studied "helix–helix interactions in glycoporin. The transmembranes domine by rotational resonance NMR. They found that the glycoporin. The primary sialoglycoprotein in human erythrocyte membranes and the Peptides with sequences that correspond to the transmembrane domain, micelles and unoriented lipid bilayers by NMR spectroscopy. They found that the peptides form dimers and they characterized packing geometry by measuring distances between specifically labelled methyl groups on sequential residues around one helical turn and backbone carbonyl groups on the other helix with rotational resonance solid-state NMR experiments".

The helix–helix interactions in glycoporin A transmembranes domine analysed by rotational resonance NMR. The glycoporin A primary sialo glycoprotein in human erythrocyte membranes and the peptides with sequences that correspond to the tansmembrane domain, micelles and unoriented lipid bilayers were analysed by NMR spectroscopy. The peptides form dimers and they characterized packing geometry by measuring distances between specifically labelled methyl groups on sequential residues around one helical turn and backbone carbonyl groups on the other helix with rotational resonance solid-state NMR experiments. High resolution p.m.r. spectrum of erythrocyte membrane fragments is obtained with soniocated dispersions. The sonic irradiation reduces the membrane fragments to miorosomal dimensions. These particles are small enough to allow rapid reorientation, permitting observation of a well-resolved spectrum if the segmental motion of the membrane components is sufficiently rapid [11,12] The temperature below 20°C the membrane spectrum is featureless and unresolved but sharpens up at 30°C and



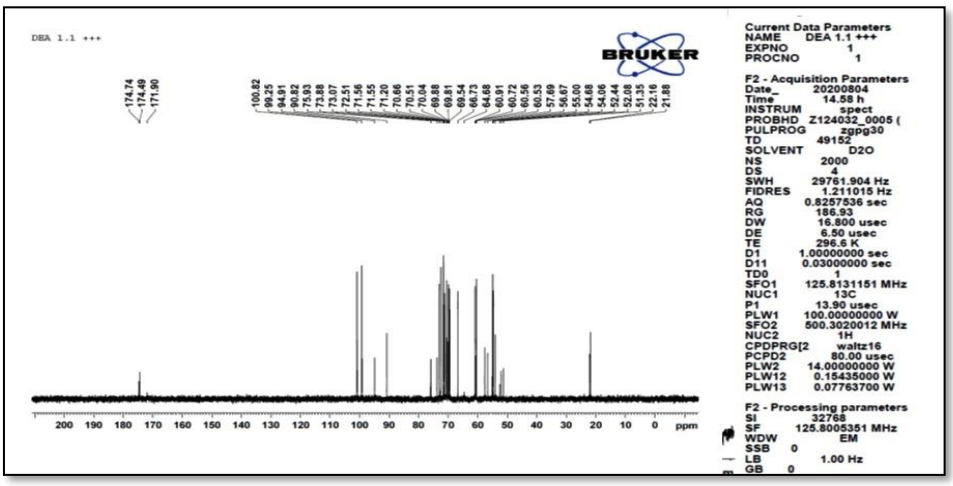


Fig. 1. The NMR spectrum of canine erythrocytic membrane glycoprotein

A: The NMR spectrum of DEA 1.1 positive membrane glycoprotein
B: The NMR spectrum DEA1.1 negative membrane glycoprotein
Note: The helix–helix interactions in glycoporinA transmembrane domine analyzed by rotational resonance NMR. The glycoporin A primary sialo glycoprotein in canine erythrocyte membranes and the peptides with sequences that correspond to the transmembrane domain, micelles and unoriented lipid bilayers were analyzed by NMR spectroscopy

above. Higher temperature considerable molecular motion increased because of a liquid crystalline arrangement of the lipids and proteins. The viscosity of blood and X-ray diffraction of an erythrocyte lipid-water system causes narrow line widths in the membrane spectrum on the basis of the segmental motion for specific proton groupings in the immediate lipoprotein of the particular chain segment. “The positional orientations of the polar groups N (CH& N.CO.CH, and CH, OC or CH,OP of protein and oligosaccharide had low microscopic viscosity to permit segmental motion of the choline and sugar protons [13-15]. The most important feature of the membrane spectrum is found in the region of 8 and 9 p.p.m. The inhibition of the (CH,) signal at 8.7 p.p.m. and the absence of the CH=CH signal at 4.7 p.p.m. are most striking indicate high local viscosity for the hydrocarbon lipid chains in the membrane”. (Rand & Luzzati, 1967, Smith et al.,1995)

“NMR is one of the pre-eminent method to obtain residue-specific dynamic information on macromolecules including membrane proteins. NMR provides dynamic information ranging from ps to s down to atomic resolution. Essential biological functions like conformational exchange, ligand or inhibitor binding, folding and unfolding and allosteric regulations in membrane proteins can be detected by employing a wide range of NMR techniques. The traditional NMR dynamics studies probe site-specific motions by measurement of longitudinal (R_1) and transverse (R_2) relaxation rates and hetero nuclear NOEs. These experiments measure motions in the ps-ns time range and provide valuable information about the protein is well-structured, flexible or completely disordered. The model-free formalism can be employed to separate internal from global motions in a residue specific manner and extract slow ms exchange processes. The extended model-free analysis of the β-barrel membrane protein OmpA revealed a rotation of the protein within the micelle. This analysis also found backbone segmental motions that increased the further the residues were from the mid-plane of the membrane. The structures of pore forming peptides, peptide hormones and antibiotics, and membrane-inserted phage coat peptides were studied since these early days by solution and solid-state NMR methods. The heteronuclear multidimensional methods have revolutionized membrane protein solution and ss-NMR in the last 15 years and permitted structural and dynamical studies to be extended to membrane proteins in the 10–40 kDa range. This range occupies a unique niche area for membrane proteins that are difficult to crystallize and that are too small to be suitable for current electron cryo-microscopy which also has seen a revolution in the last few years” [3]. The sample preparation to be a challenge with membrane proteins. The functional and other independent experiments are necessary to validate structures and to ascertain that crystallization conditions,

lipid micelles or low hydration and high protein concentrations in lipid bilayers do not distort the structures. The membrane protein NMR also plays a unique role to examine conformational transitions of partially folded membrane proteins such as those involved in intracellular and viral membrane fusion. The hybrid structural techniques are becoming increasingly popular and the combination of NMR with electron cryo-microscopy, x-ray crystallography, EPR and fluorescence spectroscopy and computation will likely find increased use in the future [16]. “Particularly promising areas in NMR studies are conformational changes of membrane proteins in response to small molecule and protein ligands and the changing lipid environment at different physiological states of cellular membranes. The exploration of the structural and mechanistic biology of membrane proteins by NMR has a bright future and bring many new exciting discoveries” [3]

4. CONCLUSION

In this preliminary study, the DEA 1.1 positive and negative membrane glycoprotein showed chemical shift with minimum spectral difference and functional group, CH3CO at the level of 20-30 ppm, RCH2Cl at the level of 35-45 ppm, RCH2OH, C=O (in acids and esters) at the level of 170 ppm were identified in both DEA 1.1 positive and negative membrane glycoprotein however indepth detailed further studies were needed in future.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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