

NMR application for precise detection and distinction of anti-DNA hydrolytic activities

Introduction

The literature suggests distinct sequence specificity between DNase 1 and ssDNA antibody in that DNase 1 prefers A-T sequences while anti-ssDNA prefers C-C, and dsDNA C-G sequences.¹ NMR proton spectra will illustrate the sequence of hydrolytic events in terms of specific motif of hydrolytic attack and monitoring cleavage sequential continuum. If there are any differences between natural/commercial DNase and anti-DNA antibodies they will be regarded as specific and distinctive entities. Some researchers believe that anti-dsDNA antibody prefers phosphate sugar bonds to nucleotides, while anti-ssDNA autoantibodies are directed more toward nucleotide sequences.^{2,3} From our work and work of others⁴⁻⁷ anti-DNA antibodies show different profiles in terms of clonality, banding patterns, and affinity binding.⁸ These could be some of the reasons for distinctive preferences for DNA structural elements, and even some other cellular protein molecules like α -actinin, in mesangial cells.⁹ On the other hand, Kim et al.,¹⁰ argued that there is no difference in sequence specificity with respect to binding and hydrolysis between anti ss- and anti-dsDNA antibodies. A careful analysis of proton shifts in place and frequency can help us to shed further light on these interactions and provide some definitive answers in this debate. Additionally, these analyses should allow precise determination of the point(s) of enzymatic attack for each of these candidates making them more distinctive.

Experimental design

NMR will also be adjusted and optimized for proton spectra shifts, as the result of nucleotide accumulation due to DNA cleavage. Knowing the primary structure of the DNA probes, (synthetic oligo nucleotide -ss) it will be possible to deduce the existing differences in specificity for the substrates and sequences between ss antibodies and DNase. The optimization of the concentration of the substrate is necessary, which according to preliminary data, is within micro molar range. Further fine tuning should be designed and executed for that purpose. Our preliminary data using micro molar concentration of the substrate (DNA) and TRIS buffer have shown that we have to optimize the buffer and concentration level, but the reaction with DNase and anti-ssDNA antibody is possible to get and follow.

Anticipated results

On the basis of preliminary data we expect to get the proton spectra of our ssDNA oligo probe and the sequence of nucleotides cleaved during its hydrolysis. We also believe that we shall at optimal enzyme/substrate ratio; temperature and pH of adjusted TRIS buffer get the most efficient hydrolysis. We anticipate, with respect to our preliminary published data^{6,7,11} that anti-ssDNA antibodies at least in the flare of lupus disease, will show hydrolytic activity distinctive with respect to kinetic parameters from commercial DNase, and so further prove that it is an intrinsic feature of anti-ssDNA lupus antibody. This will give a new prospective of anti-ssDNA antibody, as possible pathogenic molecular interlayer within lupus immunological scenario, as well as possible biomarker of lupus flare prediction. We

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actually expect that activities within and after the flare should be different, e.g., stronger during the flare indicating stronger pathogenic effect (destruction of nuclear DNA and nucleus itself). We would not expect normal donors (ND) to give hydrolytically active anti-ssDNA antibodies.¹²⁻¹⁴ If that happens, we would check for other possible signs of lupus disease, in collaboration with physicians. NMR Figure 1 1D NMR proton spectra of single stranded (ssDNA) Gololobov modified oligo 18-mer. This is a spectrum of non-hydrolyzed oligo-dT in micro molar concentration, when proton 1D NMR spectral analysis is used Figure 1.

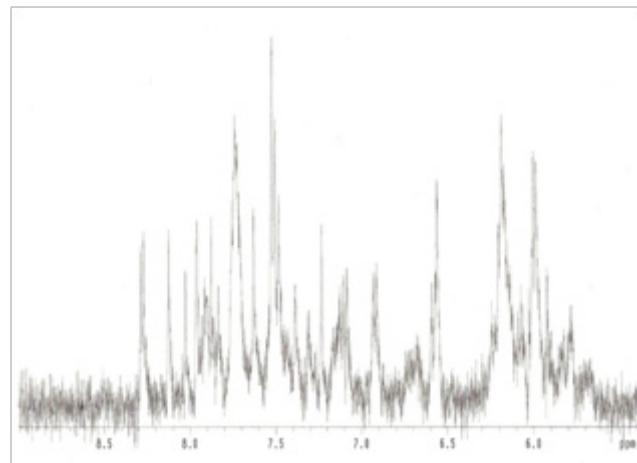


Figure 1 1D NMR proton spectra of single stranded (ssDNA) Gololobov modified oligo 18-mer.

Conclusion

The idea for NMR detection of DNA hydrolysis was that one would see shift in proton peaks as oligo is being hydrolyzed, and by analyzing proton spectra, deduce the specific site/location of hydrolytic attack and specific sequence of hydrolytic products/nucleotide cascade that follows after initial step /hydrolytic attack. The detection of differences in those parameters could strongly discriminate between DNA-se enzymatic activity and intrinsic DNA hydrolytic activity of autoimmune antibody.

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Conflicts of interest

Author declares that there is no conflicts of interest.

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