Analysis of Macromolecule Resonances in ^{1}H NMR Spectra of Human Brain

Kevin L. Behar, Douglas L. Rothman, Dennis D. Spencer, Ognen A. C. Petroff

Macromolecule resonances underlying metabolites in ^{1}H NMR spectra were investigated in temporal lobe biopsy tissue from epilepsy patients and from localized ^{1}H spectra of the brains of healthy volunteers. The ^{1}H NMR spectrum of brain tissue was compared with that of cytosol and dialyzed cytosol after removal of low molecular weight molecules (<3500 daltons) at 8.4 and 2.1 Tesla. The assignment of specific resonances to macromolecules in 2.1 Tesla, short-*TE*, localized human brain ^{1}H NMR spectra *in vivo* was made on the basis of a *J*-editing method using the spectral parameters (δ, *J*) and connectivities determined from 2D experiments *in vitro*. Two prominent connectivities associated with macromolecules *in vitro* (0.93–2.05 δ and 1.6–3.00 δ) were also detected *in vivo* by the *J*-editing method. Advantage was taken of the large difference in measured T_1 relaxation times between macromolecule and metabolite resonances in the brain spectrum to acquire 'metabolite-nulled' macromolecule spectra. These spectra appear identical to the spectra of macromolecules isolated *in vitro*.

Key words: macromolecules; proteins; *J*-editing; brain spectroscopy.

INTRODUCTION

Macromolecule resonances have been shown to underlie those of metabolites in ^{1}H NMR spectra of rat and rabbit brain tissue *in vitro* (1–6). Studies of dialyzed cytosol from brain homogenates (3) and perchloric acid extracts (5, 7, 8) have permitted several resonances to be assigned to proteins. These resonances possess shorter T_2 relaxation times compared with low molecular weight metabolites and are substantially reduced in spin-echo spectra acquired with long *TE* delay times. Recently, several studies performing localized short-*TE* ^{1}H NMR spectroscopy of normal human brain (9–13) have reported the presence of broad resonances in the region between 0.9 and 1.3 ppm. These resonances do not correspond to known low molecular weight metabolites in the ^{1}H NMR spectra of extracted brain tissue (1, 2, 4, 13) and appear qualitatively similar to resonances recently assigned to proteins in ^{1}H NMR spectra of rat brain (3). Increases in the intensity of the resonances in this region have been reported in *in vivo* ^{1}H NMR studies of demyelinating disease (10, 14, 15) and tumors (10, 16, 17). Identification of these resonances are of importance in the accurate quantification of metabolite resonances *in vivo* and the differentiation of protein resonances from those of lipids and lactate in certain pathological states.

We present here the characterization of the underlying macromolecule spectrum in short *TE* (17 ms) localized ^{1}H NMR spectra of normal human brain *in vivo* at 2.1 T. Macromolecule resonances were assigned on the basis of their connectivities by selective editing of *J*-coupled resonances, T_1, and T_2 relaxation times, and direct comparison to *in vitro* measurements of the metabolite-free cytosolic macromolecule fraction of human brain tissue. A simple method to selectively acquire the macromolecule spectrum *in vivo* is described. Portions of this work were presented recently as abstracts (18, 19).

METHODS

Preparation of Brain Tissue

Human brain tissue was obtained from patients undergoing *en bloc* resections of the temporal lobe for intractable epilepsy (12). In most cases the brain tissue was chilled on ice but not frozen and prepared for spectroscopy or homogenized within about 3 h of surgery. Tissue was cut into small strips and loaded into 5-mm NMR tubes. The cytosolic macromolecule fraction, devoid of low molecular weight metabolites and peptides, was prepared according to procedures described in a previous study (3). Human brain tissue was homogenized (5:1 vol/wt) in deionized water containing 0.5 m*M* of a protease inhibitor, phenylmethylsulfonyl fluoride (PMSF), and 0.02% NaN_3 and centrifuged for 30 min at 16,000 × *g* (4°C). This procedure was repeated twice. The combined supernatant was recentrifuged for 1.5 h at 100,000 × *g* (4°C) and the supernatant, consisting of the cytosolic fraction, was retained. A portion of the cytosolic fraction (10%) was lyophilized and dissolved in ^2H_2O for analysis by ^{1}H NMR, while the remainder of the cytosolic fraction was subjected to extensive dialysis (Spectrapor 3; 3,500 molecular weight cutoff) to remove low molecular weight metabolites (<3500 Da). The dialysis buffer consisted of 20 m*M* potassium phosphate (pH 7.0), 0.5 m*M* PMSF, and 0.02% NaN_3. The dialyzed cytosolic fraction was lyophilized and resuspended in ^2H_2O or deionized water (~1:1 vol/wt tissue wet weight) for ^{1}H NMR spectroscopy at 8.4 or 2.1 Tesla, respectively.

Human Subjects

In vivo spectroscopy was performed on normal adult volunteers of both genders. All subjects gave informed consent for the study, which was approved by the Yale Human Investigations Committee.

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From the Departments of Neurology (K.L.B., O.A.C.P.), Internal Medicine (D.L.R.), and Neurosurgery (D.D.S.), Yale University School of Medicine, New Haven, Connecticut.

Address correspondence to: Kevin L. Behar, Ph.D., C-128A Sterling Hall of Medicine, 333 Cedar Street, Yale University, New Haven, Ct 06510.

