THREE-DIMENSIONAL STRUCTURE OF THE HUMAN ANAPHASE-PROMOTING COMPLEX

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The anaphase-promoting complex (APC) is a large multi-subunit complex that has cell cycle regulated ubiquitin-protein ligase (E3) activity. Together with an ubiquitin activating (E1) and an ubiquitin-conjugating enzyme (E2) the APC catalyses the formation of polyubiquitin chains on substrate proteins such as securin and mitotic cyclins and thereby targets them for the degradation by the 26S proteasome. The human APC is composed of at least 11 subunits. For understanding why the APC is assembled of so many subunits it will be of importance to know the structure of the complex. Structural information is available only for APC10/DOC1 whose crystal structure has been solved recently. However, no crystal structure is shown for the other 10 APC subunits and crystallization of holo APC is currently impossible due to the lack of appropriate purification protocols. To gain insight to the structure of the holo APC, we purified HeLa cell APC in a homogeneous soluble form suitable for electron microscopic analyses. Based on the analysis of 13000 molecular images we obtained a three-dimensional model of the human APC at a resolution of 24 Å. The APC has a complex asymmetric structure of about 150 Å in diameter in which an outer protein wall encloses an inner cavity that could represent a reaction chamber for ubiquitination reactions. For further analyses we developed a method that allows the purification of a large APC subcomplex. Structural and biochemical analyses of this sub-complex should help to understand the function and localization of individual subunits within the holo APC.

Keywords: UBQUITINATION CRYOELECTRONMICROSCOPY CELLCYCLE

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POROUS SILICON: A NUCLEATION-INDUCING MATERIAL FOR PROTEIN CRYSTALLIZATION

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Production of high quality protein crystals of both soluble and especially of membrane proteins remains a difficult and important task. Hence there is an urgent requirement for new and improved methodology to aid crystal growth. Considerable effort has been invested in the search of substances (nucleants) that will induce efficient heterogeneous nucleation of protein crystals in a controlled manner [e.g. 1-3]. To date, nucleation has been facilitated mainly by seeding, epitaxy, charged surfaces or mechanical means. A different approach is introduced here, involving the use of a mesoporous material that is likely to induce efficient heterogeneous nucleation of protein crystals.

Keywords: PROTEIN CRYSTALLIZATION NUCLEATION PHASE DIAGRAMS

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STRUCTURAL BASIS FOR CHK1 INHIBITION BY UCN-01 AND ITS ANALOGS

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Chk1 is a serine-threonine kinase that plays an important role in the DNA damage response including G2/M cell cycle control. Stauroporine-like kinase inhibitors, UCN-01 (7-hydroxystaurosporine) and SB218078 have been shown to be potent Chk1 inhibitors that abrogate the G2/M checkpoint induced by DNA damaging agents. To understand the structural basis for Chk1 regulation and inhibition by UCN-01 and its analogs, we determined the crystal structures of Chk1-staurosporine, Chk1-UCN-01 and Chk1-SB218078 binary complexes.

The structure shows that all three compounds bind to the ATP binding pocket of Chk1, and cause only slight changes in the protein conformation. The high structural complementarity of these interactions is consistent with the potency and selectivity of this class of inhibitors. X-ray crystallography has also revealed some specific binding interactions of inhibitors to Chk1. We made a comparison between these Chk1 complexes and cyclin-dependent kinases 2 (CDK2) complexed with staurosporine, in respect of both structure and biological activity of the inhibitors to Chk1 and CDKs. Selectivity of UCN-01 towards Chk1 over CDKs can be explained by the presence of hydroxyl group in the lactam moiety interacting with the ATP binding pocket.

Keywords: KINASE, CHK1, UCN-01

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THE BIOLOGICAL MACROMOLECULE CRYSTALLIZATION DATABASE: NEW DATA, CRYSTALLIZATION PROCEDURES AND STRATEGIES

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The Biological Macromolecule Crystallization Database (BMCD) archives crystallization data from published reports for all forms of biological macromolecules that have produced crystals suitable for x-ray diffraction studies (1). It contains comprehensive information for all classes of biological macromolecules critical for the development of crystalization strategies (2,3). This data resource has evolved from a stand-alone PC database to a web-based database (http://wwwhmcd.nist.gov:8080/bmcd/bmcd.html).

The crystallization data includes all of the information necessary for reproducing the crystallization of a biological macromolecule. This includes the crystallization conditions, crystal data, comments about the crystallization procedure, and information on the biological macromolecule or biological macromolecule complex. A new website architecture that is being developed will be described along with a significant increase in the data content. Crystallization procedures, including fast screens and more general procedures, can be developed effectively using this upgraded web-based resource.

References