NMR DIFFUSION MEASUREMENTS OF COMPARTMENTALIZED AND MULTICOMPONENT BIOLOGICAL SYSTEMS

Studies of Tropoelastin, the Self Association of N-Methylacetamide, and q-Space Analysis of Real and Model Cell Suspensions

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Abstract

Molecular diffusion is an inherent feature of all fluid systems. The processes and interactions that characterize these systems are in some way dependent upon the mobility of the component molecules. Pulsed field-gradient spin-echo nuclear magnetic resonance (PGSE NMR) is a powerful tool for the study of molecular diffusion; for heterogeneous systems, such as those typically found in biology, this technique is unsurpassed in the diversity of systems that yield to its probing. The aim of the work presented in this thesis was to use an integrated NMR-based approach, in conjunction with computer modeling, for the study of molecular diffusion in compartmentalized and multicomponent biological systems.

Erythrocyte suspensions provided an ideal experimental system for the study of compartmentalized diffusion in cells. Water exchanges rapidly between the intra- and extracellular regions and, as the major constituent of the cell, provides a strong and predominant proton NMR signal. In addition, the cells are known to align in the strong static magnetic field of the spectrometer. As a consequence of these two properties, the signal intensity from a suitably designed series of PGSE NMR experiments exhibits a series of maxima and minima when graphed as a function of the magnitude of the spatial wave number vector **q**. The apparently periodic phenomenon is mathematically analogous to optical diffraction and interference and is referred to here as diffusion-coherence. It is the characterization of this phenomenon, with the aid of computer-based models, which was the focus of a major section of the work described herein.

Two quite distinct molecular systems formed the basis of the work in which I investigated diffusion in multicomponent systems. Both systems involved molecules that undergo self-association such that at equilibrium a population distribution of different oligomeric species is present. The first of these was tropoelastin, the monomeric subunit of elastin, which under certain conditions aggregates to form a coacervate. The second system was *N*-methylacetamide (NMA) which also undergoes extensive self-association. NMA oligomers have previously been studied as peptide analogues due to the presence in the monomer of a peptide linkage. In this work the aim was to use PGSE NMR diffusion measurements, in a manner that is in many ways analogous to analytical ultracentrifugation, to obtain estimates of hydrodynamic and thermodynamic parameters. Computer modeling was also used extensively in this section of work for the interpretation of the experimental data.

Main References

This thesis is based on the following papers which are ordered according to publication date and will be referred to in the text by their corresponding Roman numeral. The author's role in each paper is outlined in italics below each reference:

I. Torres A.M., Taurins A.T., Regan D.G., Chapman B.E., Kuchel P.W. (1999) Assignment of coherence features in NMR *q*-space plots to particular diffusion modes in erythrocyte suspensions. *J. Magn. Reson.* **138**, 135-143.

- *design, development, and application of computer models used to assist in the assignment of coherence features; analysis and interpretation of simulation data; involvement in the analysis and interpretation of experimental data and in all discussions of ideas presented in the paper*

II. Regan D.G., Kuchel P.W. (2000) Mean residence time of molecules diffusing in a cell bounded by a semi-permeable membrane: Monte Carlo simulations and an expression relating membrane transition probability to permeability. *Eur. Biophys. J.* **29**, 221-227.

- *design, development, and application of computer models; analysis and interpretation of data; development of theory relating membrane transition probability to permeability in collaboration with co-author*

III. Kuchel P.W., Durrant C.J., Chapman B.E., Jarrett P.S., Regan D.G. (2000) Evidence of red cell alignment in the magnetic field of an NMR spectrometer based on the diffusion tensor of water. *J. Magn. Reson.* **145**, 291-301.

- *direct involvement at all levels of project including: design and implementation of PGSE NMR experiments, discussion of ideas; development and formulation of theory; development of computer-based mathematical methods for data analysis*

IV. Toonkool P., Regan D.G., Kuchel P.W., Morris M.B., Weiss A.S. (2001) Thermodynamic and hydrodynamic properties of human tropoelastin: analytical ultracentrifuge and pulsed field-gradient spin-echo NMR studies. *J. Biol. Chem.* **276**, 28042-28050.