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^1H NMR of Human Brain Tissue and Dialyzed Cytosol at 8.5 T

^1H NMR spectra were acquired at 360.13 MHz using an AM 360 WB spectrometer (Bruker Instruments) operating at 8.4 Tesla. Samples were placed in 5-mm NMR tubes and a small aliquot of ^2H_2O was added to permit field/frequency locking. 1D ^1H NMR spectra were acquired using recycle delays of 2.28 to 2.86 s for dialyzed cytosol (macromolecules) and 7.56 s for whole tissue (metabolites plus macromolecules). The water signal was suppressed using 1 to 2 s of presaturation. FIDs were obtained with 8 or 16 K data points using a spectral width of 5000 Hz. Water suppressed 2D COSY (20) and *J*-resolved ^1H NMR spectra of the cytosolic macromolecule fraction of rat brain were obtained using previously described methods (3). Symmetrized 2D COSY spectra were acquired with a 90°-t_1–90°-t_2 pulse sequence as a matrix of 256 × 1024 data points in the time domain and zero-filled to 512 × 1024 points prior to 2D Fourier transformation. COSY spectra represent the sum of 64 FIDs for each of 256 increments in t_1. The spectral width was 4000 Hz in both the F2 and F1 dimensions. Data were processed with sine-bell weighting in both dimensions and presented as magnitude spectra. 2D COSY spectra were processed both with and without symmetrization as a precaution against generation of artifactual cross-peaks.

2D *J*-resolved spectra were acquired with a 90°-t_1/2–180°-t_1/2-t_2 pulse sequence as a matrix of 32 × 4096 data points and zero-filled to 64 × 4096 points prior to 2D Fourier transformation. *J*-resolved spectra represent the sum of 512 FIDs for each of 32 increments in t_1. The spectral width was 4000 Hz in F2 and ±31.25 Hz in F1. All 2D spectra were processed using sine-bell weighting in both the F1 and F2 dimensions.

Spectra were obtained at room temperature (22°C). Chemical shifts were referenced to sodium 3-trimethysilyl[2,2,3,3-^2H]-propionate at zero ppm.

Localized Spectroscopy and Spectroscopic Editing of Human Brain and Dialyzed Human Brain Cytosol at 2.1T

^1H NMR spectra were acquired at 2.1 T (89.4 MHz) using a modified Biospec I spectrometer (Bruker Instruments, Billerica, MA) with actively shielded magnetic field gradients (Oxford Magnet Technologies, Oxford, U.K.). Subjects were positioned supine on a patient bed within the magnet and their heads restrained in an adjustable holder. Shimming was performed by automated adjustment of first and second order shim currents based on a rapid field mapping procedure (21). A creatine line width-at-half-height of 4–5 Hz without apodization was routinely obtained in the localized spectrum. An 8-cm diameter ^1H transceiver coil was placed subjacent to the occiput. Localization was provided by a 3D ISIS (22) sequence with outer volume suppression and selective excitation as previously described (23, 24) to give a volume of 1.7 × 4 × 4 cm^3 in the occipital lobe. Water suppression was achieved by a 22-refocusing pulse (25) and a chemical shift selective 80 ms hyperbolic secant pulse (26) followed by an inversion recovery delay. For short *TE* spectra the *TE* time was 17 ms with the semi-selective refocusing pulse optimum at 2.30 ppm, a repetition time of 4.45 s and an inversion recovery delay of 1.03 s. Metabolite-nulled short-*TE* spectra were obtained by replacing the 80-ms hyperbolic secant pulse with one of 8 ms duration. Chemical shifts were referenced to creatine at 3.03 ppm.

Connectivities between *J*-coupled resonances were determined by homonuclear pulse editing (27, 28) using the spin echo pulse sequence described recently for localized ^1H NMR measurements of GABA (23). The carrier frequency of the 22-refocusing pulse of the spin-echo sequence was centered either at 1.6 or 2.1 ppm, which created intensity nulls at these chemical shifts, respectively, inhibiting *J*-modulation of the coupled spins. The *TE* time of the spin-echo editing sequence was equal to $1/(2J)$ or 68 ms for $J = 7$ Hz. Selective inversion of *J*-coupled resonances was induced by a DANTE (29, 30) pulse train (48 pulses, 26.5 ms duration) at either 1.6 or 2.1 ppm, which was phase-cycled with the refocusing pulse in order to eliminate dispersive phase refocused magnetization (31) produced by the DANTE pulse. Two subspectra are obtained: subtraction of the subspectrum obtained with DANTE inversion from one obtained without inversion eliminated all resonances not spin-coupled to the resonance(s) at the DANTE shift position. The subspectrum obtained without the DANTE represents the total resonance intensity (coupled and non-coupled spin systems) at that *TE* delay time from which the edited intensity can be compared. The macromolecule resonance intensity at 3.00 ppm was corrected for the off-resonance editing contribution of GABA 4-CH_2 at 3.00 ppm by acquiring a spectrum with the DANTE frequency positioned at 1.9 ppm, which is the chemical shift of the coupled 3-CH_2 of GABA (23). A 7% intensity adjustment factor was applied to the DANTE subspectrum prior to subtraction to compensate for imperfect selectivity (23). A repetition time of 3.3 s was used for the *in vivo* editing studies and an inversion-recovery time of 0.88 s. All edited spectra were acquired in two blocks of 128 scans (~17.8 min). FIDs were given 3 Hz exponential line-broadening and zero filled to 32 K data points. Zero order baseline correction was applied to each edited spectrum as defined between 4.0 and −1 ppm. Chemical shifts were referenced to creatine at 3.03 ppm in the nonedited subspectrum.

^1H NMR spectra of dialyzed human brain cytosol were obtained at room temperature (22°C) at 2.1 T from a 2-cm diameter sphere within a small Helmholtz coil using the same pulse sequence and B_1 strengths in order to maintain the same pulse lengths as for the *in vivo* measurements. Macromolecules were isolated from ~4.7 g of brain tissue. Approximately 80% (3.7 g equivalent weight) was redissolved in 3.7 ml water to yield a 1:1 wt/vol ratio of which 2.5 ml was loaded into the 2-cm diameter sphere. Chemical shifts were referenced to the most upfield macromolecule resonance (M1) at 0.93 ppm.

