7. Summary

The catalytic activity of ribonucleic acids is essential for probable prebiotic processes. The formation of C-N bonds is most important for the metabolism in a hypothetical RNA world. This work describes the development of a selection scheme for the isolation of a new ribozyme for the N-glycosidic bond formation between an activated sugar phosphate and a nucleobase. This catalytic RNA should be isolated from a combinatorial RNA library with the use of *in vitro* selection techniques. The principle of direct selection was extended to "direct selection with linkercoupled reactants".

This new selection scheme requires the sitespecific introduction of one reactant via a flexible linker to all RNA-pool members. Using T4 RNA ligase, a method for enzymatic introduction of synthetic linkers to the 3´end of RNA was developed. Nicotinic and orotic acid could be directly and specifically attached to the 3´end of RNA via a flexible linker.

Those molecules that catalyzed the reaction between the bound nucleobase and the activated sugar phosphate acquire automatically a cis-diol group. So the catalytic RNA molecules could be separated from inactive species via oxidation with periodate, immobilisation on a hydrazine modified solid phase and subsequent laser induced cleavage or affinity gelelectrophoresis. The RNA part of the isolated products was enzymatically amplified and subjected to further rounds of transcription, conjugation and selection. No catalytically active RNA molecules could be isolated.

RNA is predestinated for the use as a biosensor due to it´s precise molecular recognition, fast formation of structures and enzymatic function.

In this work a dynamic system was developed for the direct and nonradioactive detection of theophylline on the basis of the hammerhead ribozyme and the theophylline aptamer. The time resolved kinetic analysis of theophylline dependend ribozyme activity could be performed with the use of the "molecular beacon" technology.

Our sensor consists of a ribozyme part and double modified substrate, which is selectively cleaved in the presence of theophylline.

The increase of fluorescence was proportional with the cleavage rates and therefore an indicator of the actual theophylline concentration.

Theophylline could be detected in concentrations of 0.01 - 2 mM. Coffeine did not influence the system.

This method was developed on the basis of RNA technology and modern fluorescence detection strategies and is well suited for further refinements and improvements, and the extension to a variety of other analytes.