Heat-Bath Cooling of Spins in Two Amino Acids

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Abstract

Heat-bath cooling is a component of practicable algorithmic cooling of spins, an approach which might be useful for in vivo \textsuperscript{13}C spectroscopy, in particular for prolonged metabolic processes where substrates that are hyperpolarized ex-vivo are not effective. We applied heat-bath cooling to 1,2-\textsuperscript{13}C\textsubscript{2}-amino acids, using the \(\alpha\) protons to shift entropy from selected carbons to the environment. For glutamate and glycine, both carbons were cooled by about 2.5-fold, and in other experiments the polarization of C1 nearly doubled while all other spins had equilibrium polarization, indicating reduction in total entropy. The effect of adding Magnevist\textsuperscript{®}, a gadolinium contrast agent, on heat-bath cooling of glutamate was investigated.

1. Introduction

Heat-bath cooling \cite{1} is a key building-block of algorithmic cooling (AC), a recent spin-cooling approach originally proposed for purifying qubits in NMR quantum computers \cite{2 3}. AC intersperses heat-bath cooling (which combines polarization transfer and fast repolarization) with reversible polarization compression. By itself, polarization compression \cite{3 4} is limited due to the entropy bound (also called Shannon’s bound) \cite{5}, and further limited by the unitary bound on spin dynamics \cite{6}. However, heat-bath cooling allows AC to cool spins (spin-half nuclei) far beyond these bounds \cite{2 3 4 5 6 7 8 9}. AC, as well as its components, heat-bath cooling and polarization compression, may enhance in vivo \textsuperscript{13}C magnetic resonance spectroscopy (13C-MRS), which enables dynamic analysis of metabolic processes in living organisms \cite{11 12}.

Heat-bath cooling achieves controlled entropy extraction from suitable spin systems \cite{1} by applying one or more steps of selective-reset. Each selective-reset cools a selected slow-relaxing spin (computation spin) by polarization transfer (PT) from a more polarized reset spin, and this PT is followed by a delay. We call this idle step (the delay) a reset step. The reset spin repolarizes rapidly during that reset step so that the spin system is cooled; the reset spin may be reused via an iterative (or recursive) algorithm \cite{2 3}. For a pair of computation and reset spins, respectively \(c\) and \(r\), we denote here by \(\mathcal{R}(c, r)\) the ratio between the thermalization time of the computation spin, \(T_1(c)\), and the characteristic repolarization time of the reset spin (for liquid NMR, it is the thermalization time, \(T_1(r)\)). A large ratio, \(\mathcal{R}(c, r) \gg 1\), is required to preserve most of the enhanced polarization of the computation spin during reset steps. In addition, strong scalar J-coupling (or for solids dipolar coupling) is required, \(J \gg 1/T_2\); such that loss of polarization by decoherence during PT would be minimized.

AC produces spin-cooling using heat-bath cooling steps, wherein the spin entropy is transiently reduced (the spins are cooled) even though no physical cooling is applied to the system. Practicable cooling algorithms that yield moderate cooling already for small spin systems were suggested \cite{2 3}, and improved algorithms were recently derived \cite{9}. Heat-bath cooling, by itself, can already cool the entire spin system, bypassing the entropy bound, if, at equilibrium, the reset spin polarization, \(\varepsilon(r)\), is higher than the polarization of the computation spins, \(\varepsilon(c)\).

For larger spin systems, AC can theoretically generate hyperpolarized spins, for which the polarization is increased by several orders of magnitude \cite{2 3 4 5 6 7 8 9 10}. However, such extensive cooling faces significant obstacles and requires many selective-resets and is not expected in the near future. Other spin-cooling methods that produce hyperpolarization include dynamic nuclear polarization in the solid state followed by rapid thawing (dissolution DNP) \cite{13}, parahydrogen induced polarization (PHIP) \cite{14}, and optical pumping of noble gases \cite{15}. For small spin systems, AC is applicable with current technology, and has two important advantages over dissolution DNP, the most generally applicable hyperpolarization method: both AC and heat-bath cooling require no special apparatus and may therefore be applied directly in vivo, in particular to enhance 13C-MRS of slow metabolism in the brain; Furthermore, the polarization enhancement may be applied/replenished in vivo after several hours.