Measurements of T_1 and T_2 Relaxation Times

Spin-lattice (T_1) relaxation times of resonances in dialyzed human brain cytosol were measured at 2.1 T by an

inversion-recovery technique. T_1 values were determined at room temperature using nine delay times between 0.021 and 0.821 s. All values were calculated from peak heights and fit to the equation $M(t) = M_0^* (1 - 2 \exp[-t/T_1])$ using a simplex algorithm to iterate the parameters M_0 and $1/T_1$ for the best fit to the data. The simplex algorithm was written in Turbo-Pascal by Dr. H. P. Hetherington. All calculations were implemented on a 486 33-MHz personal computer.

For *in vivo* T_1 measurements at 2.1 T, the time between the center of the three ISIS inversion pulses and the start of the acquisition sequence was incremented over five delays between 0.068 to 0.517 s using a repetition delay time of 4.45 s. The ISIS inversion pulses were three 256 point phase swept 8 ms hyperbolic secants (26) (μ = 5, 2000 Hz bandwidth) with 10 ms interpulse delays used for gradient dephasing and rise time. To facilitate comparison with the *in vivo* data, the ISIS sequence was also used to obtain T_1 data from the dialyzed human brain cytosol at 2.1 T. In analyzing the data the ISIS inversion pulse permutations were approximated as an alternated single inversion pulse and the data fit by the equation $M = M_0 \exp(-t/T_1)$ using a simplex algorithm.

The T_2 relaxation time of the 0.93 ppm resonance was determined *in vivo* and *in vitro* from spectra acquired at five different *TE* delay times between 19.4 and 145.2 ms using a repetition time of 3.3 s. The refocusing null was placed at 2.1 ppm in order to inhibit *J* modulation due to the 2.1- to 0.93-ppm connectivity.

Free induction decays obtained from both *in vivo* and *in vitro* experiments were given 2 Hz exponential line broadening and zero-filled to 32 K data points prior to Fourier transformation. Zero order baseline correction was applied to the T_1 data between 0 and −5 ppm and extended under the spectrum. Zero and first order baseline correction was applied to the T_2 data between 2.1 and 0 ppm. The larger residual water resonance in the T_2 experiment due to the off-resonance placement of the semiselective pulse necessitated the different choice of the baseline region.

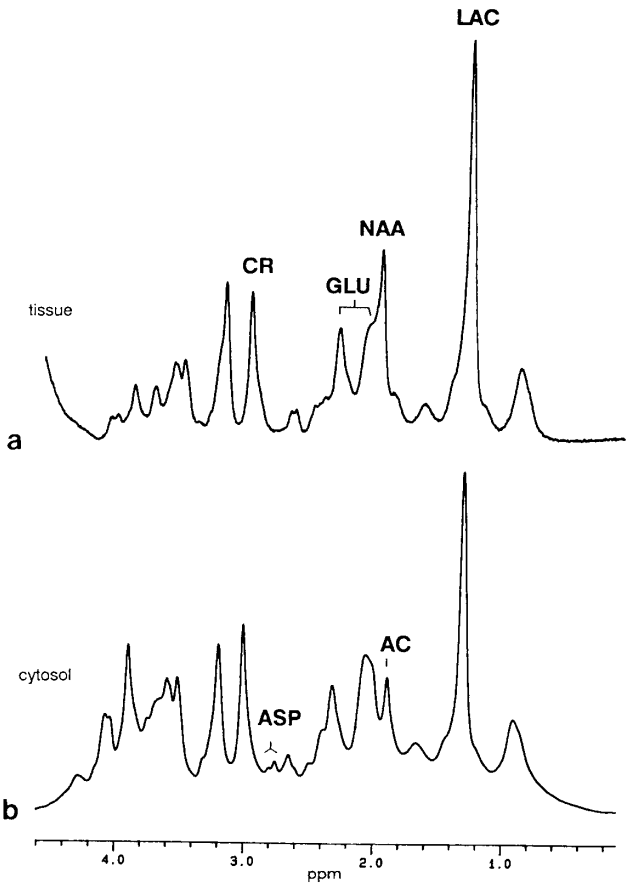

FIG. 1. (a) ^{1}H NMR spectrum of human brain temporal lobe tissue. (b) ^{1}H NMR spectrum of the cytosolic fraction of human temporal lobe following ultracentrifugation (100,000 × *g*). Spectrum B was line broadened so as to give equivalent line widths for creatine at 3.0 ppm ($\Delta\nu_{1/2}$ = 19 Hz) with the spectrum of whole tissue shown in (a). The intensity of the spectrum in (b) was normalized to that of (a) based on the integrated area of the macromolecule peak at 0.93 ppm.

