

Unveiling Extracellular Inorganic Phosphate Signals from Blood in Human Cardiac ^{31}P NMR Spectra

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ABSTRACT

^{31}P NMR spectra of the human heart are usually contaminated by signals that originate from blood. The main blood signals are 2,3-diphosphoglycerate (2,3-DPG), which overlap and sometimes obscure the signal of myocardial inorganic phosphate used to calculate intracellular pH and to monitor metabolic changes in the heart. In this work we demonstrate, first, that even without proton decoupling the resolution of such spectra can be high enough to evaluate intracellular inorganic phosphate of myocardium in about 70% of the spectra and, second, that extracellular inorganic phosphate from blood contributes a signal in the chemical shift region of the 2-phosphate signal of 2,3-DPG.

Key Words: Blood; Heart; Inorganic phosphate; ^{31}P NMR spectroscopy; pH

INTRODUCTION

^{31}P in vivo NMR spectra taken from human heart usually show not only signals from myocardium but also from blood. The main blood signals are 2,3-diphosphoglycerate (2,3-DPG) and ATP. Although 2,3-DPG is easily accessible, the ATP blood signals add to the ATP sig-

nals from myocardium, thus disturbing the correct quantification of myocardial ATP. If the 2,3-DPG/ATP ratio of blood is known, a correction of the ATP signals can be carried out to determine the integral of the ATP signals from myocardium (1–4). Usually, 2,3-DPG gives broad signals whereby the signal of the 2-phosphate of 2,3-DPG (2-DPG) overlaps and sometimes obscures the

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signal of myocardial intracellular inorganic phosphate (P_i^{in}), used for the calculation of pH of P_i^{in} (pH^{in}) (5,6). Hence, improved localization techniques confining the spectrum to myocardium, proton decoupling, and special editing techniques are applied to ensure quantification of P_i^{in} and calculation of pH^{in} (1–3,7–11). Nevertheless, we show that even in coupled ^{31}P in vivo NMR, spectral resolution can be high enough to evaluate P_i^{in} .

As a very annoying observation in well-resolved coupled ^{31}P NMR spectra from human heart, the doublet of 2-DPG shows different amplitudes (Fig. 1a). This suggests that one or even several signals are present close to or underneath the right peak of the doublet. If there are other signals, it is not possible to use the difference in the integrals of the peaks at 5.5 and 6.3 ppm as the integral of P_i^{in} , a method that has been used as a semi-quantitative approach (12,13). We demonstrate that in coupled ^{31}P in vivo NMR spectra, a signal of extracellular inorganic phosphate (P_i^{ex}) of blood (10,14) is present that can be evaluated using VARPRO (15) time domain fitting and prior knowledge.

MATERIALS AND METHODS

Measurements were performed on a Magnetom SP 63 Helicon whole body imager (Siemens AG, Erlangen, Germany) operating at 1.5 T with $^1\text{H}/^{31}\text{P}$ Larmor frequencies of 63.6/25.7 MHz. A 10-cm-diameter $^1\text{H}/^{31}\text{P}$ double resonant surface coil was used for transmission and reception. By using a two-dimensional–CSI sequence (improved version of that given in Ref. 2), we recorded ^{31}P spectra from 8×16 voxels with 41-ml volume. Only one voxel of each subject's data set was chosen. This contained the apical part of the interventricular septum and the adjacent anterior part of the left ventricular free wall (2). Due to voxel size, every spectrum showed an inevitable contamination of blood. The voxel size and shape were chosen as a compromise between reduction of blood contamination and reasonable signal-to-noise ratio. The sequence was electrocardiogram triggered, and 16 acquisitions were recorded, resulting in a measuring time of about 30 min.

Nine male control subjects were examined, two of them twice and one three times, resulting in 13 spectra (Fig. 1a). The spectra were fitted with VARPRO (15) in the time domain, using Gaussian model functions. In addition to frequency, amplitude, and linewidth of every line, the constant and the linear phase of the spectra were evaluated by VARPRO.