Significant entropy reduction was already demonstrated...
experimentally [1]. That heat-bath cooling experiment used $^{13}$C-trichloroethylene (TCE) in chloroform solution (with $\varepsilon(r) \sim 4\varepsilon(c)$), a simple model system comprising three spins - two carbons and one proton, where C1 is the quaternary carbon and C2 is bound to the proton. A paramagnetic reagent, Cr(acac)$_3$, improved both $T_1$ ratios [16], such that $R(C1, H) \sim 10$ and $R(C2, H) \sim 5$. The entire spin system was cooled beyond the entropy bound by a dual selective-reset, using the four-step pulse sequence POTENT (POLarization Transfer by ENvironment Thermalization) [1]:

1. PT #1. $H \rightarrow ^{13}$C $\rightarrow ^{13}$C (HCC) relay implemented by two refocused INEPT sequences [17, 18] using non-selective (“hard”) pulses.
2. Reset #1. A significant delay of $\sim 2T_1(H)$.
3. PT #2. Refocused INEPT from the partly repolarized proton to C2.
4. Reset #2. A significant delay of $\sim 3T_1(H)$.

After POTENT, both $^{13}$C were cooled nearly two-fold, while the proton regained most of its equilibrium polarization, such that the entire spin system was cooled. In the submitted version of [1], another heat-bath cooling experiment is described where only steps 1–3 above are performed, such that both carbons are cooled, each by a factor greater than two.

We chose to work here with glycine and glutamate, which are both neurotransmitters [19]. Glutamate has the major excitatory role in the human brain, while glycine assumes an inhibitory function in specialized neurons [19] and also acts as a cofactor for excitatory neurotransmission within the forebrain [20]. Glutamate is present in the brain at significant concentrations (as high as 10 mM) [21], and also acts as a cofactor for excitatory neurotransmission within the forebrain. Glutamate has the greater than two.

experimentally $^{13}$C polarization by about 4-fold [4, 18], and sufficiently high $R(C1, H_\alpha)$ were expected and confirmed. For each spin system, the entropy was reduced by a single selective-reset (the first two steps of POTENT), and in a different

2. Materials and Methods

NMR samples (0.5 mL) of $^{13}$C-labeled amino acids (99% $^{13}$C, Cambridge Isotope Laboratories) were prepared in D$_2$O (99.96% D), and the pH was adjusted by adding K$_3$PO$_4$ (anhydrous, 99% pure, Sigma-Aldrich) and measured using 6.4–8.0 pH sticks (0.2 units, Lypham); see samples $G$ and $E$ in Table 1. D$_2$O solutions of Magnevist$^\text{®}$ were prepared from lyophilized samples of a clinical aqueous solution (0.5M, Bayer HealthCare Pharmaceuticals). A dilute solution of Magnevist in D$_2$O (5 mM) was added directly to the NMR tubes with a micropipette in 5 µL aliquots, corresponding to incremental Magnevist concentrations of 0.05 mM, similar to a recent study of glycine relaxation [27]. Aliquots were added until $T_1(H_\alpha)$ was reduced to about 1s; see samples $E_{30}$ and $E_{40}$ in Table 1.

All experiments were performed on a Bruker Avance 500 spectrometer with a standard 5 mm BBO probe and a digital variable temperature control unit, at controlled temperatures (0.1K accuracy). $T_1$ relaxation times were measured by inversion recovery (with the standard Bruker t1ir pulse sequence), consisting of a prolonged recycle delay ($T_{1\text{ir}}$), followed by 180°, τ, 90°, and acquisition. 17 logarithmically-spaced $\tau$ values were used, such that a few were below $T_1$ and at least one delay exceeded $T_{1\text{ir}}$.