RESULTS

Characterization of Human Brain Macromolecule Resonances *in Vitro*

High resolution 8.4 T ^{1}H NMR spectra of human temporal lobe tissue and the cytosolic fraction from a tissue homogenate are shown in Fig. 1. The two spectra are qualitatively similar and differ mainly in the region between 1.9 and 2.8 ppm due to the hydrolysis of *N*-acetylaspartate to acetate and aspartate which is enhanced during the homogenization procedure (Fig. 1b). The increased level of lactate in both spectra reflects postmortem metabolism after surgical removal of the brain tissue and during its preparation for spectroscopy. As seen in the more highly resolved spectrum of brain cytosol (Fig. 2a), two broad peaks, labeled as M1 and M3, are observed among the more numerous narrow resonances and multiplets of low molecular weight metabolites. Removal of these low molecular weight metabolites and peptides (<3500 Da) by dialysis revealed the underlying macromolecule spectrum. More than seven distinct resonances are resolved between 0.9 and 3 ppm at this field strength (Figs. 2b and 2c), while additional ones form a second band between 3.7 and 4.4 ppm. Connectivities between *J*-coupled resonances were identified in 2D COSY spectra of the dialyzed cytosol as shown in Fig. 3. Cross-peaks were identified in the COSY spectra at (0.96, 2.08), (1.24, 4.23), (1.43, 4.30), (1.72, 3.00), (2.19, 2.54, 3.78), and (3.00, 3.32). Although these resonances were too broad to measure multiplicities directly, it was possible to extract both the multiplicity and apparent coupling constants for several of these resonances. As shown in the *J*-resolved spectrum (Fig. 4), a substantial portion of the apparent line width of the major resonances (M1 to M7) is due to the overlap of numerous multiplets. Apparent coupling constants were also measured for several of these resonances in the *J*-resolved spectrum (Fig. 4) and their values are given in Table 1. The ^{1}H NMR spectrum and connectivities are similar to those found in rat brain cytosol where we have tentatively assigned several of these resonances to amino acids in the more mobile polypeptide chains of

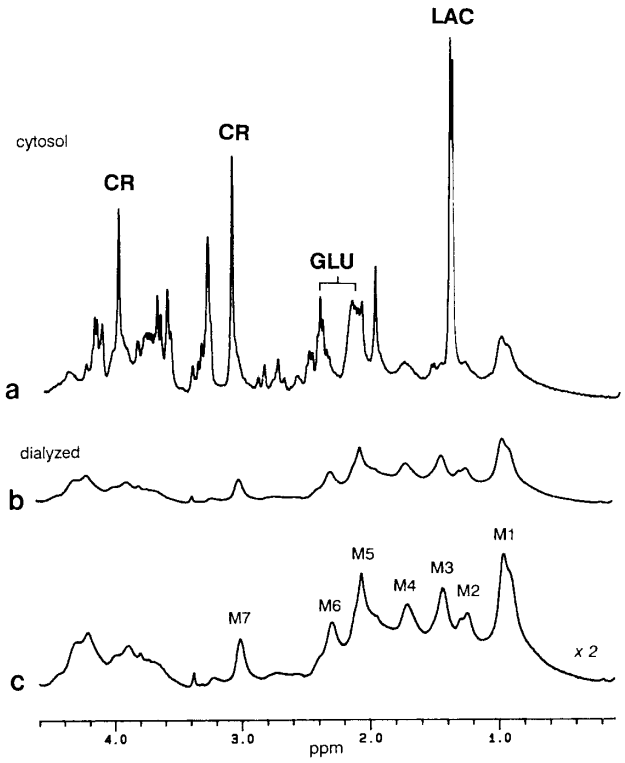

FIG. 2. (a) ^1H NMR spectrum of human cerebral cytosol following ultracentrifugation (100,000 × *g*) but prior to dialysis. (b) ^1H NMR spectrum of cerebral cytosol after extensive dialysis to remove low molecular weight metabolites and peptides (<3500 Da). The spectrum in (b) was normalized to that of (a) on the basis of the macromolecule peak at 0.9 ppm and increased ×2 for clarity (c).

proteins (3). Inspection of *J*-resolved subspectra indicated that a sharp nonphase modulated component at 2.05 ppm (and one of lesser intensity at 2.11 ppm) overlaps other resonances in the M5 region and has a relatively long T_2 of ~267 ms at 8.4 T[1] (average value of two experiments). The long T_2 and singlet structure of this resonance suggests the presence of a very mobile methyl group.

In Vivo and *in Vitro* Relaxation Time Measurements of Macromolecule Resonances at 2.1 T

As shown in Fig. 5a in a localized, short-*TE* (17 ms) ^1H NMR spectrum of human occipital lobe, two broad resonances were observed at 0.93 and 1.33 ppm (see below). The T_1 and T_2 relaxation times were measured at 2.1 Tesla for the macromolecule resonance at 0.93 ppm in dialyzed cytosol and for the resonance at the same chemical shift position in the human brain spectrum *in vivo* (Table 2). The T_1 value measured using the ISIS-based method for the peak at 0.93 ppm (M1) in the localized spectra of human brain was 250 ± 36 ms (±SD, n = 4), similar in magnitude but slightly longer than the value of 184 ms measured for the corresponding peak in the dialyzed cytosol. The T_1 of this resonance determined for dialyzed cytosol by the IR method was 221 ms indicating that the ISIS-based method underestimated the value of T_1. The T_2 relaxation time for this peak measured *in vivo* under similar conditions was 44 ± 4 ms (±SD, n = 4), which was near that measured for the corresponding peak in the dialyzed cytosol of 36 ms. The values of T_1 and T_2 measured for the macromolecule peak at 0.93 ppm are ~4 times less than the corresponding relaxation times previously reported for low molecular weight metabolites in the human brain spectrum (32–36).

[1] The 8.4 Tesla data were fit to a two-parameter single exponential equation between 128 and 496 ms (24–47 points) where the decay was mono-exponential. A rapid decay of intensity of the M5 peak for inversion-recovery delay times <128 ms were probably due to *J*-modulation effects from more quickly relaxing overlapping resonances.

