Abstracts

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ATOMIC RESOLUTION STRUCTURE OF TWO TRYPSIN INHIBITORS

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Crystallographic studies of macromolecules at atomic resolution provide unprecedented wealth of data at unprecedented detail. It is possible to relax or remove some of the stereochemical restraints that are necessary at lower resolution. The use of full-matrix refinement allows to estimate the variances in structural parameters and, in consequence, to evaluate the standard restraints themselves. The results indicate that some of the standards need revision. The peptide bond shows large deviations from planarity, and the N-Ca-C angle has a wide spread. Anisotropic displacement parameters and resolution of electron density of individual atoms make observations of unusual structural features reliable. Double conformations can be resolved, and solvent can be modeled beyond the first hydration shell. The lecture will illustrate the achievements of atomic resolution protein crystallography using the structures of two trypsin inhibitors: CMTI, of plant origin, and BPTI, of mammalian origin, determined, respectively, at 1 and 0.86 A. Although the R-factors are similar (0.11 and 0.10, respectively), there is a dramatic improvement of quality when resolution is extended from 1 to 0.86 A. There are clear H-atom difference Fourier peaks (also in water molecules) in the BPTI structure, but not in the CMTI structure. Some of them correspond to C-H…X and N-H…pi bonds. The estimated errors in bond lengths are 0.005-0.01 (BPTI) and 0.01-0.02 A (CMTI). In the CMTI structure, there is an unexpected zinc cation coordinated by four Glu groups, two of which are protonated. Two histidine residues have different tautomeric forms. In the BPTI structure, there is a double-conformation disulfide bond.

STRUCTURAL AND ENERGY COMPARISON OF ETHYLENAMINE INHIBITOR BINDING TO THE WILD TYPE AND MUTANT HIV-1 PROTEASES

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X-ray structures of wild type (resolution 2.45 Å) and mutant HIV-1 protease (A71V, V82T, I84V, resolution 2.2 Å) complexed with newly developed inhibitor Boc-Phe-[CH2CH2NH]-Phe-Glu-Phe-NH2 have been recently solved 1,2. In this contribution, the inhibitor OE binding modes to both proteases are compared. OE shows tight binding to the wild type (K_i = 1.5 nM) as well as mutant (K_i = 4.1 nM) protease and the binding modes of the inhibitor to the wild type and mutant proteases are similar at the first view. Several differences have been determined by structural and energy analysis. Conformational analysis was done to explain disorder observed in the mutant complex.

Results of structural analysis: The hydrogen bonds to catalytic aspartates Asp 25 and Asp 125 formed in the case of hydroxyethylamine inhibitors by isosteric hydroxyl group are in the case of the ethylenamine inhibitor OE replaced by hydrogen bonds from isosteric NH group. In the mutant complex, the isosteric NH group forms hydrogen bonds to both catalytic aspartates. In the wild type complex, the isosteric NH group forms a hydrogen bond to Asp 125 and an intramolecular hydrogen bond to carbonyl group of Boc.

Results of energy analysis: There is a similar distribution of interaction energies between the inhibitor OE and the protease in both complexes. The larger differences were found in interactions between the mutated residues and OE: Weaker van der Waals interactions of the mutated residues Val 84 and Val 184 with OE were found in the mutant protease. The lack of interaction energy is partly compensated by new aromatic hydrogen bond between Phe of the inhibitor in position P1 and the mutated residue Thr 182.

Results of conformational analysis: Thr 82, Val 84, Thr 182 and Val 184, i.e. all mutated residues which have direct contact with the inhibitor, were found in alternative conformations in the mutant complex. Additionally, the inhibitor can bind to the active site in two alternative opposite orientations. Energy analysis based on molecular mechanics showed that all alternative conformations of Thr 82, Val 84 and Val 184 have similar interaction energy with the inhibitor and probably there is no relation between the inhibitor orientation and the conformations of these three mutated residues. On the contrary, aromatic hydrogen bond exists between Thr 182 and OE. This interaction is energetically strongly favorable and, probably, orientation of OE binding to the protease determines definitely the conformation of Thr 182.

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COMPUTER MODEL OF DNA B - POLY(L-LYSINE) INTERACTION

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The aim of our research is to contribute to the elucidation of the structure and polyelectrolyte interaction of plasmid DNA with poly(L-lysine). Complexes of DNA with polycations were proposed as nonviral vectors for the delivery of genes. Future gene delivery vectors of this type should be designed to be able to protect DNA during transport in body fluids and the cytoplasm of the target cell. Finally the pDNA has to be released before transcription and translation of a therapeutic protein [1]. Computer modelling of coupling of a fragment of DNA double helix with the synthetic polycation, poly(L-lysine), was the first step in our attempt to describe the interaction between natural and synthetic polyelectrolytes within a polyelectrolyte complex. The starting model was based on our physico-chemical experiments where the charges of the DNA chain were compensated [2].

Preliminary computer experiments were performed with monomers bearing various positively charged groups in the side chains: primary, secondary, and tertiary amino or quaternary ammonium groups. Dimethyl phosphate was used as the model anion. *Ab initio* quantum mechanic and molecular mechanic calculations have shown the highest binding energy, that probably controls stability of the complex for the phosphate-primary amine pair.

In the next step, the system of an eleven-base-pair DNA BDAT fragment and hexamer of the positively charged L-lysine cation were introduced into the simulation procedure. The suite of Biosym/MSI programs was utilized, particularly, Insight II and Discover [3]. The cvff force field was chosen because the formal charges along the molecules were in relative good agreement with our MP2/6-31G* quantum mechanic results describing binding of positively charged L-lysine with a negatively charged dimethyl phosphate fragment. Generic distances between six amine and dimethyl phosphate groups were introduced with upper bounds of 5 Å. Steepest descent (22,000 iterations) and conjugate gradients (10,000 iterations) of potential energy minimization were stopped when the shifts in coordinates were lower than 0.01 kcal/mol.Å.

Our proposed model shows the flat poly(L-lysine) strand embedded into the major groove of the DNA BDAT molecule. The amine groups are in a good contact with the dimethyl phosphate groups with the average distance between oxygen and nitrogen atoms of 3.81(5) Å. The hydrogen bonds of poly(L-lysine) pointing to the major groove of the DNA BDAT are thus also possible.

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