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STRUCTURAL STUDY OF COLLAGEN MODEL PEPTIDE, (PRO-HYP-GLY)11 AT HIGH RESOLUTION

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Since the 7/2-helical model was proposed by our group in 1977 based on the structure of (Pro-Pro-Gly)10, the single crystal structures of model peptides have contributed to obtain important information on the triple-helical structure of collagen. In this study, we report the high resolution crystal structure of collagen model peptide, (Pro-Hyp-Gly)11 using synchrotron diffraction data (SPring-8) at 100K. The structure has been refined to an R-factor of 0.15 and an Rfree-factor of 0.19 using 1960 and 217 reflections respectively, extending up to 1.5 Å resolution on the layer lines based on the repeating period of 20 Å. Different from (Pro-Hyp-Gly)10 case, only a few satellite spots were observed above and below the strong reflections on the 20 Å layer lines. The obtained molecular conformation and hydrogen bonding schemes of peptide chain are essentially the same as those found in the previous analyses of (Pro-Pro-Gly)n (n=9,10) and (Pro-Hyp-Gly)10, which suggests that the peptide molecule in this crystal takes the 7/2-helical conformation. Compared with that of (Pro-Hyp-Gly)10 case where intensity data were collected at room temperature, a fairly large number of water molecules bound to the triple-helical structure were found in this analysis. Most of the newly found water molecules maybe attributed to the data collection temperature together with the analysis at high resolution. All the proline rings of Hyp were found to be up-puckering, while those of Pro showed both up- and down-puckering.

Keywords: COLLAGEN MODEL PEPTIDE TRIPLE HELIX

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NF-kB REGULATION BY IkB-α AND IkB-β

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The transcription factor NF-kB illustrates how complex cell signaling can lead to the temporal activation of transcription in a specific set of genes. Gene activation by NF-kB is a highly complex event and is regulated at multiple levels. Some of the regulatory checkpoints are at the levels of subunit dimerization, IkB binding and DNA binding by NF-kB proteins. We would like to know the molecular mechanism of a) preferential dimer formation by the members of the Rel/NF-kB family; b) inhibition of NF-kB functions by IkB-α and IkB-β; c) transcriptional regulation as mediated by NF-kB proteins. We have determined three-dimensional structures of several complexes relating these regulatory events of NF-kB signaling pathway. Structures of several DNA bound NF-kB dimers suggest a mechanism of DNA binding by NF-kB with high specificity. In addition, our structures also explain how specific NF-kB dimers preferentially bind to specific kB DNA. Structures of IkB-α/NF-kB and IkB-β/NF-kB complexes reveal that IkB-αand IkB-β interact NF-kB differently. These structures have provided insights that allow us to propose models of how these specific functions are achieved.

Keywords: TRANSCRIPTION FACTOR IKB BINDING NF-KB PROTEINS

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COMPOUND ACTIVE CENTER OF RIBONUCLEASE III: MOLECULAR BASIS FOR DOUBLE-STRANDED RNA CLEAVAGE

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Ribonuclease III (RNase III) from Aquifex aeolicus belongs to the family of divalent-cation-dependent endonucleases that show specificity for double-stranded RNA (dsRNA). RNase III enzymes are conserved in all studied bacteria and eukaryotes and known to transform precursor RNAs into mature RNAs and to produce small single-stranded RNAs that regulate stage-specific development and mediate RNA interference. Functionally dimeric RNase III proteins from bacteria consist of an endonuclease domain followed by a double-stranded RNA-binding domain (dsRBD). The three-dimensional structures of various dsRBDs have been previously elucidated. Here we present the crystal structures of the endonuclease domain of A. Aeolicus RNase III in its ligand-free form and in complex with Mn^{2+}. Our structures reveal a novel all-helical protein fold. The functional dimer is formed via mainly hydrophobic interactions, including a ball-and-socket junction that ensures accurate alignment of the two subunits. The protein fold and dimerization create a valley that can accommodate a dsRNA substrate. Six negatively charged side-chains at each end of this valley form a compound active center. Metal ion binding has significant impact on the formation of two RNA-cutting sites within each compound active center. On the basis of structural, genetic, biological, and biochemical data, we have constructed a hypothetical model of RNase III in complex with dsRNA, which provides the first glimpse at RNase III in action after 33 years the first RNase III enzyme was discovered.

Keywords: RNASE III, DSRNA CLEAVAGE, RNA INTERFERENCE

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THE STRUCTURE OF THE BINDING SITE OF ACETYLCHOLINE RECEPTOR AS VISUALIZED IN THE X-RAY STRUCTURE OF A COMPLEX BETWEEN α-BUNGAROTOXIN AND A HIGH AFFINITY MIMOTOPE PEPTIDE

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We have determined the crystal structure at 1.8 Å resolution, of a complex of α-bungarotoxin with a high affinity 13-residue peptide that is homologous to the binding region of the α-subunit of acetylcholine receptor. The structure was solved by molecular replacement (program MOLREP) which produced a correct solution with only ONE of several highly homologous toxins starting structures. The high affinity peptide was automatically traced with program ARP WARP. The peptide fits snugly to the toxin and adopts a β-hairpin conformation. The structures of the bound peptide and the homologous loop of acetylcholine binding protein, a soluble analogue of the extracellular domain of acetylcholine receptor, are remarkably similar. Their superpostion indicates that the toxin wraps around the receptor binding-site loop, and in addition, binds tightly at the interface of two of the receptor subunits where it inserts a finger into the ligand-binding site, thus blocking access to the acetylcholine-binding site and explaining its strong antagonistic activity.

Keywords: α BUNGAROTOXIN, ACETYLCHOLINE RECEPTOR, MOLECULAR REPLACEMENT