

## Structure Investigation of Acyl-CoA-Binding Protein from Bovine Liver using 2D $^1\text{H}$ NMR

by

Kim V. ANDERSEN, Svend LUDVIGSEN, Jens KNUDSEN\*  
and Flemming M. POULSEN†

Department of Chemistry, Carlsberg Laboratory,  
Gamle Carlsberg Vej 10, DK-2500 Copenhagen Valby, Denmark.  
Tlf. +45 31 22 10 22

\*Institute of Biochemistry, University of Odense,  
DK-5230 Odense M, Denmark.

In order to determine the three-dimensional structure in solution of an 86 residue Acyl-CoA-Binding Protein (ACBP) from bovine liver<sup>1,2</sup>, two-dimensional  $^1\text{H}$  NMR have been applied.

COSY, DQF COSY, E.COSY, Relayed COSY, TOCSY, NOESY and DQ-spectra have been recorded in 90:10  $\text{H}_2\text{O}:\text{D}_2\text{O}$  and 99.996%  $\text{D}_2\text{O}$  at two temperatures, 298K and 310K, all at pH 7.0.

The spectra have been analyzed, and 81 of the 86 spin systems have been identified. Sequence specific assignment has been obtained for 74 spin systems, revealing a secondary structure mainly consisting of  $\alpha$ -helices.

Four  $\alpha$ -helices have been identified covering residues 3-11, 22-37, 51-61 and 72-85. However one of these (helix 22-37) may consist of two smaller helices bend around residue 27.

The analysis of long-range NOE's has shown that residues in helix 2 are close to residues in each of the other helices, and that helix 1 and helix 4 are in contact. Amide proton exchange reveals that amides close to the contact region of the four helices exchange slowly, and in particular that amide protons in a stretch of 9 residues (73-81) in helix 4 exchange slowly. This observation suggests that this helix is in the interior of the protein. However, a more precise picture of the structure however is expected as a result of future structure calculations.

Figure 1 illustrates the 36 long range NOE's that have been assigned, giving the first impression of the protein folding.

1. Mogensen I.B., Schulenberg H., Hansen H.O., Spener F., Knudsen J. (1987) *Biochem.J.* 241, 189-192.
2. Mikkelsen J., Højrup P., Nielsen P.F., Roepstorff P., Knudsen J. (1987) *Biochem.J.* 245, 857-861.

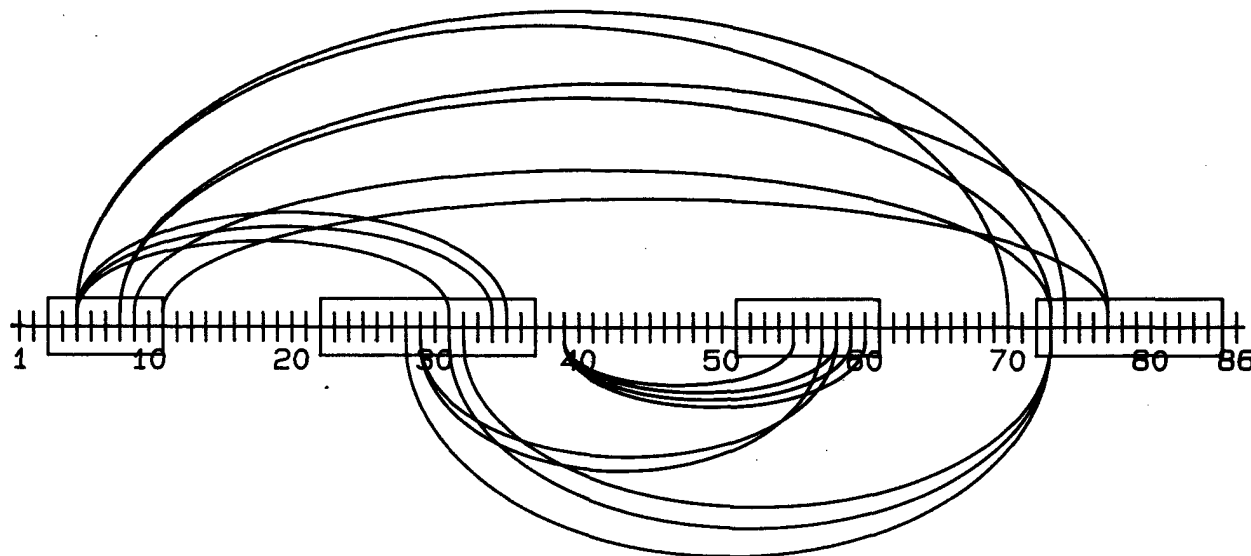


Figure 1. Long range Noe's in ACBP.