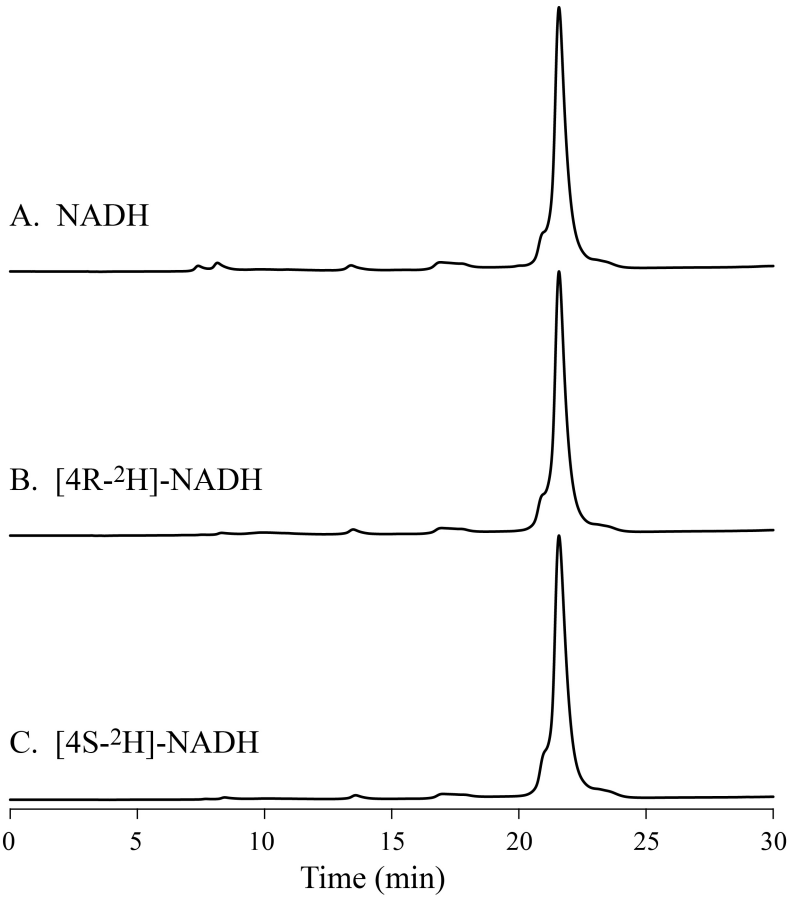
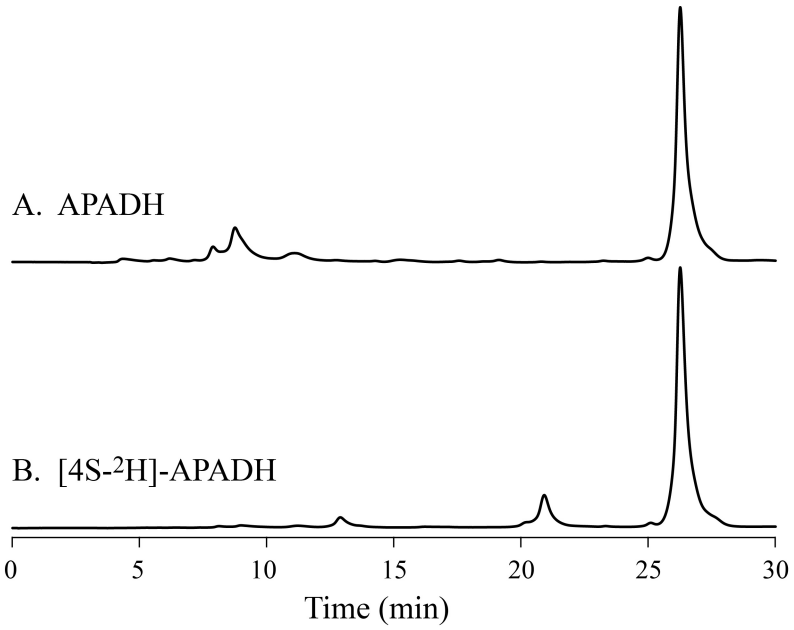
Figure S1



