Journal of Magnetic Resonance 229 (2013) 155–172

Contents lists available at SciVerse ScienceDirect

Journal of Magnetic Resonance

journal homepage: www.elsevier.com/locate/jmr

CEST: From basic principles to applications, challenges and opportunities

Elena Vinogradov [a,*], A. Dean Sherry [a,b], Robert E. Lenkinski [a]

[a] Department of Radiology and Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, TX, USA
[b] Department of Chemistry, University of Texas at Dallas, Dallas, TX, USA

ARTICLE INFO

Article history:
Received 24 September 2012
Revised 18 November 2012
Available online 6 December 2012

Keywords:
Saturation transfer
CEST
MT
NOE
diaCEST
paraCEST
lipoCEST
Spin-lock
Off-resonance $T_{1\rho}$
APT
gagCEST
glycoCEST

ABSTRACT

Chemical Exchange Saturation Transfer (CEST) offers a new type of contrast for MRI that is molecule specific. In this approach, a slowly exchanging NMR active nucleus, typically a proton, possessing a chemical shift distinct from water is selectively saturated and the saturated spin is transferred to the bulk water via chemical exchange. Many molecules can act as CEST agents, both naturally occurring endogenous molecules and new types of exogenous agents. A large variety of molecules have been demonstrated as potential agents, including small diamagnetic molecules, complexes of paramagnetic ions, endogenous macromolecules, dendrimers and liposomes. In this review we described the basic principles of the CEST experiment, with emphasis on the similarity to earlier saturation transfer experiments described in the literature. Interest in quantitative CEST has also resulted in the development of new exchange-sensitive detection schemes. Some emerging clinical applications of CEST are described and the challenges and opportunities associated with translation of these methods to the clinical environment are discussed.

1. Introduction

Contrast agents are widely used in MRI for signal enhancement. They allow better differentiation between healthy and diseased tissue, as well as better visualization of different structures. Most of the agents in clinical use today are complexes of Gd^{3+} ions that shorten the relaxation time of the free water protons. These agents are not selective, and distribute uniformly throughout the extracellular space after intravenous injection [1].

In addition to relaxation-based contrast, MRI offers a variety of contrast techniques based on the intrinsic properties of tissue, such as coupling to neighboring nuclei, chemical exchange or flow. Magnetization Transfer (MT) contrast, a technique utilizing Saturation Transfer (ST), uses a long, weak, off-resonance RF pulse to saturate a broad water signal that lies beneath a sharper bulk water signal in many tissues [2]. In early 1990s, Balaban and co-workers introduced Chemical Exchange Saturation Transfer (CEST) as a new class of contrast agents for MRI. In this approach, a slowly exchanging group possessing a chemical shift distinct from water is selectively saturated and the saturation is transferred to the bulk water via chemical exchange [3]. The method has gained and continues gaining popularity due to several attractive features. CEST allows the operator to switch the image contrast "on" and "off" via an RF pre-saturation pulse. As chemical exchange can be quite sensitive to the environment of a contrast agent, the CEST effect can be used to image important physiological parameters, such as pH [4–6] and metabolite levels [7–9]. Among numerous innovations and applications are multi-color CEST [10] and an artificial CEST gene reporter [11]. The technique can be applied for variety of ailments and metabolic disorders, such as cancer [12,13], ischemia [14], cartilage degeneration [15], just to name a few.

A number of excellent reviews have been written on CEST methods and agents [16–23]. In this review we have tried to put an increased emphasis on the physics behind CEST experiment: to put it in the context of other saturation experiments, to emphasize similarities between exchange and cross relaxation, and to highlight identity with the off-resonance spin-lock experiments. We are covering in greater depth some of the novel, alternative exchange detection techniques. In addition, we have highlighted some of the emerging clinical applications of CEST and the challenges and opportunities associated with the translation to the clinic.

2. CEST 101

2.1. Mechanism

The basic principle of CEST is straightforward, and schematically shown in Fig. 1. It relies on the presence of a solute protons resonating at a frequency different from water and engaged in

* Corresponding author.
E-mail address: elena.vinogradov@utsouthwestern.edu (E. Vinogradov).

1090-7807/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jmr.2012.11.024

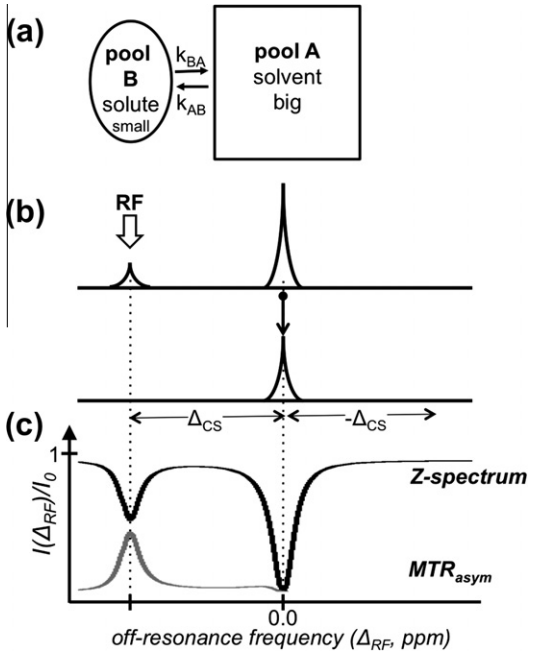

Fig. 1. Schematic of CEST experiment. (a) Pool A (solvent) is in exchange with pool B (solute). (b) Pools A and B have distinct chemical shifts, with the difference of Δ_{CS}. RF is applied on-resonance with pool B resulting in saturation transfer and signal decrease of pool A. (c) Z-spectrum: normalized water intensity (I/I_0) vs off-resonance frequency of the saturating RF (Δ_{RF}). Water resonance is assigned 0 ppm value. MTR_{asym}: Z-spectrum asymmetry vs RF off-resonance value.

