Characterization of the LysR-type Transcriptional Regulator HsdR Gene and Its Adjacent Short-chain Dehydrogenase/Reductase SDRx Gene in *Comamonas testosteroni* ATCC 11996

MSc Wenjie Gong 1. Berichterstatter: Prof. Dr. E. Maser

3α-hydroxysteroid dehydrogenase/carbonyl reductase (3α-HSD/CR) from *C. testosteroni* is a key enzyme in the degradation of steroid compounds in soil and water. Interestingly, 3α-HSD/CR gene (*hsdA*) expression can be induced by steroids like testosterone and progesterone. Thus, the regulatory mechanism of 3α-HSD/CR induction attracted our interests. Previously, it has been shown that induction of *hsdA* expression by steroids is a derepression where steroid inducers bind to two repressors, RepA and RepB, thereby preventing blocking of *hsdA* transcription and translation, respectively.

In the present study, a new LysR-type transcriptional factor HsdR for 3α-HSD/CR expression in *C. testosteroni* has been identified. The *hsdR* gene locates 2.58 kb downstream from *hsdA* on the *C. testosteroni* ATCC 11996 chromosome with an orientation opposite to *hsdA*. The *hsdR* gene was cloned and the recombinant HsdR protein was overproduced, and an anti-HsdR polyclonal antibody was subsequently prepared. While heterologous transformation systems revealed that HsdR activates the expression of the *hsdA* gene, electrophoretic mobility shift assays (EMSA) clearly showed that HsdR specifically binds to the *hsdA* promoter region. Interestingly, the activity of HsdR is closely associated with *hsdA* repression by RepA in that HsdR acts after release of RepA from the *hsdA* promoter. Furthermore, *in vitro* binding assays clearly showed that HsdR can contact with RNA polymerase. Interestingly, an *hsdR* disrupted mutant expressed low levels of 3α-HSD/CR compared to wild type *C. testosteroni* after testosterone induction. In addition, HsdR itself cannot be induced by testosterone. As a member of LysR-type regulators, HsdR may also repress it own expression. Here, electrophoretic mobility shift assays indicated that HsdR specifically binds to its own promoter, and two conserved LTTRs motifs, HsdR site 1 and HsdR site 2, are found in the *hsdR-SDRx* intergenic region. As expected, mutated HsdR expression in an *hsdR-gfp* fusion mutant and an *hsdR* gene disrupted mutant of *C. testosteroni* increased compared to the wild type strain, largely because autorepression of these HsdR mutants is prevented. This result revealed that HsdR negatively regulates its own expression. Phylogenetic analyses indicated that HsdR is related to the contact-regulated gene A (CrgA) from *Neisseria meningitidis*, which exists as an octamer. To further understand the active form of HsdR, three truncated proteins, HsdRΔN (residues 1-86 deleted), HsdRΔC (residues 221-303 deleted), and HsdRΔNC (residues 1-86 and 221-303 deleted), were constructed and purified. These deleted domains are important for the positive control of HsdR on 3α-HSD/CR expression. Western blotting indicated that HsdR may also exist as an octamer, where the central domain is crucial for the multimerization of HsdR. Unexpectedly, gel filtration chromatography showed that there are two dominant oligomers (octamer and hexamer) present for HsdR and its truncated proteins. Taken together, HsdR is a positive transcription factor for 3α-HSD/CR expression in *C. testosteroni*, and it may also negatively regulate its own expression.

In addition, a novel gene *SDRx*, which is divergently transcribed from *hsdR,* was found to be a member of the short-chain dehydrogenase/reductase (SDR) superfamily. The open reading frame of this *SDRx* consists of 768 bp and translates into a protein of 255 amino acids. Two consensus sequences of the SDR superfamily were found, an N-terminal Gly-*X*-*X*-*X*-Gly-*X*-Gly cofactor-binding motif and a Tyr-*X*-*X*-*X*-Lys segment (residues 160-164 in the SDRx sequence) essential for catalytic activity of SDR proteins. Phylogenetic analyses indicated that the novel SDRx gene codes for 7α-hydroxysteroid dehydrogenase (7α-HSD) in *C. testosteroni* which is active in steroid metabolism. Degradation of the steroids testosterone and estradiol decreased in the *SDRx* knock-out mutant. Furthermore, growth on the steroids cholic acid, estradiol and testosterone was impared in the *SDRx* knock-out strain. Combined, the novel SDRx in *C. testosteroni* was identified as 7α-HSD that is involved in steroid degradation.