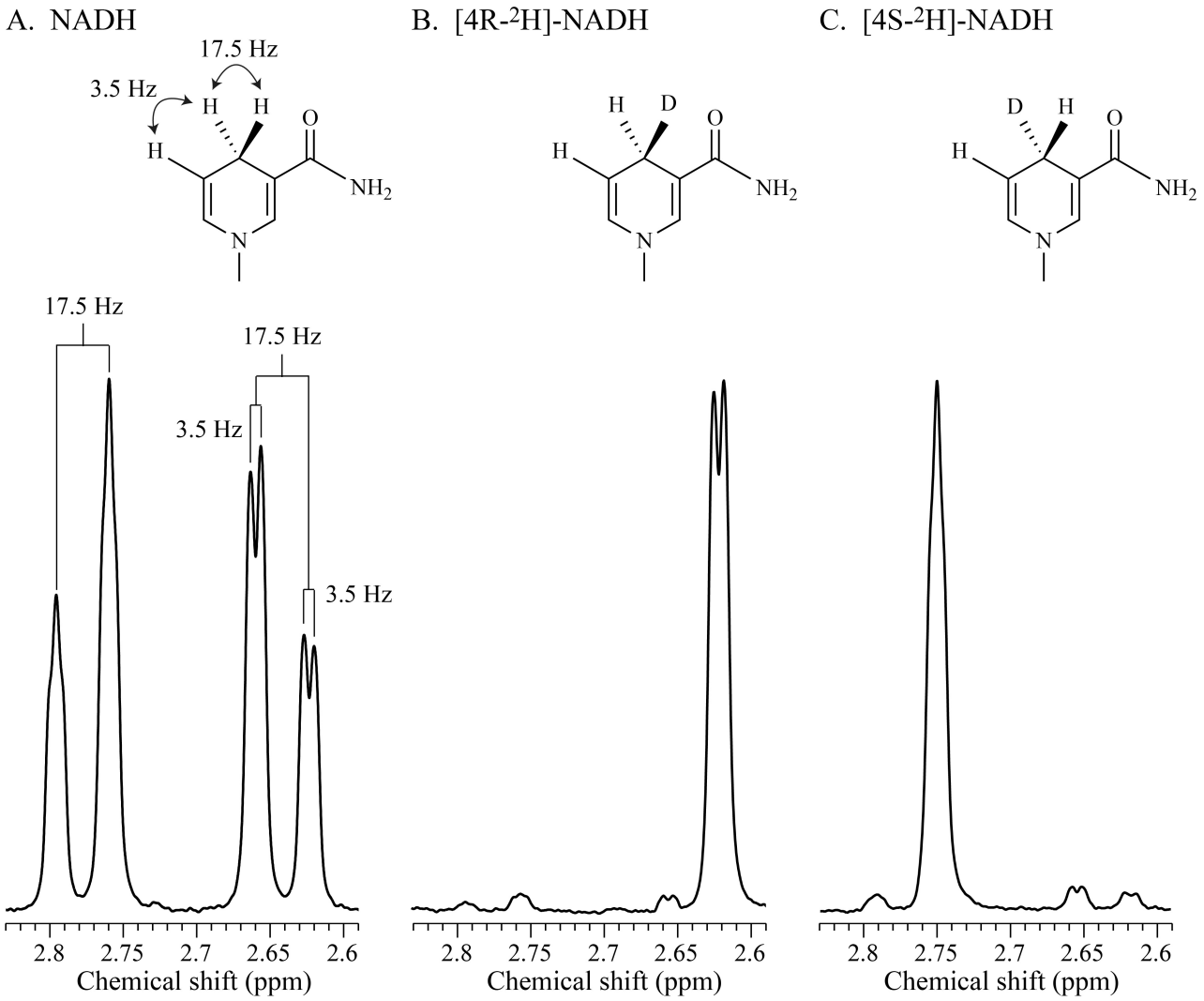
HPLC analyses of NADH, [4R-^{2}H]-NADH and [4S-^{2}H]-NADH. The 260 nm absorbance traces are shown for the elution of nucleotides from a 5 μm Nucleosil C18 column (250 mm x 3.2 mm). Conditions: injection volume 20 μL, 0.5 mL min^{-1} flow rate, 0.1 M potassium phosphate (pH 6) containing 8 mM tetrabutylammonium hydrogen sulfate with a gradient of 20 to 40% methanol in 12 min, 40 to 100% methanol in an additional 16 min, then held at 100% methanol (*1, 2*). The samples are A) NADH (produced from NAD$^+$ by glucose dehydrogenase in the presence of glucose, followed by purification by ion exchange chromatography on Q sepharose and desalting using Sephadex G-10); B) [4R-^{2}H]-NADH (produced from NAD$^+$ by horse liver alcohol dehydrogenase in the presence of 2-propanol-d_8, followed by purification by ion exchange chromatography on Q sepharose and desalting using Sephadex G-10) and C) [4S-^{2}H]-NADH (produced from NAD$^+$ by glucose dehydrogenase in the presence of glucose-d_1, followed by purification by ion exchange chromatography on Q sepharose and desalting on Sephadex G-10).