the chemical exchange process, where a proton physically moves from the solute to solvent and back. The exchanging proton pool is saturated via selective RF irradiation at the solute frequency. The saturation is transferred to the bulk water via chemical exchange thus decreasing the magnetization (and the signal) of the water (Fig. 1b). The solute is typically in a very low concentration (μM to mM range) and is not observable in the standard MR signal. However, continuous transfer of saturation serves as amplification, allowing to indirectly observe solutes at low concentration [16]. For CEST to be successful the system needs to be in the slow to intermediate regime on the NMR scale, i.e. the chemical shift difference between solvent and solute (Δ_{CS}) has to be greater (or equal) than the exchange rate ($k_{ex} = k_{AB} + k_{BA}$): $k_{ex} < \Delta_{CS}$. Notice that the definition of "slow" or "fast" is relative here, what is important is the ratio. For saturation transfer to work, the two spin pools need to be distinguishable, and we need to be able to modulate one of the lines with a minimum effect on the other; i.e., there should be separate spectral lines. This is possible in the slow exchange regime only. Hence, CEST is the most efficient in the slow exchange regime, although experiments were reported in the intermediate exchange regime as well.

For the CEST analysis, the most common metric used is the Magnetization Transfer asymmetry (MTR_{asym}), defined as:

$$MTR_{asym}(\Delta_{CS}) = \frac{I(-\Delta_{CS}) - I(\Delta_{CS})}{I_0} \quad (1)$$

where $I(\Delta_{cs})$ and $I(-\Delta_{cs})$ are signal intensities acquired with RF irradiation applied on-resonance with the exchanging pool and at the frequency symmetric around water, and I_0 is the reference signal intensity acquired without RF pre-saturation. In the following, $MTR_{asym}(\Delta_{cs})$ and "CEST effect" are used somewhat interchangeably.

Often the normalized water signal intensity is monitored vs the frequency of the off-resonance saturation: the so-called Z-spectrum [24], as illustrated in Fig. 1c.

2.2. Types of CEST agents

In the last decade a great variety of molecules were proposed to serve as CEST agents. To the best of our knowledge, at least two classifications were suggested. First relies on the nature of the solute: diamagnetic CEST (diaCEST) and paramagnetic CEST (paraCEST). We would add to it exogenous CEST agents using liposomes (lipoCEST) and nanoparticles (molecules containing hundreds of exchanging groups). Finally, there is CEST using hyperpolarized gases (hyperCEST). An alternative, more organized, classification was recently introduced [17] based on the type of exchanging species: proton exchange (endogenous and exogenous diaCEST, some paraCEST), molecular exchange (paraCEST and hyperCEST) and compartmental (liposomes and hyperCEST).

The chemical types of proton exchange groups that can act as diaCEST agents are largely confined to –NH, $–NH_2$, or –OH groups. [3,4,8,10–12,14,15,25–28] (Fig. 2a). These groups could be endogenous (i.e., present in tissue) or exogenous (i.e., introduced as a contrast agent). The endogenous contrast utilizes exchanging protons in the fast tumbling molecules, protein backbones, side chains and small peptides present in tissue (e.g. in cells or matrix). As we will discuss in the following, the groups need to have T_2 relaxation long enough to be distinguished from the broad macromolecular component in tissue. The chemical shift of the diaCEST agents is typically within 5 ppm from water. Using the slow-to-intermediate exchange condition, $\Delta_{CS} > k_{ex}$, as a rough boundary condition for CEST effectiveness, one would expect CEST to arise only for proton sites that have an exchange rates of the order of $\sim 2 \times 10^3\ s^{-1}$ or slower. This range happens to encompass the exchange lifetimes observed for many types of –NH exchange groups and, occasionally, some –OH exchange groups. However, the small chemical shift differences, Δ_{CS}, of diaCEST agents are their primary disadvantage, since saturation of such exchange groups usually results in partial saturation of the bulk water protons as well ([29], direct saturation effect). As will be mathematically shown later, CEST contrast increases with agent concentration or exchange rate. Moreover, the exchange rate dictates minimum concentration per exchanging group that could be detected. One way to increase CEST effect is to increase the number of the exchanging groups per agent. Thus, dendrimers and polymers containing multiple exchanging diaCEST proton groups were utilized as CEST agents [30,31].

The majority of diaCEST agents to date involve endogenous proton exchange types. These are attractive because nothing is injected (FDA approval not required) and CEST imaging can be performed using modifications of the existing pulse programs. Hence, diaCEST agents have a great potential to reach clinical applications in the near future. In Section 5, we will discuss some of the endogenous diaCEST methods in greater detail. In addition, there are several diaCEST applications involving exogenous injection of the agent, such as glucose imaging (glucoCEST [32,33]) and pH imaging using iopamidol [34].

As discussed above, the diaCEST effect is limited by the small chemical shift differences and, hence, relatively low exchange rates required to stay within slow-to-intermediate regime. In early 2000, exogenous paramagnetic lanthanide (III) complexes that exhibit large hyperfine shifts (on the order of 50–700 ppm) were introduced as CEST agents: paraCEST agents [5,7,35,36], Fig. 2b. The highly shifted bound water protons or the ligand's amide or hydroxyl protons can be selectively pre-saturated, and the saturation can then be transferred to free water via chemical exchange. There are a number of potential advantages of these agents compared to diaCEST. They exhibit a wide range of exchange rates (from μs to ms) while remaining in the slow-to-intermediate exchange regime on the NMR time scale [20,21,23,36–38]. The fast exchange rates should theoretically allow detection of much lower concentrations