Doctoral Thesis Abstract (University of Sydney): NMR Studies of the Uptake and Degradation of Peptides by Human Erythrocytes

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The teleologically unsatisfying hypothesis that the cytosolic exopeptidases of the human erythrocyte are merely non-functional vestiges of red-cell differentiation is critically examined. The efficacy of $^1\text{H}$ spin-echo NMR spectroscopy for monitoring the kinetics of exopeptidase-catalysed reactions in situ in human erythrocyte lysates and intact-cell suspensions is demonstrated. The ability of this technique to simultaneously monitor the substrates and products of such reactions enabled a new procedure to be developed for determining the concentrations of reactants; this involved the calculation of unique NMR extinction coefficients for each sample, which obviated the need for preparation of calibration curves. Furthermore, a new, time-efficient method was developed for non-linear regression of the integrated Michaelis-Menten equation, which is an implicit function of the substrate or product concentration, onto reactant-versus-time progress curves for the purpose of obtaining steady-state kinetic parameters for the reactions. Derivation of the sensitivity functions for the integrated Michaelis-Menten equation revealed under what initial conditions this procedure yields the most reliable parameter estimates.

Since peptides are not products of normal erythrocyte metabolism, the work presented in the thesis examined the possibility that substrates for the cytosolic exopeptidases of these cells may arise from extracellular sources. It is shown, for the first time, that a range of di- and tripeptides may enter the human erythrocyte via a natural membrane transport system(s) which are describable by simple Michaelis-Menten kinetics; all such peptides are shown to be rapidly hydrolysed upon entering the intracellular milieu. However, preliminary evidence revealed that the rate of peptide uptake by human erythrocytes declines markedly as the residue-number is increased beyond three; it is consequently suggested that there may be a critical residue-number or hydrodynamic volume which determines whether peptides are permeant to these cells.

Potential physiological roles were assigned to some of these coupled (in the kinetic sense) peptide transport-intracellular exopeptidase systems. For example, it was demonstrated that glutamate, which is impermeant to human erythrocytes, may be supplied to these cells via the absorption and subsequent intracellular hydrolysis of plasma $\alpha$-glutamyl-peptides. Calculations based on the experimentally-derived steady-state kinetic parameters for the uptake and hydrolytic processes revealed that the plasma concentration of $\alpha$-glutamyl-peptides would only need to exceed 7.3uM for this coupled system to provide enough glutamate to sustain the observed rate of intracellular glutathione synthesis.

Figure 1: A schematic representation of the putative role of human erythrocytes, and other tissues, in whole-body peptide turnover. The dotted line is meant to portray current uncertainty about the exact role of human erythrocytes in the deactivation of circulating peptide hormones.
However, it was shown that human erythrocytes play only a minor role in the turnover of plasma prolyl-peptides and that absence of erythrocyte prolidase is probably not a major factor in the aetiology of the clinical manifestations of generalised prolidase deficiency. It was demonstrated that the rates of cis-trans isomerisation of imidodipeptide substrates of prolidase can be monitored using inversion-transfer NMR spectroscopy. Data obtained from such a study, combined with progress curves obtained from $^1$H NMR experiments monitoring all reaction species in the prolidase-catalysed hydrolysis of L-alanyl-L-proline, enabled the isomeric specificity of the enzyme to be determined; it was shown that prolidase has exceptionally high, or absolute, specificity for the trans isomer of its substrates. This information, combined with data from the literature, enabled a new model of the active site of prolidase to be constructed.

Finally, it is proposed that human erythrocytes, by virtue of their extensive distribution, large numbers, and peptide and amino-acid transporting capabilities, may play an important role in whole-body peptide turnover by assimilating plasma peptides and distributing the hydrolytic products to other tissues (see Figure 1). The clinical value of the illicit transport of otherwise impermeant drugs into human erythrocytes by way of their peptide transport system(s) is also discussed.

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