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The effect of chemical cross-linking on protein structure and function

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Chemical cross-linking coupled with mass spectrometry is a powerful technique for characterization of the architecture of proteins and protein complexes. In chemical cross-linking, the residues which are in close proximity to each other may be converted into covalent bond by cross-linker. The cross-linked residues which are then efficiently identified by mass spectrometry provide experimental distance constraints which may be applied for modeling purposes.

In recent years, the popularity of cross-linking has expanded and significant progress has been made in applications and bioinformatics improvements. However, the question if chemical cross-linking distorts the protein structure has not been investigated so far. In the present study, we have looked into the effect of the cross-linking on the structural and functional properties of human carbonic anhydrase (hCA-I).

This enzyme was cross-linked with the homobifunctional cross-linkers BS3 and BS2G. After cross-linking, measurements of enzyme activity was carried out under various conditions, such as cross-linker and enzyme concentration. Furthermore, the cross-linked enzyme was analyzed by SDS PAGE electrophoresis. In order to gain a more detailed insight into the structure of cross-linked enzyme, ^{15}N labeled hCA-I was upon cross-linking analyzed using NMR spectroscopy by measuring of the ^{1}H-^{15}N TROSY spectra which were compared with the spectrum of unmodified enzyme.

Measuring of enzyme activity of cross-linked hCA-I revealed significant influence of cross-linking on enzyme activity. The chemical shift changes of the backbone amide were monitored by measuring of the ^{1}H-^{15}N TROSY. Superposition of the ^{1}H-^{15}N TROSY spectra of the unmodified and cross-linked hCA-I revealed that the relative intensities and the peak positions of several well-resolved peaks were significantly different, whereas interestingly a large majority of cross-peaks were rather slightly affected. The overall chemical shift perturbation pattern caused by BS3 is different to BS2G which confirm their different effect on protein structure, however the NMR data indicate that cross-linking of hCA-I has little effect on the protein structure.

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SW02.S8 Protein Dynamics (II-W8)

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Dynamic mechanisms of inhibiting protein–protein interactions in translation initiation for design of anti-tumor agents

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Eukaryotic translation is regulated by features of the 5′UTR and by the concentrations and state of initiation factors. mRNAs with short 5′UTR are translated efficiently, do not rely heavily on a functional initiation machinery and are thus called strong mRNAs. Weak mRNAs have long 5′UTRs, are translated poorly and rely on a strong initiation complex. Weak mRNAs code predominantly for oncoproteins, growth factors and anti-apoptotic proteins, which are thus down regulated by the nature of their 5′ UTR. Among the initiation factors, the concentration of the cap-binding protein eIF4E seems to be rate limiting. The eIF4E protein recruits the small ribosomal subunit to the 5′ end of the mRNA via interactions with eIF4G and eIF3. Elevated concentrations of eIF4E have been found in several forms of cancer. The activity of eIF4E is regulated by the 4E-binding proteins (4EBPs), which are targets of kinase in signaling pathways and are validated tumor suppressors. Other factors, such as eIF1, eIF1A, eIF5 and eIF2 regulate start-codon recognition.

Through structural and functional studies we try to understand the complex mechanism of translation initiation that are responsible for cellular homeostasis, cellular transformation in cancer and cancer stem cells due to loss of translational balance.

We hypothesized that weakening the interaction of eIF4E with eIF4G would selectively reduce the translation of oncogenes and messages for growth-promoting proteins. Using high-throughput screening we discovered small molecules that inhibit the eIF4E/eIF4G interaction. The inhibitors termed 4EGIs displace eIF4G from eIF4E but stabilize the interaction with 4EBP-1. Binding of the initial hit compounds and analogs to eIF4E was studied with NMR, X-ray crystallography and other biophysical techniques. The compounds and analogs were tested in *in-vitro* and *in-vivo* assays. The molecules exhibit activity against melanoma, breast, lung, prostate cancer and acute myelogenous leukemia (AML). The lead compounds inhibit eIF4E/eIF4G interactions in xenograft tumors in mice and reduce tumor growth. 4EGI-1 inhibits tumor expression of oncogenic proteins such as cyclin E, cyclin D1, c-myc and Bcl-2. Intra-peritoneal treatment with 4EGI-1 did not exhibit any toxic effects in mice. Recently we determined high-resolution structures of eIF4E/inhibitor complexes and discovered that the inhibitors act by an allosteric mechanism. We also obtained structures of the eIF4E complex with a large fragment of 4EBP-1, which revealed the mechanism of stabilization by 4EGI1.

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Structure and dynamics in Lac repressor-DNA interactions

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The *E. coli* lac operon is the classical model for gene regulation in bacteria. An overview will be given of our work on the lac repressor-operator system. An early result was the 3D structure of lac headpiece in 1985, one of the first protein structures determined by NMR. Our studies of the structure and dynamics of complexes of a dimeric headpiece construct with lac operator DNA have provided a detailed picture of how the various lac operator sequences are recognized. Furthermore, comparison with the non-specific DNA complex clarified how the repressor searches for its target site by sliding along random DNA and binds to the operator through a folding-coupled-to-binding transition. Surprisingly, the 1D diffusion rate obtained from NMR is much slower than that determined by single molecule fluorescence methods and cannot account for an enhanced target location by lac repressor.

We have also investigated the mechanism of allosteric coupling of the lac repressor. As all allosteric changes occur in the dimer we use a dimeric form of lac repressor (70 kD), which lacks the tetramerization domain. From ^{15}N chemical shifts of the inducer (IPTG) bound and operator bound complexes we could deduce the allosteric mechanism. Furthermore, the ternary complex with both inducer and DNA bound could be characterized. Some of the results are different from what the crystal structures suggest.