In Vivo "Metabolite Nulled" Spectra of Macromolecules

By exploiting the large difference in T_1 relaxation times between the macromolecules and metabolite resonances, it was possible to suppress peaks from low molecular weight metabolites while retaining significant macromolecule intensity by application of a broad band inversion pulse followed by a recovery delay. An intensity null for

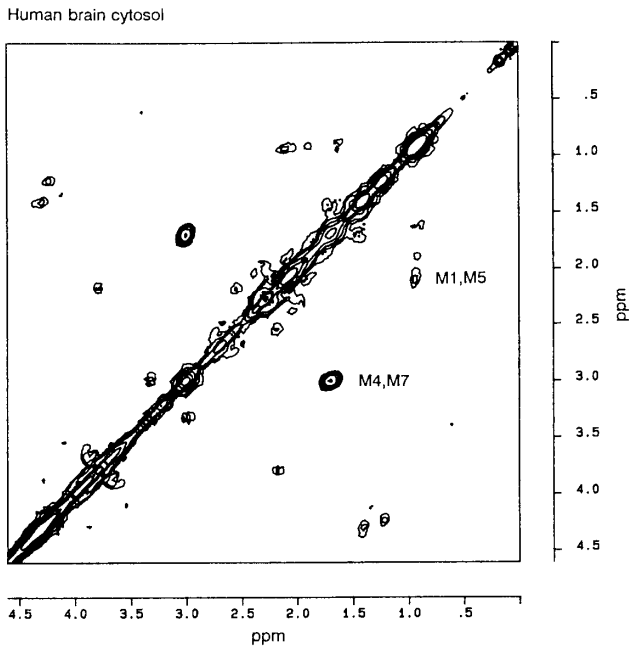

FIG. 3. 2D COSY spectrum of the macromolecules of dialyzed cerebral cytosol from human brain obtained at 8.4 Tesla. Low molecular weight metabolites were removed by extensive dialysis.

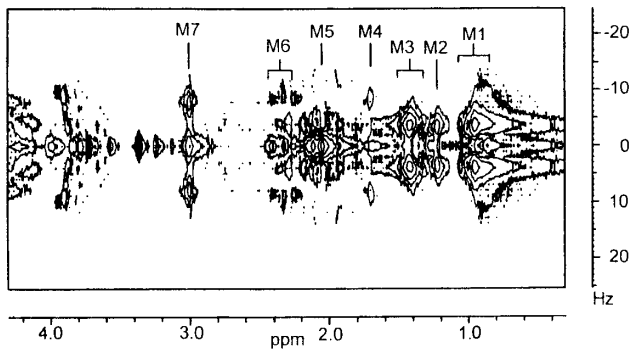

FIG. 4. 2D *J*-resolved spectrum of the macromolecules of dialyzed cerebral cytosol from human brain obtained at 8.4 Tesla.

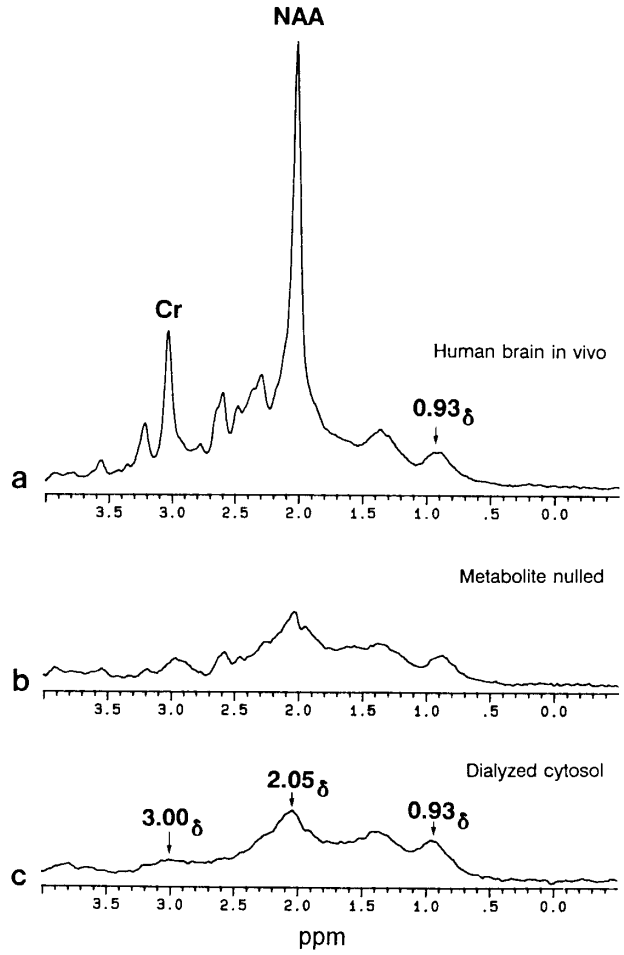

FIG. 5. Comparison of an *in vivo* short-*TE* ^{1}H NMR spectrum of human brain (a) with the metabolite-nulled spectrum (b) and the spectrum of dialyzed human cerebral cytosol obtained with the same localization sequence (c). Macromolecule resonances resolved in the *in vivo* metabolite-nulled spectrum include M1 (0.93 ppm), M2 (1.33 ppm), M4 (2.0 ppm), and M7 (3.0 ppm). Free induction decays were multiplied by an exponential function of 1 Hz prior to Fourier transformation.

metabolites occurred for a post-inversion recovery delay of 1030 ms. This delay time was chosen based on the intensity nulls of the creatine 3.03 ppm and *N*-acetylaspartate 2.02 ppm singlet resonances, which were sharp and could readily be seen relative to the broad macromolecule background. In addition, at this delay time most of the multiplet structure of the free amino acids observed between 2.1 and 2.9 ppm, which arise primarily from glutamate, glutamine, and *N*-acetylaspartate, was reduced below detection limits indicating that these resonances also have relatively long T_1 relaxation times compared with the macromolecule resonances. Figure 5b shows the macromolecule spectrum recorded *in vivo* obtained with this technique. This spectrum is similar to that of the dialyzed cerebral cytosol (Fig. 5c) and reveals for the first time the macromolecule resonances underlying the ^{1}H NMR spectrum of the human brain *in vivo*. The ^{1}H NMR spectrum of macromolecules at 2.1 T resembled that at 8.4 T, albeit less resolved, and consisted of a broad envelope with peaks at 0.93, 1.33, 2.05, 3.0, and 3.5–4.0

