A new metabolite characteristic of inflammatory MS plaques : the first MRS assignment of β-hydroxyisobutyrate in CSF

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Introduction

The need for an effective treatment of multiple sclerosis (MS) continues to motivate research into the mechanisms underlying this disease. While basic metabolic processes accompanying MS have been studied in patients by brain MRS, in vitro ^{1}H MRS analysis of cerebrospinal fluid (CSF) has been used to obtain metabolite profiles for various manifestations of MS [1-3]. In a comprehensive metabolomic study, we have recently detected increased lactate concentrations in CSF of clinically isolated syndrome (CIS) patients with vs. without inflammatory plaques [4]. We now present, for the first time, the identification of the second of two significantly altered metabolites, β-hydroxyisobutyrate (BHIB). To our knowledge, this compound has not previously been assigned in ^{1}H MR spectra of CSF or other biofluids.

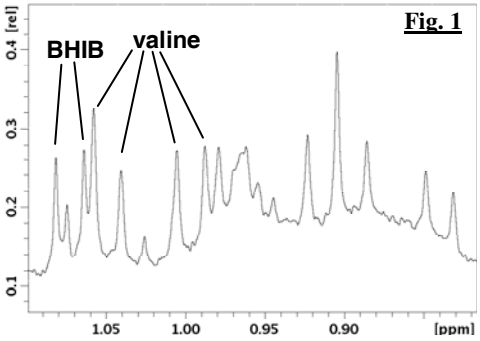
Methods

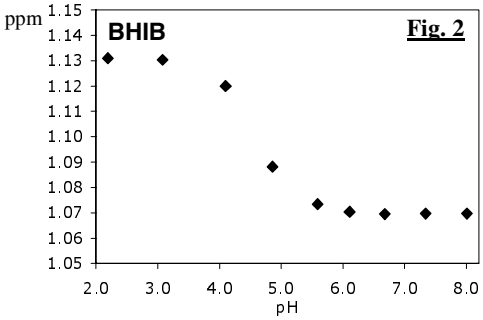
CSF was collected by lumbar puncture from CIS patients, before any treatment. For MRS signal assignment, CSF samples from 4-5 patients (ca. 8 ml total volume) were combined, concentrated by lyophilization, and prepared in D_2O for NMR analysis as described elsewhere [4]. BHIB was synthesized by hydrolyzing the ester, methyl β-hydroxyisobutyrate, in a concentrated NaOH solution. The completeness of the reaction was verified by ^{1}H MRS of the product. After BHIB spiking of a concentrated CSF sample, 1-D ^{1}H MR spectra were acquired at 28° C on an AVANCE 400 spectrometer (Bruker, Wissembourg), using TR=15 s, a 90°-pulse with water suppression, and 64K data points. A total of nine spectra were obtained at sequentially re-adjusted pH values ranging from 2.0 to 8.0 for a titration curve based on the chemical shift of the BHIB methyl signal. J-resolved and COSY spectra of a concentrated CSF sample were generated on AVANCE 400 and 500 spectrometers at 28° C. Spectra were referenced using trimethylsilyl tetradeuteropropionate (TSP-d_4) as an internal standard.

Results

Figure 1 shows the BHIB methyl signal in the high-field region of a typical CSF spectrum of a CIS patient (pH 7.0). This doublet is identical, with respect to both chemical shift and coupling constant, to peaks previously referred to as XS1.06 and XS1.08 [2], or "U" (unassigned doublet in CSF samples) [4,5].

Figure 2 shows a titration curve obtained for the BHIB methyl signal following BHIB addition to a concentrated CSF sample. This curve falls precisely on a titration curve previously measured for the unassigned doublet "U" in a pure CSF sample [5].





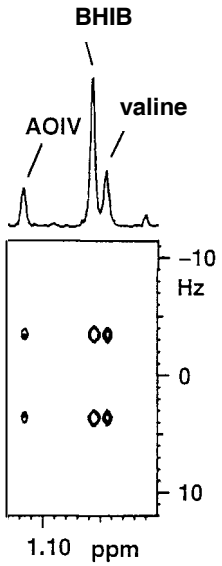


Figure 3 shows the high-field region of a J-resolved 2D spectrum of concentrated CSF at pH 7.0, including its 1D projection. The BHIB doublet at 1.07 ppm is readily recognized

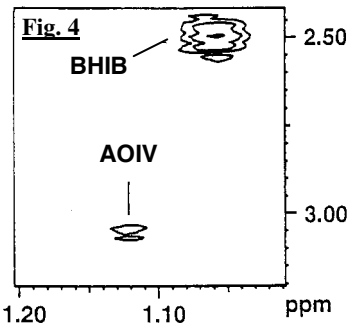
Figure 4 shows the relevant region of a COSY spectrum of concentrated CSF at pH 7.0. The correlation peak at 1.07/2.5 ppm is consistent with a previously reported cross peak for "U" [5], and with the C<u>H</u>$_3$-C<u>H</u> correlation expected for BHIB based on a 1-D ^{1}H MR spectrum of a BHIB reference solution at pH 7.0 (not shown).

For better orientation, signals adjacent to the BHIB methyl doublet are also annotated : α-oxoisovalerate (AOIV) and valine.

Discussion

BHIB was identified, for the first time, in ^{1}H MR spectra of CSF. A combination of dedicated 1D and 2D ^{1}H MR experiments was employed for signal assignment. BHIB concentrations in CSF, measured for individual patients, were on the order of magnitude of previously reported values determined by gas chromatography-mass spectrometry [6].

BHIB, whose concentration is significantly increased in CIS patients with vs. without inflammatory MS plaques [4], is a typical partial-degradation product of branched-chain amino acids released from muscle [7]. Thus, the BHIB increase may be due to increased uptake (leakage?) from blood in the presence of active plaques since BHIB is more concentrated in blood than in CSF. BHIB is a neoglucogenesis substrate; therefore, BHIB accumulation may alternatively indicate a perturbation of astrocytic gluconeogenesis due to effects caused by inflammatory plaques or reduced cerebral blood flow [8].



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