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THE EFFECT OF WATER SOLVENT ON THEORETICAL NMR SPIN - SPIN COUPLINGS IN DNA: IMPROVEMENT OF CALCULATED VALUES BY APPLICATION OF TWO SOLVENT MODELS

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The NMR indirect spin - spin coupling constants calculated in DNA base pairs are compared with one-bond $^1J(X,H)$, $^1J(C,X)$ and two-bond 2J(X,H), (X = C, N) coupling constants measured in DNA hairpin molecule d(GCGAAGC) [1]. The two theoretical models of solvent: explicit and Polarized Continuum, were introduced. Both explicit and PCM solvent model provide a similar improvement of the calculated J constants when compared to the experimental data. The mean absolute deviation between the calculated and experimental couplings is significantly reduced by solvent inclusion, from 1.7 to 1.1 Hz for guanine, from 2.4 to 0.6 Hz for cytosine, and from 2.3 to 1.6 Hz for adenine. The strongest solvent shift was calculated for the $^1J(C,H)$ coupling constants, particularly for the (C8,H8) coupling in guanine (6.1 Hz) and adenine, and the (C5,H5) and (C6,H6) couplings in cytosine. These changes in $^1J(C,H)$ coupling seem to correspond to the charge transfer from water bonding and lone pair orbitals to the guanine anti-bonding C8-H8, C8-N7, and C8-N9 orbitals. From the close agreement between the calculated and experimental coupling constants we can deduce that the C-H group of the hairpin bases is extensively hydrated even when interaction with solvent is rather weak and non - specific.

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LECTURES - MARCH 15

STRUCTURAL BASIS OF HIV-1 AND HIV-2 PROTEASE INHIBITION BY A MONOCLONAL ANTIBODY

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Since the demonstration that the Human Immunodeficiency Virus protease (HIV PR) is essential in the viral life cycle [1], this enzyme has become one of the primary targets for antiviral drug design. With the objective of probing the structural stability of HIV PR and the eventual design of potential inhibitors directed to regions other than the active site, we have examined the effects of anti-HIV-1 PR monoclonal antibodies (mAbs) on the catalytic activity of the protease [2, 3]. Monoclonal antibody mAb1696, although raised against the HIV-1 PR, inhibits the catalytic activity of both the HIV-1 and HIV-2 enzymes with inhibition constants in nanomolar range [3] and cross-reacts with peptides comprising the N-terminus of the HIV protease (residues 1 to 7). The N-terminal region is essential for dimerization of monomers and thus forming the active HIV PR dimer.

To investigate further the mechanism of HIV PR inhibition by mAb1696, a recombinant single-chain Fv fragment (scFv) that contains heavy and light chain variable domains of mAb1696 joined by a flexible linker have been prepared and used for crystallographic studies [4]. Although the crystallization of scFv1696 complexed with the HIV proteases has not yet been successful, the formation of these complexes has been demonstrated in solution. As an alternative approach to studying the antigenic recognition by the mAb 1696 at the structural level, we have crystallized complexes of scFv1696 with the respective epitope peptide fragments of the HIV protease. The three-dimensional structure of the complex formed between scFv1696 and epitope peptide PQITLWQRR (corresponding to the N-terminus of HIV-1 PR) has been solved and refined at 2.70 Å resolution [4]. The structure of the complex formed between scFv1696 and epitope peptide PQFSLWKR (corresponding to the N-terminus of HIV-2 PR) has been solved and refined at 1.88Å resolution.

Interactions of peptides with scFv1696 have been analyzed and compared in both structures. On the basis of the interactions seen in the complex, the cross-reactivity between mAb1696 and the HIV-1 and HIV-2 protease and their N-terminal peptides can be explained. The broad reactivity between mAb 1696 on one side and the HIV-1 PR and HIV-2 PR derives from the invariance or conservation

of the first six N-terminal residues of the two strains. Most of these residues are deeply buried in the antibody-binding groove and establish extensive contacts.

Using the peptide as a guide, a docking complex of a whole protease monomer was generated, which suggests that mAb 1696 inhibits the HIV PR by favouring the dissociation of the active homodimer. A dissociative mechanism of protease inhibition by 1696 is consistent with the stoichiometry of the inhibition complex, as derived from the inhibition kinetic studies [3].

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CRYSTALLOGRAPHIC STUDY OF AN ANTI -CARBONIC ANHYDRASE IX MONOCLONAL ANTIBODY M75

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Carbonic anhydrase IX (CA IX) is a cell surface protein, strongly associated with certain types of human carcinomas. The predicted protein of cloned CA IX cDNA consists of the signal peptide, proteoglycan-related sequence, carbonic anhydrase domain, trasmembrane segment and a short intracellular tail (1, 2). Until now, molecular basis of involvement of CA IX in carcinogenesis has remained unclear. CA IX is a cell adhesion molecule, its carbonic anhydrase (CA) is enzymaticaly active. Structural study of a CA IX-binding monoclonal antibody (mAb) M75, complexed with its epitope peptide may contribute toward elucidation of the role of CA IX. To achieve this goal, two parallel approaches were chosen: analysis of Fab fragment, or of a smaller scFv fragment, both containing the complete antigen binding site present in mAb M75.

Monoclonal antibody M75 was obtained (3) and proved to react excellently with native and denaturated CA IX. Using synthetic oligopeptides, the epitope of mAb M75 was localized in the proteoglycan domain of CA IX, in the region of a tandem repeat and identified as amino acids PGEEDLP (4). The Fab fragment was obtained by papain cleavage. We obtained crystals of free Fab M75 and Fab M75 complexed with two different epitope peptides. The data set for Fab M75 was collected and the structure solving is underway.

Another approach is to prepare sc Fv fragment of this antibody (described in the contribution of Vlastimil Král, Milan Fábry, Magda Hořejší, Jan Zavada, Juraj Sedláček: Molecular cloning, *E. coli* expression and purification of scFv antibody fragments of diagnostic/therapeutic interest.

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A LONG WAY TO WELL DIFFRACTING PROTEIN CRYSTALS

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In experiments of protein crystallization very often spherulites, microcrystals and needles appear as a result of not having optimal crystallization conditions. Optimization of conditions is often guided by a sense of protein, however, theoretical knowledge and practical experiences are inevitable. It is well known that the best conditions for growing crystals differ from crystal nucleation conditions. Separation of these two processes in order to obtain well diffracting crystals will be presented. The importance of protein purity and homogeneity in growing crystals will be stressed and procedures improving crystal quality will be discussed. One of the topics will concern practical aspects for preparation of protein crystals for data collection at room and cryogenic temperature. All these points will be documented by our experiences with crystallization of a number of proteins, their mutants and complexes.