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NMR Studies on the Structure of Human Annexin I

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Annexin I is a member of the annexin family of calcium dependent phospholipid binding proteins and has anti-inflammatory activity by inhibiting phospholipase A_2 (PLA_2). Recent X-ray crystallographic study of annexin I identified six Ca^{2+} binding sites, which was different types (type II, III) from the well-known EF-hand motif (type I). In this work, the structure of annexin I was studied at atomic level by using ^1H, ^{15}N and ^{13}C NMR (nuclear magnetic resonance) spectroscopy, and the effect of Ca^{2+} binding on the structure of annexin I was studied, and compared with that of Mg^{2+} binding. When Ca^{2+} was added to annexin I, NMR peak change was occured in high- and low-field regions of ^1H-NMR spectra. NMR peak change by Ca^{2+} binding was different from that by Mg^{2+} bindig. Because annexin I is a larger protein with 35 kDa molecular weight, site-specific (amide-^{15}N, carbonyl-^{13}C) labeling technique was also used. We were able to detect methionine, tyrosine and phenylalanine peaks respectively in ^{13}C-NMR spectra, and each residue was able to be assigned by the method of doubly labeling annexin I with [^{13}C]carbonyl-amino acid and [^{15}N]amide-amino acid. In ^{13}C-NMR spectra of [^{13}C]carbonyl-Met labeled annexin I, we observed that methionine residues spatially located near Ca^{2+} binding sites were significantly effected by Ca^{2+} binding. From UV spectroscopic data on the effect of Ca^{2+} binding, we knew that Ca^{2+} binding sites of annexin I have cooperativity in Ca^{2+} binding. The interaction of annexin I with PLA_2 also could be detected by using heteronuclear NMR spctroscopy. Consequently, we expect that the anti-inflammatory action mechanism of annexin I may be a specific protein-protein interaction. The residues involved in the interaction with PLA_2 can be identified as active site by assigning NMR peaks effected by PLA_2 binding.