

7 SUMMARY

Due to its toxicity, the use of daunomycin, an anthracycline antibiotic that is widely used in cancer therapy, poses a risk for everyone involved with the substance. Therefore, a strict surveillance of the working area of producers, pharmacists, and hospital staff is equally necessary as monitoring the actual dose of the drug in a patient's blood. Since a relevant proportion of administered daunomycin is excreted unmetabolised, contaminated waste water can also have an impact on the environment. For that reason, waste water analysis, especially of hospital waste water, is of great concern.

This work focussed on the selection of daunomycin-specific DNA aptamers, which were isolated from a combinatorial library with a randomised region of 40 nucleotides. Aptamers were selected using a semi-automated selection procedure that was established for aptamer generation on magnetic particles. This procedure utilised the robotic workstation BioSprint 15. To monitor the course of the selection without the use of radioactivity, two assays were developed. The “fluorescence dye-linked aptamer assay” (FLAA) allows for monitoring the enrichment of binding sequences by the use of a single-stranded DNA-specific fluorescence dye. With the “diversity assay for nucleic acids” (DANA) the decrease in diversity of an oligonucleotide pool can be observed. Combination of both assays provides information of when the selection has reached a final state and when to proceed with the separation of aptamer sequences. For the daunomycin-selection, the decrease in diversity of the nucleic acid pool clearly coincided with the increase of binding molecules.

After ten rounds of selection, 24 aptamers were isolated and, according to their sequence, could be divided into seven different groups. All aptamers proved to be high-affinity binders in FLAA experiments. A strong bias for guanine in the sequence was revealed as well as strong sequence homologies among all sequences.

The best binder identified in FLAA experiments was further characterised. Its dissociation constant was determined to be 20 nM, which makes it superior compared to other aptamers against small molecules. Most DNA aptamers to small molecules that have been described showed dissociation constants in the micromolar range. The aptamer's binding characteristics under different buffer conditions and pH values were also evaluated. The binding proved to be independent of the presence of distinct salts, as long as the concentration of counter ions was sufficient. Binding at different pH revealed an optimum curve with the best binding observed at pH 6.0. Denaturation prior to application was not necessary for an efficient binding; immobilisation via 5'-end was possible without loss of function. The aptamer proved to be specific for the anthracyclines daunomycin and doxorubicin, the C-14-hydroxy derivative of daunomycin, with the affinity for doxorubicin being even higher.

In addition, two assays based on aptamers were established for daunomycin detection. A dipstick test on nitrocellulose was developed, which allowed for the qualitative detection of 5 µM daunomycin. For the proof of principle, the colorimetric detection was achieved by manual addition of an enzyme substrate. For an easy and rapid application, however, the detection mode should be further optimised to avoid a second manual handling step. Furthermore, a competition assay in microtitre plate format was established, which permitted the detection and quantification of up to 15 nM daunomycin and

doxorubicin, respectively. Based on its sensitivity, this assay would be suitable for monitoring the daunomycin concentration in patients' serum – particularly since tests performed in 10 % foetal calf serum revealed that the aptamer remains stable in diluted serum samples at least for the duration of the assay.