The Report on Short Visit Nr. 738 in the frame of the ESF EIPAM program

The Matrix assisted laser desorption-ionization technique (MALDI) provides possibility of non-destructive ionization of large biological molecules. Although this technique is widely used as an analytical tool in biochemistry for analysis of large biological polymers like proteins or DNA, the processes behind desorption / ionization are not well understood.

In present work we have study fragmentation channels in post source decay (PSD) of oligonucleotide anions. To study fragmentation processes between selected nucleotides we have used oligonucleotides containing six nucleotides. Two thymidine nucleotides which are known to have better stability against fragmentation were placed on both ends of this chain. The studied pair connection was between the nucleotides in the middle of the chain. The measurements were done on following oligonucleotides, nucleotides are in 5' to 3' order:

1. TTCGTT
2. TTGCTT
3. TTACTT
4. TTCATT
5. TTAGTT
6. TTGATT
7. TTTTTT

The MALDI instrument used for this work is a Bruker Reflex IV equipped with a reflectron type Time Of Flight mass spectrometer. This provides a possibility of PSD analysis by using a two step mass analysis. In the first step the parent oligonucleotide anion is selected and flown through the spectrometer. During this flight it may dissociate by PSD process. On the other end of spectrometer the ions are reflected and reaccelerated and their flight time, which is mass dependent, is measured. The mass spectrum of ionic products of post source decay is obtained.

The most important fragmentation channels for oligonucleotide anions $TTXYTT^-$ have found to be rupture between bases X-Y leaving the charge on TTX or on YTT

fragment. The intensity ratios of TTX^- and YTT^- ions for oligonucleotides (1) to (6) are shown in the table 1. For oligonucleotide (7) no TTX^- fragment has been observed at laser power used.

Table 1.

Oligonucleotide $^{5'}$TTXYTT$^{3'}$	TTX fragment	YTT fragment	Intensity YTT^- / TTX^-
TTCGTT	TTC	GTT	1.63
TTGCTT	TTG	CTT	1.35
TTACTT	TTA	CTT	0.80
TTCATT	TTC	ATT	4.21
TTAGTT	TTA	GTT	0.77
TTGATT	TTG	ATT	4.42

From obtained intensity ratios we can see that for oligonucleotides (1) and (2) the charge is left preferably on YTT fragment. For oligonucleotides (3) to (6) where adenine is present, the charge is left preferably on fragment containing adenine. Especially preferable has been found production of ATT^- anion.

Another channels observed are loss of one base from X or Y nucleotide without braking the backbone chain.

Table 2.

Oligonucleotide $^{5'}$TTXYTT$^{3'}$	Base loss from X	Base loss from Y	Intensity loss X / loss Y
TTCGTT	M-C	M-G	1.0
TTGCTT	M-G	M-C	1.0
TTACTT	M-A	M-C	0.4
TTCATT	M-C	M-A	2.6
TTGATT	M-G	M-A	3.6

We have observed that loss of cytosine and guanine from oligonucleotide anion (1) and (2) have approximately the same intensity. For oligonucleotides (3), (4) and (6) the loss of adenine is preferred. Here, however, for further evaluation more precise measurements with better accuracy are necessary. The obtained results will be published.
This visit gave me a unique opportunity to get experience with the MALDI technique and also to make personal contacts. The agreements about further cooperation have been done.

In Bratislava, 27.8. 2005 Michal Stano