Table 1
Chemical Shifts and Coupling Constants of Macromolecule Resonances Detected in Human Brain

Peak	δ, ppm	*J*,[a] Hz	Multiplicity[a]	Connectivity
M1	0.93*	7.3	d	2.05*
M2	1.24	6.9	d	4.23
M3	1.43	7.3	d	4.30
M4	1.72*	—	m	3.00*
M5	2.05		s	
	2.11		s	
	2.05*	—	—	0.93*
	2.19	—	—	2.54, 3.78
M6	2.29	—	m	
M7	3.00*	7.8	t	1.72*
	3.00	—	—	3.32

Parameter values were determined from COSY and *J*-resolved spectra of dialyzed human cerebral cytosol at 8.4 Tesla. An asterisk (*) depicts peaks and connectivities also observed in edited spectra *in vivo* at 2.1 Tesla; all others were observed at 8.4 Tesla.
[a] These values should be interpreted as "apparent" values due to the potential effects of strong coupling.
The appearance of a "dash" in the table indicates that the coupling constant and/or multiplicity was not determined.

ppm. Figure 6 shows ^{1}H NMR metabolite-nulled spectra obtained from four subjects demonstrating the consistency of the macromolecule envelope in normal human brain. As shown in Fig. 7, when the metabolite-nulled spectrum was subtracted from the short-*TE* spectrum, the intensity of the macromolecule resonance at 0.93 ppm was effectively removed as was most of the intensity between 0.9 and 1.9 ppm. The residual intensity at 1.33 ppm after subtraction had a doublet structure with ~7 Hz separation suggesting a contribution from lactate.

In Vivo and *in Vitro* Editing of Macromolecule Resonances at 2.1 Tesla

Connectivity information was obtained at 2.1 T by use of a homonuclear editing method because this method could be readily applied *in vivo*. Homonuclear edited spectra of the dialyzed human brain cytosol revealed *J*-couplings between the peaks at 0.93 and 2.1 ppm and between the peaks at 1.6 and 3.0 ppm when the DANTE inversion was placed at 2.1 or 1.6 ppm, respectively (Figs. 8a and 8b). These connectivities correspond to those between peaks M1–M5 and M4–M7 as depicted in the 2D COSY spectrum of dialyzed cytosol shown in Fig. 3. In order to further characterize the *in vivo* macromolecule spectrum, connectivity information was obtained from localized edited spectra of five adult volunteers using a localized homonuclear-editing method (23). The frequencies of the DANTE inversion pulses were chosen to test the 0.93–2.05 δ and 1.6–3.00 δ connectivities established in the 2D COSY spectra and the results of the editing experiments on dialyzed human brain cytosol. As shown in Figs. 8c and 8d, application of the DANTE inversion at 2.1 ppm yielded a peak at 0.93 ppm similar in line shape to that measured in the dialyzed cytosol. When the DANTE inversion was placed at 1.6 ppm, a peak appeared in the difference spectrum at 3.01 ppm also similar to that observed *in vitro*. The ratio of the intensities of the edited 3.00 and 0.93 ppm resonances were 0.90 ± 0.18 *in vivo* (±SD, n = 5) and 0.80 for the

Table 2
Relaxation Times and Relative Intensities of Macromolecule Peaks Detected in the ^1H NMR Spectrum of Human Brain and Dialyzed Cerebral Cytosol at 2.1 Tesla

	0.93δ Peak (M1)		Relative areas*		
Experiment	T_1 (ms)	T_2	0.93δ edit / 0.93δ tot	3.00δ edit / 0.93δ tot	3.00 δ edit / 0.93δ edit
In Vivo: (n = 4)	250 ± 36	44 ± 4	0.42 ± 0.04	0.38 ± 0.11	0.90 ± 0.18
In Vitro: (n = 1)					
ISIS	184	36	0.22[b]	0.23[b]	0.80[c]
IR	221				

Values are given as the mean ± SD.
[a] Values represent ratios of peak areas measured in ISIS-localized spectra of human brain (*in vivo*) or dialyzed cerebral cytosol (*in vitro*). T_1 values for resonances in the dialyzed cytosol were determined at room temperature with an ISIS-based method and with an inversion recovery (IR) method.
[b] The values of the ratios were 16–22% lower in the nonlocalized edited spectrum.
[c] The value was determined from the respective areas of the edited resonances in spectra without the ISIS sequence in order to improve the signal-to-noise ratio.

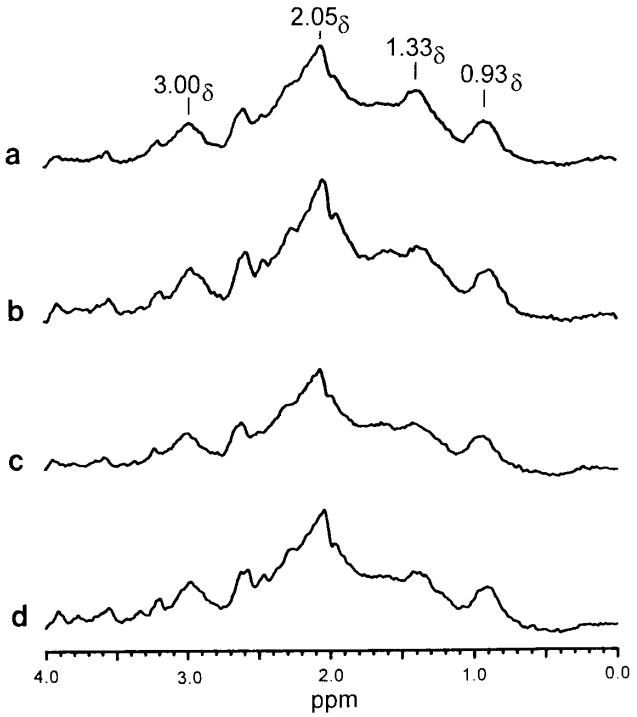

FIG. 6. Localized metabolite-nulled ^1H NMR spectra obtained from the occipital lobe of four different subjects. Resonance assignments are based on the spectrum of dialyzed cerebral cytosol. Free induction decays were multiplied by an exponential function of 1 Hz prior to Fourier transformation. Spectra are depicted on the same absolute vertical scale.

