Transmembrane pH gradients in tumor cells: observations using 19F NMR of promising new reporter molecules

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Introduction: pH plays an important role in tumor proliferation, metabolic control, and response to therapy. In particular, it has been shown that the tumor cell transmembrane gradient influences the biodistribution and efficacy of many chemotherapeutic agents. We have been developing novel pH indicators and report progress in the development of useful ^{19}F NMR reporter molecules. We had shown that 6-fluoropyridoxol (FPOL, a vitamin B6 analog) could be used to measure transmembrane pH gradients in blood and perfused hearts (*Curr. Med. Chem.* 6, 481, 1999), but it doesn't enter into tumor cells. We have now examined new indicators to investigate transmembrane pH gradients. 6-fluoropyridoxamine (FPAM) is a close analog of FPOL: it requires sophisticated organic synthesis, but the substance has a large chemical shift response to pH ($\Delta\delta_{acid\text{-}base}$ = 10.1 ppm) and a pKa = 7.05. Like FPOL, FPAM shows two signals in whole blood and perfused hearts.

Methods: Wild-type and stably transfected cells (Morris Hepatoma, Chinese Hamster Ovary) were cultured in RPMI 1640 medium plus 10% fetal bovine serum, 100 IU/ml penicillin and 100µg/ml streptomycin at 37 °C under 5% CO_2. The cells were trypsinized and suspended in TRIS-EDTA (pH 8.2) buffer with a cell concentration around 10^7/ml. Initial experiments were performed at 14.1 T with a capillary of sodium trifluoroacetate (NaTFA) as an external chemical shift reference and more recently data have been obtained at the more relevant field of 4.7 T.

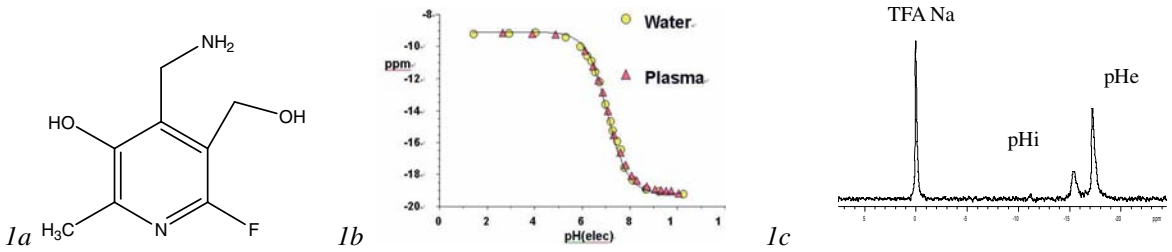

Fig 1. a) The chemical structure of FPAM, b) the titration of FPAM in water and plasma, c) intra and extra signals when FPAM was in whole blood, with NaTFA as chemical shift reference.

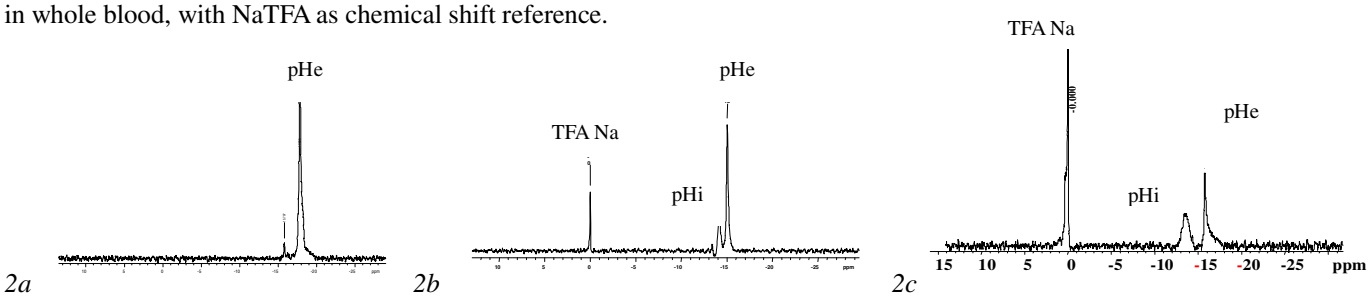

Fig 2. FPAM added to 10^7 Hepatoma cells, respectively a) MH-DmdNK1 cells at 14.1 T, b) MH-TK cells at 14.1 T and c) MH-TK cells at 4.7 T.

Results: Addition of FPAM to suspensions of Morris Hepatoma MH3924A, MH-DmdNK1 cells, or Chinese Hamster Ovary Cells in buffer gave one dominant signal(extra cellular) with only a hint of second signal. By contrast for Morris Hepatoma MH-Tk cells two signals attributed to intra and extra cellular compartments were rapidly observed. Chemical shifts indicated pH values 6.82 and 7.20. The larger upfield signal suggested it was the extracellular buffer and this was confirmed by electrode. Well resolved signals could be observed at both 14.1 and 4.7 T.

Conclusion:

FPAM gave two signals in cultures of Morris hepatoma cells transfected to express thymidine kinase (TK) allowing transmembrane pH gradient to be assessed. In other cells only minimal intra cellular signal was detectable. We are currently investigating whether the TK expression is responsible for cellular uptake of FPAM. ^{19}F NMR pH indicators could be particularly useful since they may be observed simultaneously with various drugs such as 5-fluorouracil and many novel physiological and gene reporter molecules. We are now initiating studies using FPAM to investigate measurements of transmembrane pH gradients *in vivo*.

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