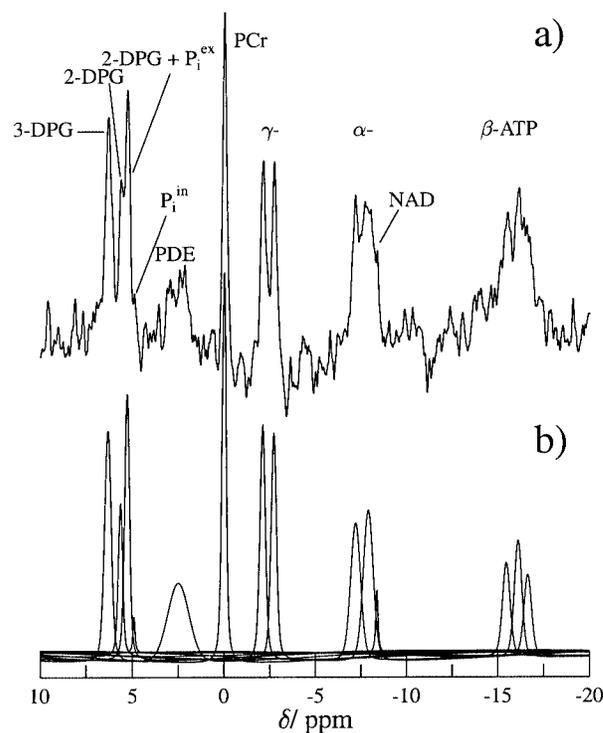


Figure 1. (a) ^{31}P two-dimensional–CSI NMR spectrum from the heart of a male control subject obtained without proton decoupling within 28 min from a 41-ml voxel. Note the different intensities of the “doublet” of 2-DPG. For assignment, see text. (b) The fitted individual model functions of the spectrum. The fit was done with the specifications and the prior knowledge of fit 0, that is, without P_i^{ex} . The constant and the linear phase were calculated by VARPRO. NAD, nicotinamide adenine dinucleotide.

The common specification and prior knowledge for the VARPRO analysis were as follows (compare Fig. 1b):

1. Seven signals were fitted to the multiplets of ATP (two signals for α - and γ -ATP each, three signals for β -ATP). The prior knowledge included equal linewidth within each multiplet, but the linewidths of different multiplets are not linked.
2. One signal was fitted to phosphocreatine (PCr), which was used as chemical shift reference ($\delta(\text{PCr}) = 0.0$ ppm).
3. One signal was fitted to the nicotinamide adenine dinucleotide signal. If the fit with nicotinamide adenine dinucleotide did not converge, this signal was omitted.



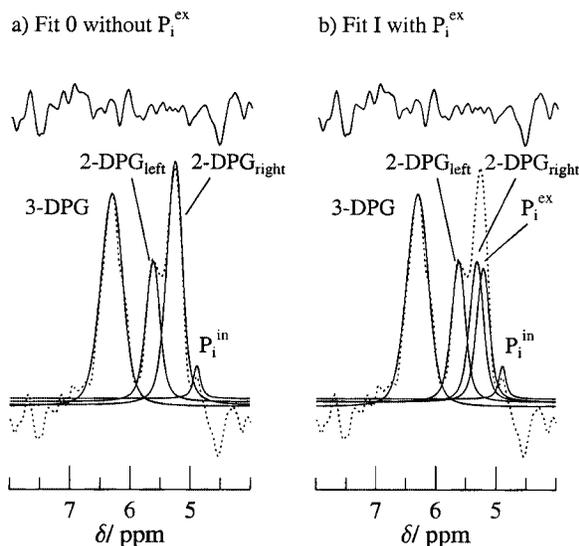


Figure 2. Shown is the region between 4 and 8 ppm of the spectrum in Fig. 1 for fit 0 (a) and fit I (b), respectively. Given are the experimental spectrum (dotted line), the individual model functions of the VARPRO fit (solid line), and the residual (difference between the measured spectrum and the fitted model; solid line above model and fit). In a the intensities of the right-handed signal of 2-DPG and P_i^{ex} are completely covered by 2-DPG_{right}. In b the two fitted signals of P_i^{ex} and 2-DPG_{right} share this intensity. Furthermore, the two signals for 2-DPG form a real doublet according to the physical requirements.

