Measuring tissue pH heterogeneity by ^{31}P NMR spectroscopy

N. W. Lutz[1], Y. LeFur[1], and P. J. Cozzone[1]
[1]Dept. of Medicine La Timone, Marseille, France

Introduction

Pathological processes are frequently induce variations in tissue pH. Thus, reliable measurement of intra and extracellullar pH (pH$_i$, pH$_e$) should be very useful for the characterization of tissue metabolism. Ideally, the pH measurement method of choice should be able to *simultaneously* determine pH$_i$ and pH$_e$ *in vivo*. ^{31}P MRS-based methods have been introduced many years ago for pH$_i$ (chemical shift, δ, of endogenous P$_i$ [1]), and for pH$_e$ (δ of exogenous 3-aminopropylphosphonate, APP [2]), notably for application in tumor animal models [3,4]. However, the consequences of pH heterogeneity have not been considered in these pH calculations. We suggest here three ^{31}P MRS-based pH calculation methods that take into account the characteristics of the pH distribution under consideration.

Methods

Tumors were induced in the thighs of nude mice by subcutaneous inoculation of 1-2x10^{6} ras-transformed CCL39 hamster fibroblasts. Four weeks post inoculation tumors were subjected to ^{1}H MRI and ^{31}P MRS under anesthesia using a Biospec 4.7 T imager/spectrometer, following i.p. injection of 1.0 ml of a 245 mM solution of APP at pH 7.4. Following reference images covering the entire tumor, ^{31}P MRS spectra were acquired using a surface coil and 5 to 7 outer-volume saturation bands for localization (TR = 8s, SW = 80 ppm, NS = 500-640). For phantom studies, aqueous APP solutions were adjusted to pH 6.5 or 7.4, and were subjected to imaging and ^{31}P MRS spectroscopy. Images and spectra were processed using our IDL-based DISPIMAG and CSIAPO software, respectively, as well as Topspin software from Bruker.

Results and Discussion

In most CCL39 tumors, the APP ^{31}P MRS resonance was broad, asymmetrically shaped, and/or exhibited more than one maximum (Fig. 1, top; spectrum processed with Lorentzian-Gaussian line shape transformation for resolution enhancement). This indicates strong pH$_e$ heterogeneity, which is confirmed by the pH curves based on this APP signal (Fig. 1, bottom); the black (pink) curve represents the pH distribution before (after) correction for non-linearity between δ and pH. In some tumors, two or more pH peaks are extremely overlapped (Fig. 2, "C34"). The currently established technique for obtaining tissue pH from ^{31}P NMR spectra consists of (i) determining the pH value corresponding to the highest point of the calculated pH curve, and (ii) presenting this value as "the" intra or extracellular tissue pH. However, it is obvious from Figs. 1 and 2 that the maximum pH$_e$ value of ca. 6.5 and 7.0, respectively, does not correctly represent the average pH$_e$ of the tumor in question.

Figure 1

Figure 2

CCL39 ("C34")
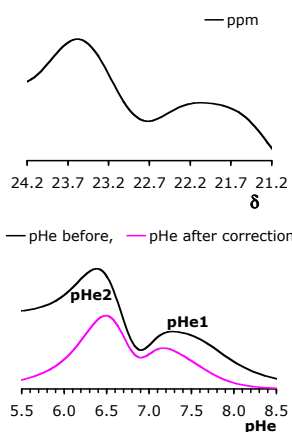
In fact, the right part of the first pH curve (Fig. 1, bottom) indicates the presence of significant tumor regions with pH$_e$ > 6.5. Similarly, the left part of the second pH curve (Fig. 2) indicates the presence of major tumor regions with pH$_e$ < 7.0. Thus, to obtain a pH$_e$ value that is representative of the entire tumor volume observed, the full pH$_e$ distribution must be taken into account. This is best done by calculating a weighted average over the entire pH profile, i.e. each point of the pH curve is weighted according to its height. We applied the standard equation for calculating a weighted average (***first method*** proposed, eq. 1):

$$\overline{pH_e} = \frac{\sum_{k=a}^{n} (pH_{e,k} \times I_k)}{\sum_{k=a}^{n} I_k} \qquad \text{eq. 1}$$

pH$_{e,k}$ is the pH value for a given point k of the digitalized pH$_e$ curve; a and n are the first and the last curve points, respectively, used for weighted-average $\overline{pH_e}$ calculation; and I$_k$ is the height of curve point k. An analogous equation holds for pH$_i$.
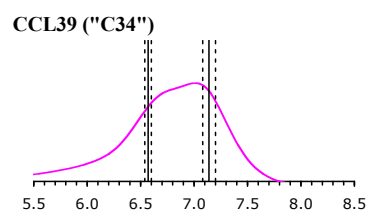
This $\overline{pH_e}$ calculation method is independent of the pH$_e$ curve shape. In fact, pH$_e$ curves can be considered apparent pH$_e$ histograms. They contain all pH$_e$ information as measured by the APP chemical shift, but curve shapes are also influenced by factors unrelated to tissue pH (natural linewidth, magnetic-field heterogeneity, and line broadening due to [1]H-^{31}P coupling, unless proton decoupling is employed). As with all histograms [5], skewness, kurtosis and multimodality can be analyzed to characterize pH distribution. In addition, if two or more distinct pH peaks can be clearly discerned, the pH of each of these peak maxima can be determined (***second method*** proposed). In Fig. 2, the solid vertical lines represent mean values of the two principal pH$_e$ values (pH$_{e1}$ and pH$_{e2}$) from a group of 7 CCL39 tumors measured, the broken lines represent standard errors. For severely overlapping curves (Fig. 2), pH$_{e1}$ and pH$_{e2}$ are necessarily estimates. Apart from the positions of the pH$_{e1}$ and pH$_{e2}$ maxima, also the areas under these two peaks can be determined, for instance by deconvolution (data not shown). The relative sizes of the pH$_{e1}$ and pH$_{e2}$ areas are a measure of the underlying relative tissue volumes, provided that the tissue APP concentration is basically independent of pH$_e$ (***third method*** proposed). The validity of the proposed methods has been tested with phantom ^{31}P MRS spectra based on varying proportions of APP at pH 6.5 and 7.4 (data not shown).

References

1. JR Griffiths et al. (1981) *Biosci Rep* 1:319-325. 2. RJ Gillies et al. (1994) *Am J Physiol* 267:C195-C203. 3. RA Graham et al. (1994) *Am J Physiol* 266:R638-R645. 4. N Raghunand (2006) *Methods Mol Med* 124:347-364. 5. Engin. Statistics Handbook, http://www.itl.nist.gov/div898/handbook