Short Communication

\(^{13}\)C-NMR studies of membrane lipid-protein interactions upon protein heat denaturation

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Summary

Spinach chloroplast membranes were studied by natural abundance carbon-13 nuclear magnetic resonance (\(^{13}\)C-NMR) spectroscopy in their normal state and after heat denaturation of membrane proteins. The membrane proteins were denatured by raising the temperature of the sample to 67 °C for 5 minutes [YashRoy, R.C. (1991) J. Biochem. Biophys. Methods 22, 55–59]. Line-broadening of \(^{13}\)C-NMR resonances arising from the 1st (carbonyl), 7th, 9th and 12th carbon atom of fatty-acyl chains with reference to the carbonyl (C-1) group shows increased immobilization of lipid fatty-acyl chains at these locations, obviously caused by changes in interactions between membrane lipids and proteins upon heat denaturation of membrane proteins.

Key words: Protein denaturation; Membrane; Lipid-protein interaction; \(^{13}\)C-NMR

A recent report [1] from this laboratory revealed that lipid-protein interactions in biological membranes could be studied by heat denaturation of membrane proteins by employing spin label electron spin resonance (ESR) spectroscopy. The present brief communication extends this approach to the use of \(^{13}\)C-NMR spectroscopy for the study of membrane lipid-protein interactions.

Chloroplast membranes were prepared from fresh spinach leaves [2,3]. The concentration of chloroplast membrane was kept at a chlorophyll [4] level of 5 mg per ml. The \(^{13}\)C-NMR spectra were recorded using a Bruker CXP-300 NMR spectrometer at a \(^{13}\)C frequency of 75 MHz and locked on deuterium with tetramethylsilane as standard [5]. The spectrometer was equipped with a variable temperature (±0.2 °C) control unit. The \(^{13}\)C-NMR spectra were recorded at 30 °C.

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in dark. For heat denaturation of membrane proteins, the sample temperature was raised to 67 °C for 5 min and lowered to 30 °C before recording the NMR spectra [1].

Fig. 1 shows the $^{13}$C-NMR spectra of chloroplast membranes at 30 °C in dark (normal: lower spectrum B; heat denatured: upper spectrum A). The spectrum was interpreted according to YashRoy [5,6], wherein it was shown that the fatty-acyl resonances from the $^{13}$C-NMR spectra of chloroplast membranes are assignable largely to linolenic acid (most abundant fatty acid in chloroplast membranes) carbons. This pattern of assignment persists even after enrichment of chloroplast membranes with $^{13}$C [7].

Comparison of the heat-denatured chloroplast membranes (upper) spectrum with the control (lower) spectrum in Fig. 1 shows a loss of intensity of certain resonances due to line broadening. Among these, the broadening effect is most marked for resonances at 173.36, 29.7, 128.2 and 130 ppm assigned to 1st (carbonyl), 7th (single-bonded), 12th and 9th (both double-bonded) carbon atoms, with reference to the carbonyl group of the linolenic fatty-acyl chains of the membrane lipids, respectively. Line broadening of these resonances is due to the slowing down of molecular motions, apparently because of altered interaction of proteins with fatty-acyl chains of membrane lipids. The fatty-acyl segments which are more markedly involved in these interactions are located between 1st and 12th carbon atoms of the fatty-acyl chains, thus corroborating the earlier ESR studies [1].

A possible explanation for enhanced lipid-protein interactions upon protein heat denaturation of biological membranes has already been given [1] as due to an unfolding of secondary and tertiary structures of the membrane proteins giving rise to altered lipid-protein interactions caused by (1) exposure of the previously unexposed groups from the molecular interior of the protein and (2) increase in
molecular size of protein with unfolding, thereby resulting in enhanced restriction on the movement of membrane fatty-acyl chains. Unlike spin label ESR, the $^{13}$C-NMR spectroscopy does not require incorporation of any extraneous probe into the sample; therefore, the present report may represent a somewhat more realistic picture than that provided by the spin labelling studies [1].

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References