digesting *S fibuligera* glucoamylase isolated from an original producer

The *Schwanniomyces occidentalis* glucoamylase-encoding gene *GAM1* was isolated from a λ Charon4A genomic library using synthetic oligo probes *GAM1* gene encodes a polypeptide of 958 amino acids which has no homology to all other glucoamylases which are sequenced thus far and have highly conserved regions in common (Dohmen *et al* 1990) On the other hand GAM glucoamylase shows a high sequence similarity to *S fibuligera* α-glucosidase According to the classification of glycosyl hydrolases based on amino acid sequence similarities Gam glucoamylase from *Sch occidentalis* belongs to family 31 The tertiary structure of hydrolases belonging to this group has not been determined, yet *Sch occidentalis* glucoamylase and *S fibuligera* α-glucosidase would be an excellent model for investigation of differences in the mode of action of α-glucosidases and glucoamylases on substrates having different lengths

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Molecular Dynamics Simulations of Nucleic Acids: A) Molecular Dynamics simulations of the Oligonucleotide with the Modified Phosphate/phosphonate Internucleotide LINKAGE

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Key words: molecular dynamics simulations, oligonucleotides

Impact of the internucleotide linkage modification by inserting a methylene group to the P-O bond, on the modified oligonucleotide binding ability to the natural DNA strand was studied by molecular dynamics simulations (Barvík Jr *et al* 1998)

The concept of SNAIGE ("Synthetic Nucleic Acids Interfering Gene Expression") represents a perspective approach in chemotherapy, promising to inhibit selectively unwanted gene expression by creation of a helical complex with target mRNA or DNA. This method is expected to become more efficient than inhibition of the resulting protein products by classical drugs (Wagner 1994).

Oligonucleotides with natural chemical composition have been, however, found as unsuitable for in vivo applications because of their insufficient resistance against endonucleases: they are degraded with a half-life around 20 min. That is why novel oligonucleotides with various chemical modifications are proposed, synthesised and tested (Wagner 1994).

Molecular dynamics simulations (MDS) allow chemists to predict or at least to estimate properties of newly proposed species and to decide for which of them the preparation technology should be developed.

The investigated model system consisted of two undecamer chains – natural and modified. The modified chain was built from 11 deoxyadenosine units connected by alternating natural (phosphate) and modified (phosphonate) internucleotide linkage with the natural one at the 5'end. The alternating linkage was chosen as the most coherent case with the tentatively synthesised species. Deoxythymidine undecamer was used as the natural complementary strand.

The Biosym software package represents a sophisticated tool for performing MDS of natural nucleic acids and proteins. The Amber force field does not contain explicitly the parameters needed to describe modified parts of the oligonucleotide structure (Cornell *et al* 1995). As the automatic filling up of the missing parameters by the Biosym leads to evidently wrong results, the force field was enlarged (Barvík Jr *et al* 1998).

We started with wide set of initial conformations, constructed by setting torsion angles (3) in the -O-PO2-CH2-O- chains of internucleotide linkages to all possible combinations of -g (-60°), g (60°), t (180°) orientations.

Our MDS simulations (Barvík Jr *et al* 1998) demonstrated that the oligo-deoxyoligonucleotide chain with alternated natural and modified (in the manner described above) internucleotide linkage, can create a stable double-helical complex with a natural oligonucleotide counterpart (it's melting temperature is remarkably above 300 K). It confirms predictions of the published studies dealing with a single modified linkage (Štrajbl and Florián 1996, Mojzeš *et al* 1995, Štěpánek *et al* 1997), that the nonisostericity of the modification is compensated by an increase of flexibility and so the compatibility with natural nucleic acids should not be lost.

The conformationally richer and more flexible internucleotide linkage leads, however, to a less stable conformation of the oligonucleotide duplex. While the unmodified (thymidine) strand possessed a B-type secondary structure, the conformation of the modified (adenosine) strand was not completely stable at 300 K.

The -ggt conformation, that was found as the most favoured for the oligonucleotide duplex, was also obtained as the most stable in the case of MMP model chain, when the influence of a polar environment was considered (Štrajbl *et al* 1997) (gas phase global energy minimum of the MMP differs (-ggg) (Štrajbl and Florián 1996).

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B) Molecular Dynamics Simulations of the UACG Tetraloop in the very short Hairpin

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The structure of RNA is as varied as its functions The single-stranded RNA molecule often folds back on itself to form double helical stem capped by a loop of non-Watson-Crick paired or unpaired nucleotides of various sizes, which are thought to provide tertiary recognition sites for both proteins and nucleic acids

The four-base loops cap many double-helical structures in rRNA Although 256 different tetraloop sequences are possible, nearly 70% of all the four-base loops in rRNAs are either UNCG or GNRA (where N is any nucleotide and R is a purine), with extraordinary high melting temperatures in comparison with similar RNA sequences (Woese *et al* 1990)

The goal of our MDS was to make clear remaining questionable structural features of the UACG tetraloop in the very short hairpin (Abdekalfi *et al* 1997), for which an