The POTENT pulse sequence [1] and its components described above were used with two main modifications; First, the INEPT pulse sequences in steps 1 and 3 were implemented with spin-selective low-power pulses for $^{13}$C excitation and inversion, which had shapes of E-BURP1 and I-BURP1 [28], respectively, and a duration of $t = 1$ ms. Second, during the PT from C2 to C1 in step 1, WALTZ-16 [29] proton decoupling was employed.

Detailed pulse sequences are illustrated in Fig. 2. The HCC relay (Fig. 2a) includes delays for the two INEPTs in step 1 ($d_4$ and $d_7$), and a variable refocusing period, $d_5$, set to be $d^4/k$ depending on the number, $k$, of α protons [18]; For glutamate, $d_5=1.21$ ms ($d_4=1.71$ ms, $d_7=4.681$ ms), while for glycine $d_5=0.39$ ms ($d_4=1.79$ ms, $d_7=4.742$ ms), where $d_5=d_4/k−1/2$ ms, and the shortening (by $1/2$ ms) compensates for the refocusing I-BURP1 pulse on C1. The HCC+WAIT pulse sequence (Fig. 2b) employed a long repolarization delay, $d_3 \sim 7T_1(H_\alpha)$. The delay $d_7$ was optimized (around $1/4J_{CC}$) in 0.01 Hz intervals to maximize the integral of C1 after HCC+WAIT. For POTENT (Fig. 2c), the first repolarization delay, $d_2 \geq 2T_1(H_\alpha)$, allowed significant cooling of C2 by the following refocused INEPT. The final delay, $d_3$, was of the order of $T_2(H_\alpha)$ to eliminate small distortions, and not for proton repolarization. Thus, the resulting POTENT is
truncated. For sample $E^*_M$, in addition to truncated POTENT, we also applied (truncated) POTENT$^+$, where $d_2$ was followed by a 90° pulse on C2, resulting in uniform lines. The delay $d_14$ in Pt #2, step 3 of POTENT, was $d_4 - \frac{1}{2}$ (again shortened to compensate for the soft pulse I-BURP).

A single scan was acquired for the samples in Table 1. In order to obtain the proton spectrum (when cooling the entire spin system), the HCC+WAIT sequence was repeated under the same conditions, while acquiring the proton spectrum (see Fig. 2c). The NMR data were apodized with small (0.3 Hz) exponential line broadening function prior to Fourier transformation. The spectra were phase-corrected according to the equilibrium spectra, and polarization enhancements were calculated from peak integrals.

3. Results

3.1. $T_1$ Measurements

To affirm that $^{13}$C$_2$-labeled amino acids are suitable for heat-bath cooling, we determined the longitudinal relaxation times (Table 1) for glycine, sample $G$, and glutamate, sample $E$, at room temperature (24.0°C). As expected, C1 had much slower relaxation, resulting in $T_1$ ratios of $R(C1,H_a) \sim 10$, while $R(C2,H_a) \sim 1.5$ (see Table 2).

For glycine in D$_2$O (sample $G$), a long relaxation time was found for the carbonyl, $T_1(C1) = 31.6 \pm 0.5$ s, similar to the relaxation time recently reported for an aqueous solution of 1.2-$^{13}$C$_2$-glycine (16% D$_2$O). $T_1(C1)_{eq} = 27.3 \pm 1.2$ s, obtained under similar conditions (2). For C2 and the $\alpha$ protons of glycine, we found much more rapid relaxation: $T_1(C2) = 3.75 \pm 0.05$ s, in good agreement with the aqueous solution, $T_1(C2)_{eq} = 4.0 \pm 0.2$ s (27) and $T_1(H_a) = 2.72 \pm 0.02$ s.

For glutamate (sample $E$), shorter relaxation times were found, $T_1(C1) = 13.03 \pm 0.04$ s, somewhat similar to an aqueous solution, $T_1(C1)_{eq} = 10.2 \pm 0.8$ s (39), $T_1(C2) = 1.96 \pm 0.02$ s and $T_1(H_a) = 1.29 \pm 0.02$ s. The relaxation times of the methylene protons were similar to $H_a$ (see Table 1).