Figure S2



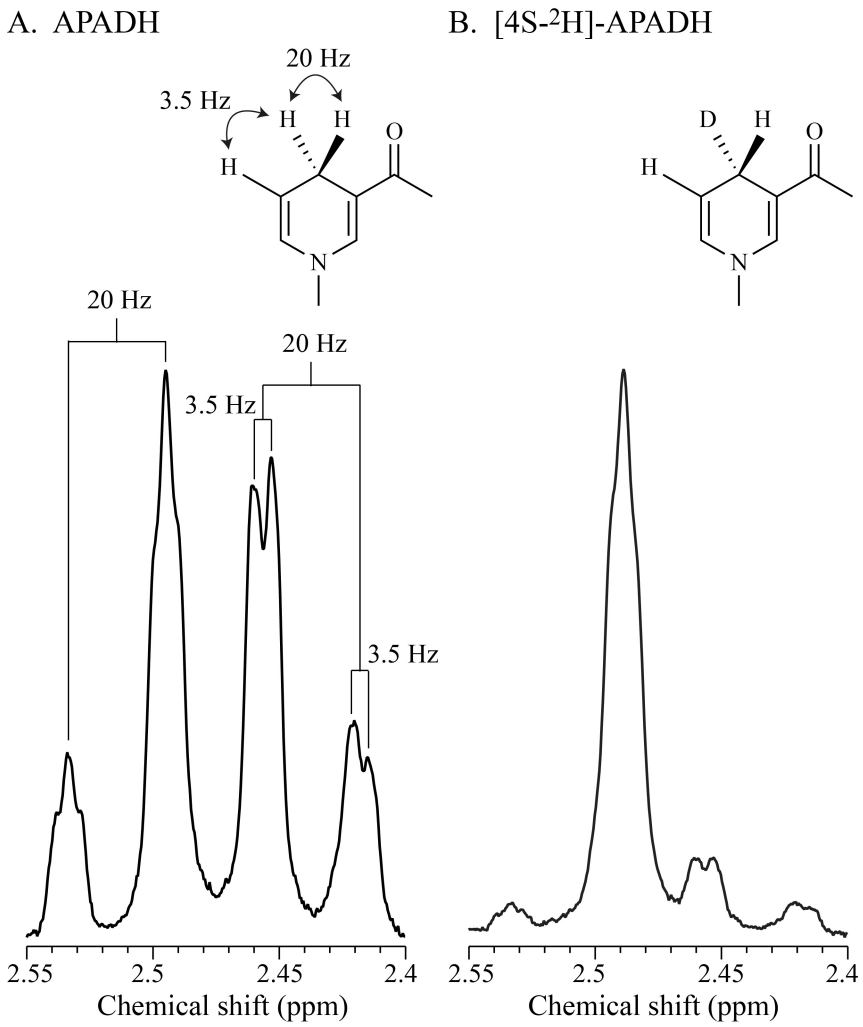
HPLC analyses of APADH and [4S-^{2}H]-APADH. The 260 nm absorbance traces are shown for the elution of nucleotides from a 5 μm Nucleosil C18 column (250 mm x 3.2 mm). Conditions: injection volume 20 μL, 0.5 mL min^{-1} flow rate, 0.1 M potassium phosphate (pH 6) containing 8 mM tetrabutylammonium hydrogen sulfate with a gradient of 20 to 40% methanol in 12 min, 40 to 100% methanol in an additional 16 min, then held at 100% methanol (*1, 2*). The samples are A) APADH (produced from APAD$^+$ by glucose dehydrogenase in the presence of glucose, followed by purification by ion exchange chromatography on Q sepharose and desalting using Sephadex G-10) and B) [4S-^{2}H]-APADH (produced from APAD$^+$ by glucose dehydrogenase in the presence of glucose-d_1, followed by purification by ion exchange chromatography on Q sepharose and desalting using Sephadex G-10).

