Detection of cerebral NAD^+ by *in vivo* ^{1}H NMR spectroscopy

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Introduction – Nicotinamide adenine dinucleotide (NAD^+) and its reduced form, NADH, have central roles in cellular metabolism and energy production as electron-accepting/donating coenzymes. In addition, NAD^+ is increasingly being recognized as a net substrate for a range of reactions related to gene expression, calcium mobilization, aging, cell death and the timing of metabolism via the circadian rhythm. Recently, it was demonstrated [1] that NAD^+ and NADH could be detected quantitatively by ^{31}P NMR spectroscopy at high magnetic field strength *in vivo*. Here we present ^{1}H NMR spectroscopy as a simple, but robust alternative for the *in vivo* detection of NAD^+.

Methods – All experiments were performed on an 11.74 T Magnex magnet interfaced to an Agilent Direct Drive spectrometer. *In vivo* and *in situ* experiments were conducted with a 14 mm diameter surface coil and a five-turn, 25 mm diameter solenoidal coil of 45 mm length, respectively. For *in situ* NAD^+ T_1 and T_2 measurements rats were euthanized by focused beam microwave irradiation ([2] FBMI, 4.5 kW, 1.1 s), followed by removal of extracranial tissues and magnetic susceptibility matching with Fluorinert FC-43 [2]. Spatial localization was based on LASER [3] executed with 0.75 ms or 3 ms SLR 90° pulses centered at 4.7 and 9.1 ppm for non-selective and selective excitation, respectively. The non-selective experiments were supplemented with VAPOR water suppression [4]. T_1 relaxation was measured with an inversion recovery method, whereas T_2 relaxation was measured by varying the LASER echo-time.

Results – Fig. 1A/B shows ^{1}H NMR spectra from rat brain *in vivo* acquired with (A) VAPOR water suppression and (B) with frequency-selective excitation of the [6.8 … 11.4] ppm spectral region. In the absence of water perturbation (Fig. 1B) the downfield region displays three clear resonances at 8.8, 9.1 and 9.3 ppm originating from the NAD^+ nicotinamide protons. Suppression of the water signal in combination with chemical exchange and cross relaxation leads to a large depression of the NAD^+ resonances (Fig. 1A). The interaction between NAD^+ and water protons was also observed during the T_1 measurements *in situ* (Fig. 1C/D). Selective inversion of NAD^+ without perturbing the water protons gives NAD^+ T_1 relaxation times between 200 and 330 ms (Fig. 1C). When the recovery of longitudinal magnetization is probed with a non-selective inversion pulse the NAD^+ T_1 relaxation times are lengthened to between 1000 and 1200 ms (Fig. 1D). The NAD^+ T_2 relaxation times constants were determined to be between 50 and 70 ms. Using the water as an internal concentration reference the *in vivo* NAD^+ concentration in the cerebral cortex was determined at 296 ± 28 μmol/L.

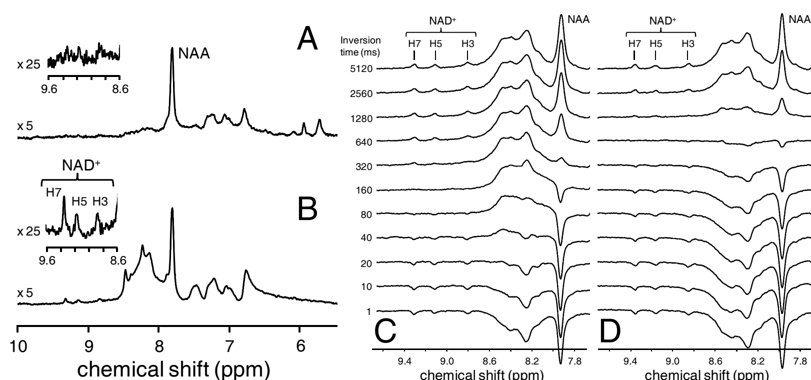

Figure 1: (A/B) ^{1}H NMR spectra from rat cerebral cortex *in vivo* (45 uL, TR/TE = 5000/15 ms, 768 averages) acquired with (A) VAPOR water suppression and (B) selective excitation of the [6.8 … 11.4] ppm spectral region. Only when water perturbation is avoided (B) are the NAD^+ nicotinamide H3, H5 and H7 resonances clearly visible. (C/D) T_1 measurements on rat brain *in situ* (256 uL, TR/TE = 5120/15 ms, 640 averages) acquired with (C) selective and (D) non-selective excitation and inversion pulses. Without water perturbation (C) the NAD^+ resonances pass the zero-crossing around 160 ms. The NAD^+ T_1 relaxation times are noticeably lengthened in the presence of water perturbation as is evidenced from the NAD^+ signal zero-crossing at circa 640 ms.

Discussion – Here we have presented a novel technique for the *in vivo* detection of NAD^+. The acquisition method is simple (LASER with frequency-selective excitation), relatively insensitive to magnetic field inhomogeneity and can even be performed at lower magnetic fields. The spectral interpretation is straightforward as the three NAD^+ resonances are free from spectral overlap. To ensure full NAD^+ signal intensity it is critical that water perturbation is minimized. Unlike the ^{31}P NMR method [1], the presented ^{1}H NMR method cannot detect NADH. However, the wide availability of proton-capable MR systems together with the ease-of-use makes ^{1}H NMR-based NAD^+ detection a competitive method for *in vivo* applications.

[1] M. Lu et al, Magn Reson Med (2013) doi: 10.1002/mrm.24859 [2] R.A. de Graaf et al, J Neurochem 109, 494 (2009) [3] M. Garwood et al, J Magn Reson 153, 155 (2001) [4] I. Tkac et al, Magn Reson Med 41, 649 (1999) This research was supported by NIH grants R01-MH095104 and P30-NS052519.