[1]H NMR Diffusion Spectroscopy of Oxymyoglobin in Perfused Rat Heart

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Introduction

Myoglobin serves as an oxygen storage protein and also plays a role of facilitated oxygen diffusion. The contrary predictions of the facilitated diffusion hypothesis between the oxygen-carrying capacity of Mb and the low solubility of free oxygen in the cell have been argued for years (1-3). Therefore, an accurate determination of myoglobin diffusion in the cell has become a critical point to the facilitated diffusion model. The visible ^{1}H NMR signal from oxymyoglobin in myocardial tissue provides an opportunity to examine the cellular diffusion property. This work is to give confident values which can test the hypothesis of facilitated oxygen diffusion.

Materials and methods

Male Sprague-Dawleys rats (350-400 g) were anesthetized by an intraperitoneal injection of pentobarbital sodium and heparinized by injection into the femoral vein. The heart was isolated and perfused using a modified Langendorff technique. The perfused heart was placed in a 20-mm NMR tube and inserted into the 400 MHz NMR spectrometer. A modified pulse-field gradient stimulated echo sequence was used to measure the ^{1}H signal attenuation caused by diffusion under KCl arrest condition.

Results

Fig.1 displays an example of the stacked spectra for signal attenuations of oxyMb Val-E11 γ-methyl at -2.9 ppm. This recent experiment shows that myoglobin diffuses isotropically in the cell at 22 ^{o}C and the average of self-diffusion coefficient of Mb is 4.24×10^{-7} cm^2/s, which is close to the calculated value by us of the rotational correlation time (4) and Stokes-Einstein equation for rotational diffusion (Table 1). Moreover, our preliminary data collected at 35 ^{o}C give the self-diffusion coefficient 7.62×10^{-7} cm^2/s, which reveals that the often used value in earlier calculations of the magnitude of facilitated diffusion was not overestimated.

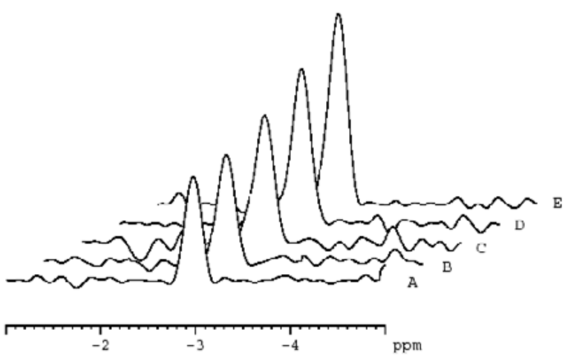

Fig.1 . ^{1}H NMR 400 MHz diffusion-weighted spectra from the perfused rat heart under KCl arrest condition at 22 ^{o}C. The signal attenuation depends upon the applied gradient strength: (A) 84.2 gauss/cm, (B) 74.8 gauss/cm, (C) 56.1 gauss/cm, (D) 37.4 gauss/cm, and (E) 4.7 gauss/cm

Table 1 Translational diffusion coefficients of Mb in muscles

Sample	*Diffusion coef.* ($\times 10^7$ cm^2/s)	*r*
Rat myocardium at 22 ^{o}C		
NMR diffusion experiment		
in the gradient X direction	4.12±0.40	0.986
in the gradient Y direction	4.51±0.38	0.990
in the gradient Z direction	4.08±0.19	0.997
Value calculated from rotational Correlation time 25 ^{o}C (4)	5.00	

Values were calculated from best curve fit of data points in Fig. 4. The radius of myoglobin is 17.5 A. Error is ± standard error. *r* is the linear regression correlation coefficient.

Summary

The matched diffusion coefficients of Mb from direct and indirect (rotational correlation time) methods indicates the mobility of Mb is just a little slower in tissue than in solution. Furthermore, raising the temperature from 22 ^{o}C to 35 ^{o}C promotes the self-diffusion coefficient about factor 1.8. That means the intracellular viscosity changes dramatically as increasing temperature to body temperature. Further temperature-dependent experiments will provide an evidence to say if this oxygen carrying protein also plays an important role of oxygen transporter or not.

References

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