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Doctoral Thesis Abstract: ^{19}F NMR of Erythrocytes: 'Split Peak' Phenomenon, Membrane Potential and Membrane Transport

Arron S. L. Xu

Fluorinated solutes such as difluorophosphate (DFP), monofluorophosphate (MFP), hexafluorophosphate (HFP), and trifluoroacetate (TFA) all showed well-resolved ^{19}F NMR resonances when they were added to erythrocyte suspensions. The broader resonances from intracellular solutes were shifted to *high* frequency with respect to their extracellular counterparts.

The ^{19}F NMR chemical shifts of the above-mentioned compounds were shifted to high frequency in the presence of proteins. An increase in temperature also led to a shift of the ^{19}F NMR resonances to high frequency. Results from this work support the hypothesis that the disruption of hydrogen bonding between the fluorine atom and solvent water atoms, by hydrated haemoglobin, is the principal physical basis for the 'split peak' phenomenon seen with erythrocyte suspensions.

The well-resolved ^{19}F NMR resonances of DFP enabled its transmembrane mass-distribution to be determined directly from an erythrocyte suspension. At transmembrane electrochemical equilibrium, the distribution of DFP was governed by the membrane Donnan potential. The membrane potential measured using DFP was independent of the concentration of the probe molecule, and the haematocrit of the suspensions within a large range.

A novel adaption of a ^{19}F NMR magnetisation-transfer technique was derived to measure the rapid membrane transport of DFP. The transport was shown to be mediated exclusively by band-3. The transport was temperature dependent; the 'break-point' temperature of the equilibrium efflux was ~31°C. Under similar conditions, the ratios of the influx rates for solutes at a concentration of 20 mM were DFP : hypophosphite : F^- : Cl^- were 1.0 : 1.5 : 33.0 : 68.1.

Department of Biochemistry
The University of Sydney
Sydney NSW 2006
Australia

The membrane-transport of TFA in human erythrocytes was significantly slower than DFP. By differentiating the inhibition brought about by a number of compounds, including stilbene disulfonates, α-cyano-4-hydroxycinnamate, *p*-chloromercuriphenylsulfonic acid, and N-ethylmaleimide, band-3 was found to be the predominant transporter of TFA uptake into human erythrocytes. A small fraction of the uptake was mediated by the monocarboxylate transporter. Under physiological conditions, transport via simple diffusion via the lipid of the membranes was negligible.

The ^{19}F NMR spectrum showed well-separated quartets arising from beryllofluorides BeF_2, BeF_3^- and BeF_4^{2-}. This phenomenon facilitated the study of the multiple equilibra associated with the complexes in a solution. In erythrocyte suspensions, the ^{19}F NMR spectra showed resonances from the intracellular populations of the complexes shifted to higher frequencies relative to their extracellular counterparts. The erythrocyte membrane-transport of the complexes was completely inhibited by stilbene disulfonates; the results suggested that band-3 was the exclusive transporter for BeF_3^- and BeF_4^{2-}, and intracellular BeF_2 arose as the result of the re-distribution of the various intracellular complexes via the multiple equilibra.

The ^9Be NMR resonances of the complexes were, a quintet, a quartet and a triplet for BeF_4^{2-}, BeF_3^- and BeF_2, respectively, and they overlapped extensively. ^9Be NMR resonances of intra- and extracellular solutes were not resolved. ^9Be NMR decoupling simplified the ^{19}F NMR spectrum. The ^{19}F NMR magnetisation transfer among various complexes in either *cis* or *trans* compartments indicated interconversion among the different species in the *cis* compartment, and the transmembrane exchange occurred within sub-minute time scale.

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