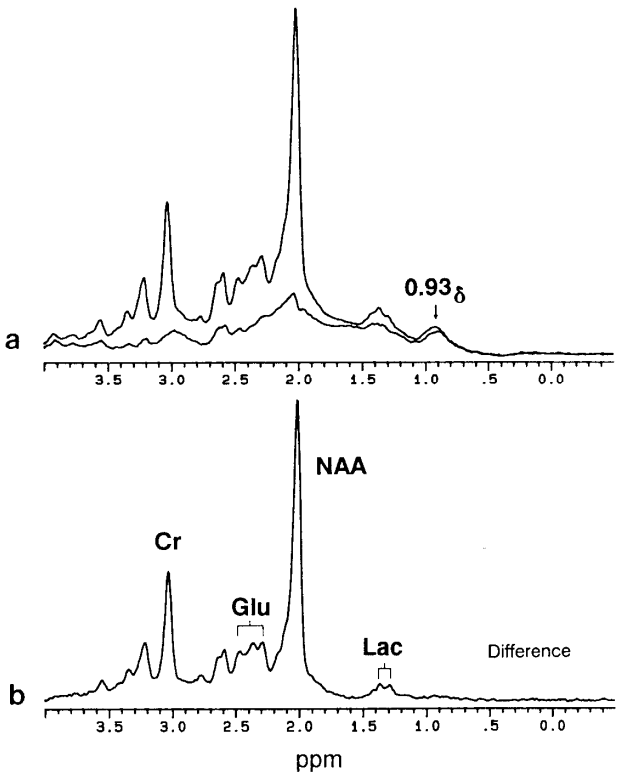

FIG. 7. Short-*TE* (17 ms) and metabolite-nulled ISIS localized ^1H NMR spectra obtained from the human cerebrum *in vivo*. Free induction decays were multiplied by an exponential function of 1 Hz prior to Fourier transformation. The difference spectrum (c) obtained by subtraction of (b) from (a) largely eliminated the macromolecule peaks (M1) at 0.93 ppm and M2 at 1.33 ppm. The flat baseline that results from the subtraction indicates that other macromolecule peaks were eliminated as well.

macromolecules of dialyzed cytosol. In the ^1H spectrum obtained *in vivo*, the intensity of the edited 0.93 ppm resonance (with the subspectrum obtained without the DANTE inversion) was 42 ± 5% of the total intensity of the "parent" peak (M1) at 0.93 ppm (±SD, n = 4).

DISCUSSION

The present study indicates that macromolecule resonances underlie those of metabolites in the localized ^1H NMR spectrum of human brain and represent an intrinsic component of the brain spectrum. The close similarity between the connectivities and coupling constants measured in 2D COSY and *J*-resolved spectra of dialyzed human cerebral cytosol in this study and that from rat brain reported in a previous study (3) strongly suggests that several of the background nonmetabolite resonances in normal human brain arise from proteins. By analogy with assignments of similar resonances in the rat brain spectrum, the macromolecule peaks between 0.9 and 2.4 ppm in the ^1H NMR spectrum can be assigned tentatively to

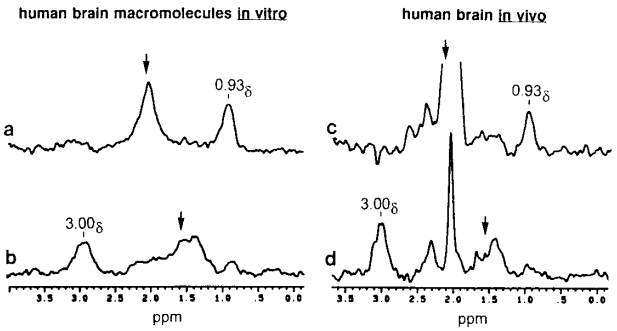

FIG. 8. Edited ^{1}H spectra of macromolecule resonances in human brain *in vivo* and in a solution of dialyzed human cerebral cytosol. The spectra were acquired with the same editing pulse sequence at 2.1 Tesla. (a, b) Edited spectra of dialyzed cytosol of human brain. (c, d) Edited spectra of human brain *in vivo*. In the spectra shown in (a) and (c), the frequency of the DANTE was centered at 2.1 ppm (arrow) which yielded a resonance at 0.93 ppm in the difference spectrum. In spectra B and D, the DANTE was centered at 1.6 ppm (arrow) which yielded a resonance at 3.01 ppm in the difference spectrum. The chemical shifts, line widths, and relative intensities of the *in vivo* edited resonances were similar to those obtained in the solution of dialyzed cerebral cytosol.

