ANNUAL REPORTS ON NMR SPECTROSCOPY

Volume 36

- NMR study of active sites in paramagnetic haemoproteins
- Empirical versus non-empirical evaluation of secondary structure of fibrous and membrane proteins by solid-state NMR: a practical approach
- Xenon NMR

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Preface

It is a great pleasure for me to introduce volume 36 of *Annual Reports on NMR Spectroscopy* which consists of reports from three very different areas of molecular science. Each of which serves to demonstrate the widespread importance of NMR spectroscopy.

The first report by Y. Yamamoto deals with the NMR Study of Active Sites in Paramagnetic Haemoproteins, Empirical versus Non-empirical Evaluations of the Secondary Structure of Fibrous and Membrane Proteins by Solid-state NMR are reviewed by H. Saitô, S. Tuzi and A. Naito and finally, C. I. Ratcliffe reports on Applications of Xenon NMR.

I am very grateful to all of these authors for the very considerable efforts which they have invested in the preparation of their manuscripts and for delivering them punctually. Such devotion is necessary for the continued success of this series as is the generous support and assistance provided by the production staff at Academic Press (London).

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May 1997

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Contents

NMR Study of Active Sites in Paramagnetic Haemoproteins

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NMR studies of paramagnetic haemoproteins are reviewed with special emphasis on characterization of both structural and dynamic properties of haem active site. In the past decade, the development of NMR methodologies for detecting the connections between hyperfine shifted signals has contributed greatly to establishing systematic and reliable strategies for the signal assignments of paramagnetic haemoproteins. The use of reconstituted haemoprotein is a characteristic of the study of b-type haemoproteins and NMR studies on reconstituted myoglobins and haemoglobins are described in some detail. Additionally, nonequivalence in haem electronic structure between the two

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different subunits in human adult haemoglobin enables one to characterize the individual subunits in intact tetramers using NMR, and the advantages in studying tetrameric haemoglobin by NMR are also described.

1. INTRODUCTION

The use of paramagnetic ions as extrinsic shift and relaxation probes for investigating the structure of biological molecules in solution has not been as fully exploited as originally expected. This is due to the presence of rapid averaging of nonspecific interaction between the ions and molecules, which complicates the interpretation of NMR parameters.[1] Paramagnetic metalloproteins, however, already contain internal paramagnetic ion(s) at specific site(s) of the molecule. Consequently, taking advantage of the properties of unpaired electron(s), resonances arising from nuclei located in the close proximity of the paramagnetic centre exhibit hyperfine shifts and hence appear outside of the diamagnetic envelope where signals due to the protein overlap severely. Hyperfine shifted signals are extremely sensitive to structural properties of molecules, as has been fully described elsewhere.[1–20] NMR studies of paramagnetic metalloproteins have provided a wealth of information for characterizing structure–function relationships of proteins.[12–15,21,22] One of the major drawbacks in the NMR study of paramagnetic molecules is obviously fast nuclear relaxation. Since there is no way to slow down the relaxation without losing the hyperfine shift, we have to accept it. Paramagnetic-induced relaxation substantially diminishes the development of the nuclear Overhauser effect or coherence in two-dimensional (2D) NMR. All the connectivities that should be observable if the molecule were diamagnetic are not always expected to be detected. However, the major 2D experiments are found to be surprisingly effective in detecting both scalar and dipolar connectivities.[23–25] The applicability of various NMR methodologies to paramagnetic molecules possessing a wide range of shifts, line widths and T_1 values has been examined in detail from both the experimental and theoretical points of view.[9,23–39]

In the present chapter, NMR studies of paramagnetic haemoproteins are reviewed with special emphasis on characterization of both structural and dynamic properties of the haem active site. As in the previous review by Satterlee,[12] an initial attempt is made to assign the signals of the paramagnetic haemoprotein to specific nuclei in the molecule by isotope labelling or comparison with model systems. This is restricted to the resonances arising from coordinated ligands.[40] In the past decade, the development of NMR methodologies for detecting the connectivities between hyperfine shifted signals has contributed greatly to the establishment of systematic and reliable strategies for signal assignment.[26,41–53] In particular, the observation of the dipolar connectivity between the resonances of paramagnetic haemoproteins