Predicting CEST properties of Polypeptides

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INTRODUCTION

Chemical Exchange Saturation Transfer (CEST) agents are a new type of non-metallic contrast agents in which contrast depends on the chemical exchange rate of the agent protons with water[1-5]. Recently, Sherry, Aime and coworkers produced a series of small paramagnetic agents with custom tailored exchange rates to produce maximal contrast[4-6]. Polypeptides have shown to produce large contrast[2] in the micromolar range, and in principle could also be tailored to maximize the exchange rate. Previous studies of protein folding have produced equations which predict the exchange rates in proteins [7-9]. In this study, we test these equations predicting exchange rates for use in the design of optimal CEST contrast agents and verify the calculations using a set of commercially available polypeptides.

METHODS

Theory: The HX (X = O or N) exchange rates were predicted based on the neighbors to the left and right using the combined base-catalyzed and acid-catalyzed equation produced by Englander and coworkers[7]:

$$k_{ex} = k_{A,ref}(A_l \times A_r)[H+] + k_{B,ref}(B_l \times B_r)[OH-] + k_{W,ref}(B_l \times B_r)$$

where: $k_{A,ref}$, $k_{B,ref}$, and $k_{W,ref}$ are the acid, base and water rate constants for the appropriate reference peptide, A_l, A_r are the specific acid rate factors for the neighboring peptides to the left and right, and B_l, B_r are the specific base factors for the neighbors. This equation can be used for both backbone amide protons (HN) and sidechain exchangeable groups (NH_2, OH, SH), by adjusting the reference rates. In addition, this can be adjusted for temperature as well, by adjusting each rate according to the expression:[8]

$$k_{A,B,W}(T) = k_{A,B,W}(293)\exp(-Ea[1/T-1/293]/R)$$

where Ea is the activation energy. The MT contrast increases exponentially with chemical exchange rates as can be seen by the expression in Snoussi et al. [3], provided a sufficiently strong saturation field such that the exchangeable group is fully saturated is assumed. This saturation strength will increase for the higher exchange rates.

Materials: Poly-L-Lys (PLL, 30 kD), Poly-L-Arg (35 kDa), poly-L-Glu (17.5 kD) and the hetero-polypeptides Poly (Glu-Lys-Lys-Lys-Lys)$_n$ (Mw 200 kD) and Poly (Trp-Lys-Lys-Lys-Lys)$_n$ (Mw 38 kD) were purchased from Sigma and diluted to the equivalent concentration of amino acids, which for 35kD polypeptides would be 100uM in pH 7.4 0.01M PBS buffer. *NMR Experiments:* The experiments were all run on an 11.7T Bruker Avance system using a triple axis gradient NMR probe. All experiments were performed at 310K. *Pulse Sequences:* Z spectra (relative water saturation as a function of saturation frequency) were taken on these compounds with the saturation transfer sequence consistomg of a saturation pulse with variable offset, power and time followed by a 10 μs $\pi/2$ pulse,-Grad-20μs π pulse-Grad- acquire. The hard 10μs and 20μs pulses were centered on the water resonance.

RESULTS AND DISCUSSION

We recently showed that enhancements by factors as large as 500,000 can be achieved for PLL, in which a large concentration of rapidly exchangeable amide protons (k_{ex} values of about 300 Hz) is present per molecular weight unit (e.g. 4.78 amide protons/kD for PLL) [2]. In order to investigate whether these rates could be predicted, we looked at the Z-spectra for a series of homopeptides and heteropeptides and tested how well the equations above could predict the contrast. The table below shows our results (at the optimal power level) vs. predicted exchange rate, which correlate quite well, with, as mentioned above, the contrast increasing exponentially with exchange rate. For Poly-L-Arg, which has the two fastest exchangeable groups, the concentration of the agent is too high and as a result significant back exchange occurs reducing the contrast. However, reducing the concentration of the agent would reduce this back exchange. The contrast was measured at an optimal saturation power for each agent. In addition, for the heteropeptides there are multiple exchange rates predicted, which we averaged in the table. Several design principles are evident, for example while Poly-L-glutamate produced only 0.1% contrast, addition of Lys or Arg residues, which are positively charged increase this rate substantially. In addition to the charge effects, certain residues such as tryptophan sterically hinder proton exchange as can be seen in the table and as a result lower the contrast. Protection factors from protein folding are also important to consider. Additionally, we are able to see a 0.5% contrast with 500 nM of 35 kD Poly-L-Arg saturating the HδN group and further improvements are feasible!

Peptide	K_{ex} (Hz)[a]	Contrast(%)[b]
Poly-L-Arg (HδN)	3335	48.4
Poly-L-Arg (HαN)	500	27.4
Poly-L-Lys	300	14.7
Poly-L-Trp:Lys(1:4)	241	4
Poly-L-Glu:Lys(1:4)	233	3.3
Poly-L-Glu	54	0.1

a. Predicted using above equations
b. Determined experimentally

CONCLUSIONS

We have carried out a study to determine whether it is feasible to predict exchange rates and thus contrast in a series of peptides using relatively simple equations. Using these equations we are able to predict the proper trend of contrast. In addition, we introduce several new agents one of whose exchange rates is fast enough to produce nanomolar contrast. These principles should prove useful for future in vivo studies designing and employing CEST agents.

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