4. Two signals were fitted to the phosphodiester region. If the fit with two phosphodiester region signals did not converge, as in the example shown in Fig. 1, only one signal was fitted to the phosphodiester region.
5. One signal was fitted to the 3-phosphate of 2,3-DPG (3-DPG). The triplet that further splits into doublets (5,10) was not resolved well enough for fitting.
6. For 2-DPG and the inorganic phosphates P_i^{in} and P_i^{ex} , two different fit approaches (fit 0 and fit I) were used:
 - a. Fit 0: One signal for P_i^{in} , none for P_i^{ex} , and no prior knowledge for the two signals of 2-DPG (Fig. 2a).
 - b. Fit I: Two signals for P_i^{in} and P_i^{ex} and equal linewidth and equal intensity, that is, a real doublet as prior knowledge for the signals of 2-DPG (Fig. 2b). No further prior knowledge

was applied and especially no frequencies were fixed.

RESULTS

Figure 1 shows a coupled ^{31}P NMR human cardiac spectrum and the corresponding VARPRO fit results. Figure 2 shows the 4 to 8 ppm section of Fig. 1, the model functions, and the residual of fit 0 and fit I. Table 1 provides the resulting mean values and SDs for 2,3-DPG and $P_i^{\text{in/ex}}$. Irrespective of the fit method, P_i^{in} gives a pH^{in} of 7.1 and has an integral relative to PCr of 0.17. The chemical shift of P_i^{ex} corresponds to a pH^{ex} of 7.4 and its relative integral is 0.30. Fit 0 converged in 12 of the 13 spectra of eight of nine control subjects. In eight spectra from six control subjects it was possible to fit P_i^{in} (62% of the cases). In other words, four spectra of three persons could not be fitted with fit 0.

Fit I converged in 7 (five control subjects) of 13 spectra, which means in 54% of the cases the determination of P_i^{ex} was successful. In all but one of them, also the fit of P_i^{in} converged. The fit results using fit 0 for the seven spectra that could also be fitted with fit I are shown as Fit 0* in Table 1. The fit with P_i^{in} converged only in five of these seven spectra, which means altogether it was possible to fit P_i^{in} in 9 of 13 spectra or in 69% of the cases. The fit results of all signals not shown in Table 1 did not change significantly with different fits. It is noteworthy that the fit results of P_i^{in} were not affected by including P_i^{ex} : The chemical shift did not change and the differences in the intensity were below the error values given by the Cramér-Rao values of the VARPRO fit.

DISCUSSION

Altogether, evaluation of myocardial P_i^{in} was successful in 9 of 13 spectra obtained without ^1H decoupling. In the other four cases, either the P_i^{in} intensity was too small or unfortunate noise signals hampered fitting. Nevertheless, the detrimental effects of overlapping signals from blood contamination could be reduced far enough to evaluate P_i^{in} in 69% of the spectra. The chemical shift and the derived pH are in agreement with values taken from literature (1,2). Hence, there is no need to use an uncertain subtraction method to obtain the intensity of P_i^{in} (12,13), besides the fact that—in addition—it does not provide the pH^{in} .

This high resolution, obtained even without proton decoupling, demands identification and quantification of



