Four haloalkane dehalogenase genes of different origin were used to construct a hybrid gene: *dhlA* cloned from *Xanthobacter autotrophicus* GJ10 [4], linB from *Sphingomonas paucimobilis* UT26 [5], *dhaA* from *Rhodococcus rhodochrous* NCIMB13064 [6] and *dhmA* from *Mycobacterium avium* N85 [7]. The technique called Degenerate Oligonucleotide Gene Shuffling was used for *in vitro* recombination of four different genes [8]. Altogether twelve hybrid genes were constructed using one pair of degenerate oligonucleotides.

For preliminary characterization, hybrid proteins were expressed in *Escherichia coli* BL21(DE3) and tested in the resting cells assay for activity towards six halogenated aliphatic compounds. Four out of twelve hybrid proteins keep good expression and ten proteins showed obvious catalytic activity. Comparison of relative activities determined for the hybrid enzymes with the activities of wild type enzymes suggests that constructs do not possess novel substrate specificities.

All hybrid genes were cloned to pET-32(a) vector to support high level of expression an stability of hybrid proteins. His-taggged tail was introduced to C-terminus of hybrid proteins. All hybrid haloalkane dehalogenases were successfuy expressed in fusion with thioredoxin in host cells *E. coli* HB101. Optimization of purification conditions on Ni-NTA agarose and cleavage of hybrid protein-thioredoxin complexes is under progress.

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TOWARDS TRUE SSTABILIZATION ENERGIES OF H-BONDED AND STACKED DNA BASE PAIRS

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The study of binding energies of Adenine...Thymine and Guanine...Cytosine base pairs in vacuo was aimed to get an information on relative order of different structure motives on the energetical scale with special focuse on differences between H-bonded and stacked structures. We also aimed to find a general way to reliable interaction energies of the weakly bonded complexes.

Structures taken from previous MD/quench studies were fully optimized at RI-MP2 [1,2] level with TZVPP [5s3p2d1f]/[3s2p1d] basis set. To approach complete basis set (CBS) limit, convergency of both HF energy and MP2 correlation energy was studied employing augmented correlation-consistent basis sets aug-cc-pV(D,T,Q)Z. Is was found that molecular interaction energies close to the CBS limit may be obtained by 2-point extrapolation [3] using aug-cc-pVDZ and aug-cc-pVTZ basis sets.

To account for higher order correlation effects convergency of CCSD(T) MP2 correction term (E_{corr}^{MP2} - E_{corr}CCSD(T)) was investigated. For the formamide… .formamidine complex (Fig. 1) which is a model for adenine..thymine interaction MP2 and CCSD(T) correlation interaction energies were evaluated with various basis sets up to aug-cc-pVTZ (Fig. 2). It was shown that unlike the correlation energy itself the CCSD(T) MP2 difference is almost basis set independent. Rather accurate values were obtained with relatively small 6-31G*(0.25) and cc-pVDZ(0.25,0.15) basis sets. Because the latter one performs well also for stacked complexes [4] it can be recommended for evaluation of the term of extended complexes possessing both H-bonded and stacked structures.

Interaction energies of the DNA base pairs obtained by combination of the CBS extrapolations of MP2 interaction energies and the CCSD(T) MP2 correction are in good agreement with experiment.

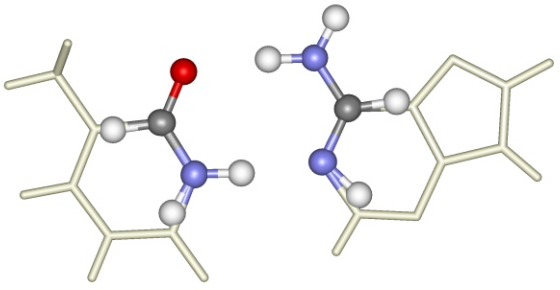

Figure 1

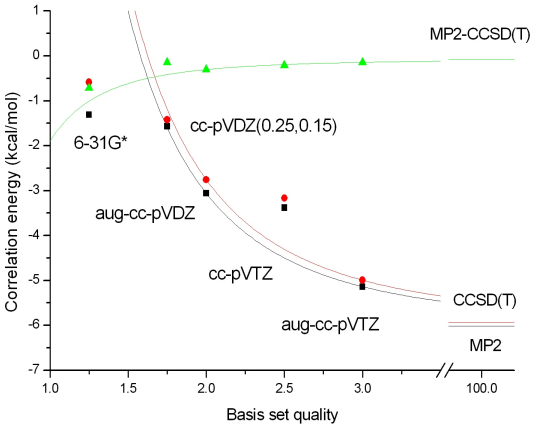

Figure 2

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POTENTIAL ENERGY SURFACES OF GUANINE - CYTOSINE BASE PAIR AND RELATED TAUTOMERS: MOLECULAR DYNAMICS AND AB INITIO STUDY

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Introduction:

The structure of DNA is determined among other factors by interactions between nucleic acid (NA) bases: guanine (G) , cytosine (C), adenine (A) and thymine (T). A theoretical study of the interaction is important for understanding of stabilizing forces in DNA and RNA. The interaction of NA bases in a vacuum in now being studied in experimental laboratories [1-4] and a knowledge of the potential energy surfaces is essential for an interpretation of experimental results. This information can be obtained by performing correlated *ab initio* calculations in combination with molecular dynamics/quenching technique (MD/Q) [5-6].

Methods:

1. *Molecular dynamics/ quenching calculations* were carried out in the NVE canonical ensemble (Constant number of particles, volume and energy) employing Cornell *et al.* AMBER force field [7], which gives results comparable with *ab initio* data [8]. Due to comparable stability of several cytosine and also guanine tautomers, all possible combinations of these tautomers should be considered. Only the most stable (stabilization energy higher than 18 kcal/mol) and populated (population greater than 5%) structures of base pairs were taken for further *ab initio* calculations.

2. *Ab initio calculations*. The geometries, interaction and tautomerization energies of base pairs were determined on RI-MP2 level employing TZVPP (5s3p2d1f/ 3s2p1d) basis set.

Results:

In all cases planar H-bonded structures are the most stable and most populated ones. The T-shaped and stacked structures are about several kcal/mol less stable (typically 5-10 kcal/mol) than the structure of the global minimum and will not be probably detectable by experimental technique.

Among all possible combinations of tautomers the highest stability shows canonical Watson-Crick (WC) structure (-26.9 kcal/mol) followed by the same binding pattern with N7 keto tautomer of guanine Also other binding patterns of ketoguanine-ketocytosine tautomers are very stable. The structures of other combinations of tautomers are usually less stable (about 4-5 kcal/mol) than the WC pair, including ketoguanine-enolcytosine structure observed in the experiment [2]. An exception is an enolguanine - ketocytosine nonplanar structure with surprisingly high stability (-25.3 kcal/mol), but due to unfavorable geometry and stability of the enolguanine tautomer itself, this structure will not be probably detectable.

Summary:

We have presented a powerful technique for scanning of potential energy surfaces of nucleic acid base pairs, which can be used for analysis of experimental results. It is demonstrated that the use of standard procedure based on chemical feeling and experience is not sufficient and several mainly unusual structures can be omitted.

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