- *design, development and application of computer models used in testing hypotheses; design and implementation of PGSE NMR experiments; analysis and interpretation of diffusion and simulation data; involvement in all discussion of ideas relating to the project*

V. Regan D.G., Kuchel P.W. (2002) Simulations of molecular diffusion in lattices of cells: insights for NMR of red blood cells. *Biophys. J.* **83**, 161-171.

- *design, development and application of computer models; design and implementation of PGSE NMR experiments; analysis and interpretation of experimental and simulation data; formulation of theory in collaboration with co-author*

VI. Regan D.G., Kuchel P.W. (2002) Simulations of NMR-detected diffusion in suspensions of red cells: the 'signatures' in *q*-space plots of various lattice arrangements. *Eur. Biophys. J.*, (in press**)**.

- *design, development and application of computer models; analysis and interpretation of simulation data; formulation of theory in collaboration with co-author*

VII. Regan D.G., Chapman B.E., Kuchel P.W. (2002) PGSE NMR Diffusion study of the self-association of *N*-methylacetamide in carbon tetrachloride. *Magn. Reson. Chem.*, (in press**)**.

- *design, development and application of computer models; design and implementation of PGSE NMR experiments; analysis and interpretation of experimental data; formulation of theory in collaboration with co-authors*

Preface

Here I describe the investigation of diffusion in compartmentalized and heterogeneous systems. The work entailed combining the experimental results from pulsed field-gradient spin-echo nuclear magnetic resonance (PGSE NMR) experiments with those obtained with computer simulations of the systems under investigation.

Erythrocyte (red blood cell; RBC) suspensions provided the cellular model for the study of diffusion in compartmentalized systems and computer models were developed to simulate these samples. Simulations were also performed on a more idealized system in which the cells were oblate-spheroids. Both systems were simulated in the context of PGSE NMR experiments. Central to this work were the concepts of *q*-space and diffusion-coherence. We have shown that diffusion-coherence phenomena can be interpreted, by means of *q*-space analysis, to provide unique information about cell suspensions. This information includes cell dimensions and spacing, membrane transport rates, and the geometrical arrangement of cells in the suspension. The computer models assisted in the assignment of coherence features to particular modes of diffusion (Paper I), and were used to explore and interpret some of the many complex 'signatures' present in *q*-space data (Papers V and VI). They were also used to develop a theory which relates membrane transition probability to membrane permeability in the context of the simulations (Paper II).

The diffusion tensor is a useful indicator of diffusion anisotropy in heterogeneous systems and in this work we showed that it provided additional confirmation of erythrocyte alignment in the static magnetic field of the NMR spectrometer (Paper III). Furthermore, it contains information relating to the arrangement of cells in the suspension (Paper VI).

Two quite different multicomponent systems were studied using PGSE NMR diffusion measurements. The first of these was tropoelastin, the monomeric subunit that is cross-linked in elastic fibers, which forms a coacervate under certain condition of temperature, pH, and salt concentration. This work was carried out to provide complementary data to those obtained using analytical ultracentrifugation and which suggested that soluble tropoelastin existed as two distinct isoforms (Paper IV). Computer modeling was again used to test this theory against various possible overall protein conformations.

The second multicomponent system that was studied was *N*-methylacetamide (NMA) in carbon tetrachloride. NMA is known to self-associate through the formation of hydrogen bonds in a manner that is both temperature and concentration dependent. The aim of the work was to use PGSE NMR to extend the temperature and concentration range beyond that available to analytical ultracentrifugation, for estimating hydrodynamic and thermodynamic parameters of multicomponent systems. A computer model was developed around the Kirkwood-Riseman theory of macromolecular diffusion, and was used in conjunction with PGSE NMR diffusion measurements to estimate equilibrium constants and oligomer population distributions (as a function of NMA concentration) on the basis of an attenuative model of indefinite self-association (Paper VII).

An historical perspective and a general outline of the studies described in Papers I-VII is given in the Introduction (page vii). Sections 1-6 cover topics central to these papers and provide more in-depth background information than is supplied by them. Sections 7 and 8 contain summaries of the results obtained and the conclusions drawn from them, respectively. Finally, it is noted that this, like all scientific endeavors, is work in progress and Section 9, Future Directions, sets out what I believe to be the important steps that should be taken to advance the areas of research dealt with in this thesis. Indeed, some of this work is already underway and data have already been obtained which will appear in future publications but are beyond the scope of the present treatise.

Contents

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