Addition of Magnevist to glutamate, sample $E_M$, decreased all $T_1$ relaxation times. Heating glutamate with Magnevist to physiological temperature (37.0°C), sample $E^*_M$, increased all $T_1$ relaxation times by about 40%. In both cases the resulting $T_1$ ratios were similar to sample $E$, see Table 2.

3.2. Cooling the Spin Systems of Glycine and Glutamate

Our goal here was to reduce the total entropy of each amino acid spin system, thereby bypassing Shannon’s bound (for similar calculations see Ref. 1). Both spin systems $G$ and $E$ were cooled at room temperature by a single selective-reset, which consisted of an HCC relay that increased the signal of C1 by a factor of about 3, followed by a significant delay, $d_3 \sim 7T_1(H_a)$. The pulse sequence HCC+WAIT (Fig. 2c) includes two refocused INEPT sequences and employs proton decoupling during the carbon-carbon PT, similar to an earlier sequence (31). C1 retained an enhancement factor of about 1.9 ($1.88 \pm 0.01$ for glycine, $1.89 \pm 0.01$ for glutamate), such that the total entropy decreased by $\sim 2.6 \epsilon^2/\ln 4$ (resulting from $2.6 \sim 1.9^2 - 1^2$), where $\epsilon$ is the equilibrium $^{13}$C polarization. The protons polarized back to equilibrium (within integration error $\lesssim 1\%$) and C2 returned to $1.00 \epsilon \pm 0.01\epsilon$, see Figure 3 suggesting that elementary heat-bath cooling is sufficient to go beyond the entropy bound. In terms of effective spin temperatures, obtained by taking the quotient of the equilibrium temperature and the cooling factor (as done in Ref. 1), C1 was cooled to $\sim 156K$–$158K$, while the other spins were at the equilibrium temperature of 297.0 ± 0.1 K.

For glutamate in the presence of magnevist (sample $E_M$), the HCC+WAIT sequence produced similar cooling as for sample $E$. $C1$ was cooled by a factor of about 1.9, while the other spins regained their equilibrium polarizations.

3.3. Heat-bath Cooling of C1 and C2

A potentially useful application of heat-bath cooling is to enhance, simultaneously, two labeled carbons by applying the POTENT pulse sequence (1 with no final delay; Fig. 2c). For both amino acids (samples $G$ and $E$), C1 and C2 were cooled to a similar extent (about 2.4-fold, see Table 2) when the first delay was $d_2 \sim 2.3T_1(H_a)$. The very short final delay ($d_3 \approx 1s$) was used only to eliminate small distortions. The spin temperatures after cooling were $\sim 120K$–$130K$.

For glutamate with Magnevist (sample $E_M$), application of steps 1–3 of POTENT resulted in cooling factors of about 2.5 and 2.3 for C1 and C2, respectively, similar to sample $E$, reflecting the similarity in $T_1$ ratios for both samples (see Table 2).

Application of POTENT to glutamate at physiological temperature of 37.0°C (sample $E^*_M$) cooled both carbons by a factor of about 2.5, quite similar to the cooling obtained at room temperature described above, see bottom part of Table 3. Interestingly, improved cooling at physiological temperature is obtained by POTENT$^+$ (Fig. 2c), where an additional 90° pulse was applied to C2, in order to provide uniform net PT (equal intensity for the lines of the C2 multiplets (13)), see Fig. 4. This sequence allowed a two-fold reduction in the final delay ($d_3$ of the order of $T_2(H_a)$) by transforming erroneous density-matrix elements into non-observable coherences; consequently, for $d_2 = 2.0T_1(H_a)$, C1 and C2 were cooled by factors of about 2.60 and 2.65, respectively.