Table 1*Fit Results of VARPRO*

	Fit 0 Without P_i^{ex}	Fit 0* Without P_i^{ex}	Fit I With P_i^{ex}
P_i^{ex}			
n			7
I/PCr · 100			30.28 ± 9.86
δ /ppm			5.24 ± 0.01
pH ^{ex}			7.41 ± 0.02
P_i^{in}			
n	8	5	6
I/PCr · 100	15.06 ± 8.14	16.51 ± 9.94	17.19 ± 8.95
δ /ppm	4.88 ± 0.05	4.89 ± 0.05	4.90 ± 0.05
pH ⁱⁿ	7.07 ± 0.04	7.08 ± 0.04	7.08 ± 0.04
2-DPG			
n	12	7	7
I/PCr · 100	86.24 ± 20.44	90.37 ± 25.22	59.59 ± 22.60
δ /ppm	5.46 ± 0.03	5.45 ± 0.01	5.49 ± 0.01
2J /Hz	8.38 ± 1.54	8.24 ± 0.56	6.63 ± 0.98
3-DPG			
n	12	7	7
I/PCr · 100	74.95 ± 16.76	77.72 ± 15.79	77.50 ± 17.16
δ /ppm	6.32 ± 0.02	6.31 ± 0.03	6.31 ± 0.03

Values are means ± SD, except n . The chemical shift of 2-DPG is the mean of the two signals. The values in column “Fit 0*” are the spectra fitted with fit 0 that could also be successfully fitted with fit I. n , number of fittable spectra; I/PCr · 100, intensities relative to PCr; δ /ppm, chemical shift; 2J , coupling constant of the 2-DPG doublet.

other usually hidden signals. A closer examination of spectra (e.g., as depicted in Fig. 1) suggests the presence of such a signal that we identify as originating from the P_i^{ex} of blood (shown in rabbit blood [10] and in cardiac spectra taken from sheep [14]). The fitting of this signal requires the prior knowledge of fit I, which is based on the fact that the 2-DPG gives a doublet with equal line-width and equal intensity. This constricting prior knowledge is necessary, because the data analysis works close to the edge of detectability and resolution of the spectra. A critical analysis of the obtained results mostly confirms the qualification to use this prior knowledge: The fitted chemical shifts were not affected by the different fit approaches, suggesting that the P_i^{ex} signal did not interfere with the correct positioning of 2,3-DPG and P_i^{in} . As a remarkable fact, the value for the chemical shift of P_i^{ex} —evaluated without any prior knowledge for this peak—corresponds to a pH of 7.41, which is in excellent agreement with literature data on blood pH (9,16). The value of the coupling constant of 2-DPG found in vivo in humans is smaller than in vitro values given in the literature (10 Hz [5] and 9.0 Hz [10], respectively). This difference may be caused by different chemical surrounding, pH,

temperature, and so on compared with the in vivo situation.

Concerning the fitted intensities, the residual of fit 0 and fit I (Fig. 2, a and b), which are nearly identical, allows two conclusions. First, P_i^{ex} is almost completely covered by the right line of 2-DPG in fit 0, because there is no remaining signal in the residuum (Fig. 2a). This is further confirmed by the decline in the amount of 2-DPG from fit 0 to fit I (Table 1). Second, the P_i^{ex} signal, fitted in fit I, is a real signal, because there is no “negative” signal in the residuum that would suggest VARPRO fitted a purely artificial signal into the noisy baseline (Fig. 2b). A further argument for a real signal is the accordance of the obtained pH^{ex} with values found in literature (9,16). It is noteworthy that the intensity of P_i^{ex} is about two times the intensity of P_i^{in} in our measurements, which strongly emphasizes the necessity of its consideration in semiquantitative methods (12,13).

Unfortunately, the resolution was not good enough to fit the triplet of doublets of 3-DPG, although almost all spectra show a slightly visible separation. Hence, this multiplet was fitted by just one signal. A further problem of fitting 3-DPG is the presence of phosphomonoesters



(2) in the region of about 6.8 ppm, which were not fitted due to insufficient knowledge about the metabolites contributing to the signal. The signal integral of 3-DPG is about 30% greater than the integral of 2-DPG, which most likely arises from different relaxation rates.

In conclusion, P_i^{in} of myocardium can be evaluated in about 70% of the cases even in coupled ^{31}P NMR in vivo spectra taken from human heart. The normally hidden signal of P_i^{ex} of blood can be unveiled using prior knowledge and time domain fitting. The detection of P_i^{ex} extends our knowledge about human cardiac ^{31}P NMR spectroscopic investigations and may improve quantitative analysis.

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