methyl and methylene resonances of protein amino acids, such as leucine, isoleucine, and valine (M1), threonine and alanine (M2–M3), lysine and arginine (M4 and M7), glutamate and glutamine (M5–M6), and the less well-defined band of resonances between 3.5 and 4.5 ppm to α-methine protons. In the *J*-resolved spectrum of dialyzed cerebral cytosol, the resonance at 0.93 ppm (M1) can be deconvoluted into overlapping doublets, one component of which is *J*-coupled to another resonance at 2.05 ppm as shown in the COSY spectrum. The multiplet structure for the 2.05 ppm region is difficult to discern in the J-resolved spectrum due to the large number of multiplets (apparent doublets or triplets) in this region. These multiplets were much lower in intensity as compared with those arising from M1 or M7. An intense connectivity was also observed between resonances at 1.6 ppm (M4) and 3.00 ppm (M7), where the latter has been tentatively assigned to ϵCH_2 of lysine (3). The resonance at 3.00 ppm may also include contributions from cystine based on the 3.00- to 3.32-ppm connectivity observed in COSY spectra *in vitro* (Fig. 3 and Table 1). A sharp resonance, which overlaps M5 at 2.05 ppm, was identified as a singlet based on its lack of phase modulation in *J*-resolved subspectra at 8.5 T (not shown). Based on its chemical shift position, it could represent the ϵ-CH_3 of methionine in proteins (37) or an *N*-acetylated *N*-terminal amino acid of brain proteins.

Comparison of spectra obtained *in vivo* at 2.1 T with spectra obtained from dialyzed cytosol provided evidence that resonances from cytosolic macromolecules contribute to the *in vivo* human brain spectrum. A broad resonance at 0.93 ppm was resolved from other major metabolites in the localized short-*TE* spectra of human brain at 2.1 T (Fig. 5). *J*-based editing experiments performed in human cerebrum clearly demonstrated that the connectivity between 0.93 and 2.1 ppm established in the COSY spectra of dialyzed cytosol at the higher field also appeared in the spectrum *in vivo*. A similar connectivity was observed in 2.1 T spectra obtained with the same editing sequence on a solution of dialyzed human brain cytosol (Fig. 8). The intensity of the *J*-edited resonance at 0.93 ppm in the localized short-*TE* ^{1}H spectrum of human brain represented ~42% of the total peak intensity (~22% in the dialyzed cytosol) observed in the non-edited spectrum. If the 0.93 ppm peak arose from the several, closely spaced ~7 Hz doublets depicted in the *J*-resolved spectra *in vitro*, then we would expect the edited resonance(s) to comprise nearly 100% of the total intensity when given the high efficiency of the editing sequence, as measured for solutions of coupled resonances. Therefore, our measured value of the edited-to-total ratio at 0.93 ppm of 0.42 ± 0.04 suggests that other resonances (some with different coupling patterns) contribute to this position also. The difference between the in vivo and in vitro percentages in their edited intensities may reflect differences in the T_2 relaxation experienced in the cellular environment *in vivo*. An alternative explanation for the 0.93 ppm resonance *in vivo* is that it arises from lipids; however, the major fatty acyl glycerides (38, 39) do not possess the 0.93- to 2.05-ppm connectivity observed here. Although a unique methyl-methylene coupling of linolenic series fatty acids has been reported in excised rat brain on the basis of a similar connectivity in chloroform-methanol extracts (40), the line shape of the 0.93 ppm resonance and relative intensities of the peaks at 1.3 and 0.9 ppm are consistent with aqueous soluble macromolecules of the dialyzed cytosol.

Spectroscopic editing clearly revealed a resonance at 3.00 ppm (M7) which could not be observed in the conventional ^{1}H spectrum of brain due to overlap with the CH_3 resonances of creatine and phosphocreatine and the γCH_2 of GABA. This resonance was shown to originate from macromolecules by its clear presence in the metabolite-nulled spectrum and the dialyzed cytosol.

The *in vivo* relaxation times of the resolved macromolecule peak(s) at 0.93 ppm were similar to the values determined for corresponding resonances in the dialyzed cerebral cytosol (Table 2). The values for T_1 of 250 ms and T_2 of 44 ms measured for the macromolecule peak at 0.93 ppm *in vivo* are substantially shorter than values reported for metabolite resonances (32–36). Consistent with reported T_1 values for metabolites of 1100 to 1400 ms (32–34), the use of a post-inversion delay time of 1030 ms in the present study resulted in a null in the intensity of most of the metabolite resonances observed in the ^{1}H spectrum at 2.1 T while leaving the peak at 0.93 ppm near its full intensity (Fig. 7). The chemical shift and line shape of the 0.93 ppm resonance in the metabolite nulled spectrum were similar to that observed in the dialyzed human cytosol (Fig. 5).

This study shows that macromolecule resonances contribute substantial intensity to the spectral region between 0.9 ppm and water in the ^{1}H NMR spectrum of human brain. Quantitation of metabolite resonances from *N*-acetylaspartate, glutamate, glutamine, GABA, lactate, and *myo*-inositol will receive proportionately larger contributions from macromolecules as the total echo delay times in ^{1}H spin-echo pulse sequences are reduced. With

the exception of the well-resolved peaks at 0.93 and 1.33 ppm, all other macromolecule peaks are obscured by the more intense metabolite resonances in the ^{1}H NMR spectrum *in vivo*. As shown in Fig. 7, subtraction of the metabolite-nulled (macromolecule-only) spectrum from the conventional, short-*TE* spectrum almost completely eliminated the 0.93 ppm resonance (and other macromolecule resonances) resulting in a flat spectral baseline. The difference spectrum highlights the significant intensity that macromolecules contribute to ^{1}H spectra of human brain acquired with short-*TE* periods. The use of the "metabolite-nulling" method and its converse in conjunction with spectral editing will be *very* useful in the quantification of macromolecule and metabolite resonances in short-*TE* ^{1}H NMR spectra *in vivo*. The method is superior to deconvolution techniques in that line width differences between metabolites and macromolecules are not very large.

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