4. Discussion

4.1. Heat-bath Cooling of Amino Acids for In Vivo Spectroscopy

The simultaneous cooling of both labeled carbons at physiological conditions (sample $E^*_M$) suggests that heat-
bath cooling is suitable for 13C-MRS, which detects and quantifies 13C-labeled metabolites in vivo, in particular at specific locations within the brain \[12\]. For glutamate, it was recently shown that the INEPT pulse sequence can be adapted to in vivo 13C-MRS and used for brain spectroscopy with WALTZ decoupling \[22, 32\]. Heat-bath cooling sequences that combine these elements may therefore be readily adapted to localized in vivo spectroscopy.

Simultaneous cooling of both \(^1\)C of 1,2-labeled amino acids by the POTENT pulse sequence may enhance the detection of such isotopomers in vivo. For glutamate, both C2-labeled and 1,2-labeled isotopomers are produced in cerebral metabolism of 1,2-\(^{13}\)C\(_2\)-labeled and \(^{12}\)C-labeled isotopomers are produced in cerebral metabolism of 1,2-\(^{13}\)C\(_2\)-glucose \[12\]. In this case, application of POTENT in vivo is expected to cool C2 in both single and double-labeled isotopomers by means of the final PT (see Fig. \[2\]). Quantitative analysis may be facilitated by a suitable setting of \(d_2\), such that both \(^1\)C of the double-labeled isotopomer are cooled to a similar extent, as shown in Table \[3\].

Heat-bath cooling of the entire spin system as done here is a preliminary step towards AC, which also demonstrates an important concept (in this case on important biomolecules): On the one hand, reversible polarization compression can cool carbons of the two amino acids further, beyond just reaching the polarization of the sensitive nucleus; On the other hand, heat-bath cooling enables entropy reduction, which is not attainable by polarization compression.

### 4.2. Algorithmic cooling of amino acids

Combining heat-bath cooling and compression provides AC that can, in theory, cool one or more spins far beyond the entropy bound \[2\]. In reality, finite \(T_1\) ratios limit the attainable cooling \[10, 32\]. On the route to useful AC, one could employ various other amino acid isotopomers, such as 2,3,4-\(^{13}\)C\(_3\)-glutamate observed in vivo \[34\] or 1,2-\(^{13}\)C\(_2\),\(^{15}\)N-N-acetyl aspartate (NAA). NAA, a major metabolite, is present in the brain at low concentrations and was detected in vivo by 13C-MRS \[11, 12, 34\]. For this molecule, the amide proton constitutes a second reset spin that is polarized \([8]\). \(^{13}\)C of \(^{12}\)C-labeled metabolites may be cooled effectively by AC (potentially using Magnevist). Heat-bath cooling in the near future, and AC later on, may enhance spectroscopy of slow metabolic processes, particularly in the brain where hyperpolarization is not effective. These methods are safe, require no additional equipment, and may be replenished.

### 5. Conclusion

Heat-bath cooling of the backbone carbons of glycine and glutamate may be readily applied in vivo, and could also be applicable to other amino acids (and similar metabolites). Going beyond heat-bath cooling, various suitably labeled isotopomers of amino acids and similar metabolites may be cooled effectively by AC (potentially using Magnevist). Heat-bath cooling in the near future, and AC later on, may enhance spectroscopy of slow metabolic processes, particularly in the brain where hyperpolarization is not effective. These methods are safe, require no additional equipment, and may be replenished.

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Figure 1: Amino acids used in heat-bath cooling experiments.

(a) HCC relay

(b) HCC+WAIT

(c) POTENT / POTENT

Figure 2: Pulse-sequences for heat-bath cooling of amino acids. Narrow black bars denote 90° non-selective pulses, while E-BURP1 and I-BURP1 represent 1 ms spin-selective BURP pulses [29]. (a) The HCC relay was implemented by two refocused INEPT sequences. (b) A significant delay (d3 ≈ 7T1(H2O)) following the relay restored the polarization of all protons. (c) Alternatively, both carbons were cooled by truncated POTENT sequences, where POTENT+ includes an additional E-BURP1 90° pulse (highlighted) on C2 after d2. Acquisition (after 90° pulse) is performed for both nuclei (b) or only for 13C (c). In figure (c) we denote the pulse sequences as POTENT/POTENT+ because for long d3 the proton would repolarize (step 4 of POTENT).
Figure 3: Cooling beyond Shannon’s bound. $^{13}$C spectra (proton spectra in insets) of glycine (left, sample G) and glutamate (right, sample E) before (bottom) and after (top) the HCC+W AIT experiment. C1 was cooled by a factor of $\sim$ 1.9, while the protons fully repolarized and C2 regained its equilibrium polarization.

