Applications of homonuclear two-dimensional (2D) NMR experiments to studies of polypeptides and proteins are investigated. The interpretation of these experiments is illustrated with studies of glucagon bound to perdeuterated dodecylphosphocholine micelles.

The following 2D 1H NMR techniques have been applied to studies of proteins: 2D J-resolved spectroscopy, 2D correlated spectroscopy (COSY or SECSY), and 2D nuclear Overhauser enhancement spectroscopy (NOESY). These techniques are described and some special problems which may arise in the measurements, the data handling, and the interpretation of protein 2D NMR spectra are discussed.

In order to get a better basis for the interpretation of 2DJ spectra of proteins, the influence of strong spin-spin coupling was investigated. The 2DJ spectra of the 20 common amino acids were measured and simulated. On the basis of this data characteristic features of strong coupling are described.

The individual resonance assignments in the spectrum of a polypeptide or of a protein are a fundamental step of the use of NMR for studies of such compounds. The combined use of COSY and NOESY spectra presents an efficient, reliable, and generally applicable method for assigning resonances along the polypeptide backbone. These "sequential assignments" are based entirely on NMR data and the known amino acid sequence. A detailed explanation of this method is presented on the basis of the spectrum of glucagon bound to fully deuterated micelles. In this spectrum all backbone and Cb-protons, with the exception of the N-terminal amino group and the amide proton of Ser 2, were individually assigned. Furthermore, all non-labile amino acid side chain protons were assigned, with the sole exception of the Cg-methylene protons of Gln 20 and Gln 24.

The resonance assignments obtained for glucagon were used to determine distance constraints between specific protons. From NOESY experiments with different mixing times, ca. 260 distance constraints between specified locations along the polypeptide were obtained. This data was used as the input for a distance geometry algorithm, that generates possible three-dimensional structures from upper and lower limits for interatromic distances. These structures were used to study the conformation of micelle-bound glucagon.