Figure S3



NMR spectra of NADH, [4R-^{2}H]-NADH and [4S-^{2}H]-NADH. The NMR spectral regions between 2.59 ppm and 2.83 ppm, containing the peaks from the C-4 protons of NADH (A), [4R-^{2}H]-NADH (B) and [4S-^{2}H]-NADH (C) are shown. The peak splittings are displayed on the NADH spectrum: the 17.5 Hz splittings are due to $^2J_{H\text{-}H}$ (geminal) coupling between the two C-4 protons; the 3.5 Hz splittings are due to $^3J_{H\text{-}H}$ (vicinal) coupling between the C-4S proton and the aromatic C-5 proton (*3*). Only the $^3J_{H\text{-}H}$ (vicinal) coupling is observed in [4R-^{2}H]-NADH and both couplings are absent in [4S-^{2}H]-NADH; the low intensity split peaks probably represent contamination by NADH. Conditions: 100 µL of 2 mM nucleotide in 20 mM Tris-HCl (pH 7.7) + 10% D_2O, measured on a Bruker DRX500 spectrometer at 500 MHz and 300 K. Chemical shifts are reported relative to TMS.

Figure S4



NMR spectra of APADH and [4S-^{2}H]-APADH. The NMR spectral regions between 2.40 ppm and 2.55 ppm, containing the peaks from the C-4 protons of APADH (A) and [4S-^{2}H]-APADH (B) are shown. The peak splittings are displayed on the APADH spectrum: the 20 Hz splittings are due to $^2J_{H\text{-}H}$ (geminal) coupling between the two C-4 protons; the 3.5 Hz splittings are due to $^3J_{H\text{-}H}$ (vicinal) coupling between the C-4S proton and the aromatic C-5 proton (*3*). Both couplings are absent in [4S-^{2}H]-APADH, and the low intensity split peaks probably represent contamination by APADH. Conditions: 100 µL of 2 mM nucleotide in 20 mM Tris-HCl (pH 7.7) + 10% D_2O, measured on a Bruker DRX500 spectrometer at 500 MHz and 300 K. Chemical shifts are reported relative to TMS.

References

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2. Yakovlev, G., and Hirst, J. (2007) Transhydrogenation reactions catalyzed by mitochondrial NADH-ubiquinone oxidoreductase (complex I). *Biochemistry 46*, 14250-14258.
3. Oppenheimer, N. J., Arnold, L. J., and Kaplan, N. O. (1971) A Structure of pyridine nucleotides in solution. *Proc. Natl. Acad. Sci. USA 68*, 3200-3205.