Figure 4: Simultaneous cooling of both backbone carbons of glutamate at physiological temperature with Magnevist (sample $E'_M$) using POTENT+, see Table 3 for details. Spectra are at equilibrium (bottom) and after cooling (top).
Table 1: $T_1$ values (in seconds) of $^{13}$C-labeled glycine and glutamate in D$_2$O ($\sim$300 mM). Samples $E_M$ and $E'_M$ contain 0.05 mM Magnevist. Errors are standard deviations of 3–10 single scans.

| Sample | Amino acid | $T_1$ [K] | $T_1$ (C1) | $T_1$ (C2) | $T_1$ (H$\alpha$) | $T_1$ (H3) | $T_1$ (H4) |
|--------|------------|-----------|------------|------------|-----------------|------------|------------|
| $G$    | glycine    | 297       | 31.6 ± 0.5 | 3.75 ± 0.05 | 2.72 ± 0.02     |            |            |
| $E$    | glutamate  | 297       | 13.03 ± 0.04 | 1.96 ± 0.02 | 1.29 ± 0.02     | 1.001 ± 0.006 | 1.281 ± 0.003 |
| $E_M$  | glutamate  | 297       | 10.2 ± 0.1$^b$ | 1.84 ± 0.02 | 1.10 ± 0.01     | 0.920 ± 0.003 | 1.160 ± 0.001 |
| $E'_M$ | glutamate  | 310       | 14.36 ± 0.06 | 2.66 ± 0.04 | 1.50 ± 0.01     | 1.270 ± 0.004 | 1.606 ± 0.004 |

$^a$Molar ratios of K$_3$PO$_4$ to amino acid were 0.5 for glycine (pH 8) and 0.8 for glutamate (pH 7.5).

$^b$For sample $E_M$, only two measurements were taken for C1.

Table 2: $T_1$ ratios for the samples in Table 1.

| Sample | Magnevist[mM] | $R$(C1, H$\alpha$) | $R$(C2, H$\alpha$) |
|--------|---------------|---------------------|---------------------|
| $G$    | 0             | 11.6 ± 0.3          | 1.38 ± 0.03         |
| $E$    | 0             | 10.1 ± 0.2          | 1.52 ± 0.04         |
| $E_M$  | 0.05          | 9.3 ± 0.2           | 1.67 ± 0.03         |
| $E'_M$ | 0.05          | 9.6 ± 0.1           | 1.77 ± 0.04         |

Table 3: Simultaneous cooling of both backbone carbons by POTENT without (top two rows) and with added Magnevist. In the last row, POTENT$^+$ was used (see Fig. 2c and text). Results are averages and standard deviations of at least 3 single-scan measurements.

| Sample | $d_2/T_1$(H$\alpha$) | $d_3$(s) | C1 factor$^c$ | C2 factor$^c$ |
|--------|-----------------------|----------|---------------|---------------|
| $G$    | 2.2                   | 1.0      | 2.32 ± 0.06   | 2.52 ± 0.09   |
| $E$    | 3.1                   | 1.0      | 2.45 ± 0.08   | 2.29 ± 0.04   |
| $E_M$  | 1.8                   | 1.0      | 2.45 ± 0.08   | 2.49 ± 0.04   |
| $E'_M$ | 2.7                   | 0.5      | 2.61 ± 0.01   | 2.65 ± 0.01   |

$^a$See Fig. 2.

$^b$The ratio between signals with and without cooling.

$^c$With the delay used for POTENT ($d_2 = 2.7$s), POTENT$^+$ cooled C1 and C2 by respective factors of about 2.5 and 2.7.