

Homonuclear Two-Dimensional ^1H NMR of Proteins. Experimental Procedures

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Experimental techniques used for homonuclear 2D ^1H NMR studies of proteins are described. A brief survey of the general strategy for structural studies of proteins by 2D NMR is included. The main part of the paper discusses guidelines for the selection of experimental techniques, the elimination of artifacts and unwanted peaks in protein 2D ^1H NMR spectra, suppression of the solvent line in H_2O solutions, experimental parameters, numerical data processing before and after Fourier transformation, and suitable presentations of complex 2D NMR spectra.

I. INTRODUCTION

During the past five years it was demonstrated that well-resolved, informative two-dimensional (2D) ^1H NMR spectra of proteins in solution can be recorded with commercially available high-resolution NMR equipment (1-5). The fundamental experimental schemes of the experiments which have most profitably been used for studies of proteins are shown in Fig. 1 (1-3, 6-10). With the use of 2D NMR nearly complete individual proton resonance assignments have been obtained for several small proteins (11-19) and 2D NMR investigations of static and dynamic aspects of the conformations of these proteins in solution are in progress (e.g., 20-24). With the exception of some reports on conceptually new procedures (8, 25-33), our earlier publications contain little information on experimental details of the 2D NMR measurements. The present paper describes salient points from our practical experience gained when optimizing the experimental conditions for homonuclear 2D ^1H NMR with proteins.

While the scope of the present article is limited to technical aspects which have been of particular relevance for homonuclear ^1H experiments with proteins, much of this information should be of interest also for studies of other classes of large molecules. Several reviews are already available which survey the procedures used for the study of low molecular weight compounds (e.g., 34-36).