In vivo ^{31}P NMR diffusion spectroscopy of phosphocreatine and ATP in rat skeletal muscle

Robin A. de Graaf, Arnaud van Kranenburg, Klaas Nicolay

Department of *in vivo* NMR, Utrecht University, The Netherlands

Introduction

Pulsed field gradient NMR techniques enable the accurate measurement of molecular displacement through diffusion [1]. The measurement of diffusion coefficients *in vivo* is complicated by a variety of factors, including the presence of diffusion barriers like cellular membranes. However, in turn these barriers and their effect on the measured (apparent) diffusion coefficients (ADC) may provide important information on cellular compartment sizes. The ^{31}P NMR based measurement of the diffusion coefficient of the metabolically important ATP molecule is complicated by the short T_2 relaxation time (~ 30 ms), extensive J evolution and the low gyromagnetic ratio and sensitivity of the ^{31}P nucleus. Here we present a sensitive method based on adiabatic RF pulses to measure the diffusion of ATP on rat skeletal muscle *in vivo*, such that the important phosphocreatine (PCr)/ATP energy buffering system can be studied in more detail.

Materials and methods

All experiments were performed on a Varian/SIS Co spectrometer interfaced to a 4.7T Oxford magnet equipped with a 220 mT/m gradient insert (risetime = 300 µs). A double-turn surface coil (Ø 2.0 cm) tuned to ^{31}P (80.984 MHz) was used for transmission and reception. The surface coil was positioned around the hindleg muscle of anaesthetized, mechanically ventilated Wistar rats. The adiabatic pulse sequence (Fig. 1) generates a selective stimulated echo (TR = 5 sec, TE = 20 ms, NEX = 128), such that significant diffusion times can be obtained with minimal T_2 weighting. The effects of J evolution were eliminated by selective refocusing of α- and γ-ATP resonances with SSAP, an adiabatic analogue of a jump-return pulse [2]. Diffusion weighting was achieved with a pair of trapezoidal shaped magnetic field gradients (δ = 9.3 ms, ε = 0.3 ms). In order to detect diffusion anisotropy, unidirectional diffusion sensitization was used (six b values between 100 and 4000 s.mm^{-2} for all TM values). Diffusion restriction was investigated by varying TM between 37.5 and 1200 ms.

Results

Fig. 2 shows two typical *in vivo* ^{31}P NMR diffusion datasets with unidirectional diffusion weighting approximately parallel (left) and perpendicular (right) to the main muscle fiber direction (TM = 75 ms). Clear diffusion anisotropy is observed for PCr, but also for both ATP resonances, indicating the feasability of *in vivo* ATP diffusion measurements with the sequence of Fig. 1. Three separate, orthogonal diffusion sensitized experiments were performed. To eliminate any rotational dependence of the diffusion measurement, the calculated ADC values were averaged, which corresponds to the trace of the diffusion tensor. Fig. 3 shows the TM dependence of the average diffusion coefficient of the three observed ^{31}P NMR resonances, indicating diffusion restriction for both PCr and ATP. Furthermore, the free diffusion coefficient of both PCr and ATP, as estimated by extrapolating TM to zero, closely approximates that in an isotropic solution at the same temperature, indicating that *in vivo* metabolite diffusion is not fundamentally different [3, 4].

Conclusions

Here we have presented a new, selective, stimulated echo sequence based on adiabatic RF pulses, which is capable of measuring the diffusion of ATP *in vivo*. The refocusing of ATP J evolution and the high sensitivity of combining adiabatic RF pulses with surface coil detection, allows a quantitative evaluation of diffusion anisotropy and restriction of ATP. Such measurements provide important, basic information on the regulatory characteristics of mammalian energy metabolism.

References

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Figure 1

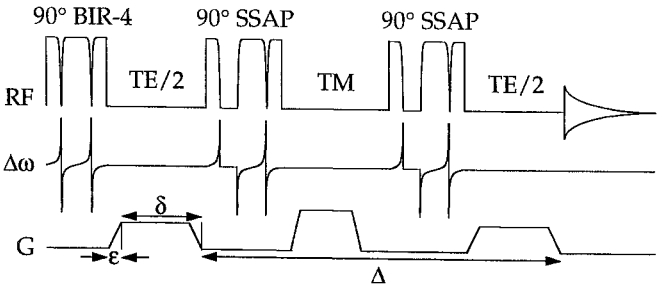

Figure 2

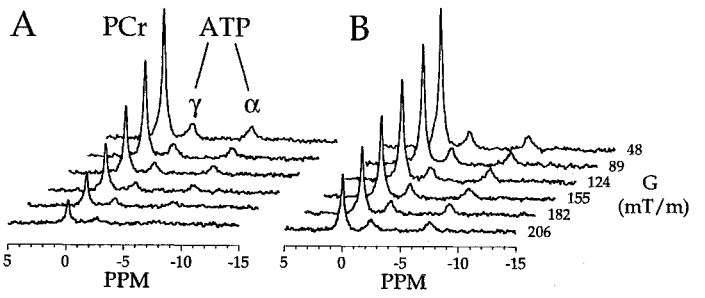

Figure 3