Steroid-induced regulation of 3α,20β- and 3β,17β-hydroxysteroid dehydrogenase activity in wild type and mutants of *Streptomyces hydrogenans*

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Abstract. The effect of estradiol, hydrocortisone and progesterone on 3α,20β- and 3β,17β-hydroxysteroid dehydrogenase (HSD) in mutants of *Streptomyces hydrogenans* was compared to the steroid response of the wild type. Mutants were defective in arginine biosynthesis and/or aerial mycelial formation and lacked both enzymes or only 17β-HSD. Some 17β-HSD mutants had lost the ability to be induced by estradiol, by progesterone or by both. Some 20β-HSD mutants had lost the ability to be induced by hydrocortisone, by progesterone or by both. Non-inducibility of 17β- and 20β-HSD by progesterone was not co-ordinate. An additional study of the growth phase-dependent enzyme activity of the wild type after induction with estradiol, hydrocortisone and progesterone was performed.

Key words: Enzyme induction — Steroid transformation — Mutagenesis — Aerial mycelial formation — Arginine auxotrophy — *Streptomyces hydrogenans*

To extend our knowledge about steroid-dependent enzyme induction in this microorganism, the effects of estradiol, hydrocortisone and progesterone on 17β- and 20β-HSD in mutant strains of *S. hydrogenans* were investigated.

Materials and methods

Strains and culture conditions. A wild type isolate of *Streptomyces hydrogenans* ATCC 19631 (Lindner et al. 1958) designated as strain HY 0 and several of its mutants were used. The strains were maintained on oatmeal agar (Wacker et al. 1970) or as lyophilized conserves. Cells were grown in batch cultures in complete medium (g/l; yeast extract, Difco, 5; Bacto peptone, Difco, 5; glucose, 10; NaCl, 2.9; KH₂PO₄, pH 7.4, 2.7) on a rotary shaker at 30 °C and 150 rpm. Aliquots of an over-night culture were used to inoculate cultures containing 500 ml complete medium with a dilution ratio of 1:50. Growth was checked by measuring the absorbance at 420 nm. Minimal agar (g/l; yeast nitrogen base w/o amino acids, Difco, 7; glucose, 10; NaCl, 10; Bacto agar, Difco, 20; KH₂PO₄, pH 7.4, 5) and minimal agar supplemented with amino acids, purine and pyrimidine bases (1 mM) were used to identify prototrophs and auxotrophs.

Isolation and characterization of the mutants. Stationary phase *S. hydrogenans HY 0* cells grown in complete medium were washed twice in 70 mM NaCl, 40 mM Na₂HPO₄, 20 mM KH₂PO₄, pH 7.4. Aliquots were removed, centrifuged and resuspended in the same buffer containing acridine orange (AO), ethidium bromide (EBR), ethyl methane-sulfonate (EMS) or N-methyl-N'-nitro-N-nitrosoguanidin (MNNG). After 2 h at 30 °C the cells were harvested, washed four times, diluted and plated onto YPD agar (Esposito et al. 1969). Auxotrophs and mutants defective in aerial mycelial formation (Amy; Redshaw et al. 1979) were characterized by replica plating on minimal agar or by transferring on oatmeal agar, respectively. Penicillin G (50 μg/ml minimal medium) was used to enrich auxotrophs during a 30 h incubation. Dilution, plating and replica plating were repeated several times to obtain pure mutant clones. Spontaneously formed mutants were derived from 3–6 weeks-old oatmeal agar cultures after an additional incubation on YPD agar for 10–15 days. Reversion analysis was performed with 10⁸–10⁹ colony forming units on minimal agar (auxotrophs) and oatmeal agar (Amy-mutants).

Enzyme induction. Steroids dissolved in a final volume of 2 ml ethanol/DMSO (9:1, v/v) were added to the 500 ml
cultures 4 h after inoculation in the following final concentrations: estradiol 150 μM, hydrocortisone 300 μM and progesterone 150 μM. Control cultures received the same volume of steroid free solvent. Cells were harvested 3 h later by centrifugation, washed twice in 10 mM Tris-HCl, pH 7.4, 10 mM KCl, 1.5 mM MgCl₂ and stored at -80°C. Growth phase-dependent 17β- and 20β-HSD activity was analysed in the same way, with the exception that the steroids were added to the cultures at different times.

**Assays.** About 1.5 g frozen cells were washed twice in 10 mM Tris-HCl, pH 7.4 and were resuspended in 3 ml of the same buffer containing 1 mM NaN₃. 17β- and 20β-HSD were assayed in the S₁₆₅-supernatant of ultrasonic cell homogenate (Betz et al. 1976) according to Markert and Trüger (1975) and Wacker et al. (1970). Protein was quantified according to Lowry et al. (1951).

**Results**

*Growth phase-dependent 17β- and 20β-HSD activity in the wild type.* Growth phase-dependent 17β- and 20β-HSD activity in *S. hydrogenans* HY 0 was examined during a period of 30 h. Basal 17β- (Fig. 1 A) as well as 20β-HSD activity (Fig. 1 B) was not essentially altered during this period and no significant effects of hydrocortisone on 17β-HSD (Fig. 1 A) and of estradiol on 20β-HSD (Fig. 1 B) were observed. The strongest induction of 17β-HSD by estradiol was obtained during early log-phase (4 to 7 h after inoculation; Fig. 1 A). Estradiol-dependent 17β-HSD activity was reduced from ca. 950 to about 500 μkat/kg during the stationary phase. In contrast, progesterone-induced 17β-HSD activity was mostly enhanced during a relatively short period of late log-phase (Fig. 1 A). A maximum activity of ca. 750 μkat/kg was obtained. Hydrocortisone- and progesterone-dependent 20β-HSD activity reached its maximum at the same time during late log-phase (Fig. 1 B).

**Amy-mutants and auxotrophs.** Unusual high frequencies of mutants with the phenotype Amy⁻ and Arg⁻ were obtained spontaneously, as well as after treatment with different chemical mutagens (see Table 1). Four auxotrophs besides Arg-mutants could also be isolated (2× Leu⁻, 1× Met⁻, 1× Ilv⁻). With the exception of the Ilv⁻-phenotype (not listed), which reverted with a frequency of \( \leq 10^{-7} \) to Ilv⁺, no reversion was observed.

**17β- and 20β-HSD mutants.** Spontaneous loss of 17β- and 20β-HSD was examined with 116 colonies following growth on oatmeal yeast peptone dextrose (YPD) agar as described above. 14% of these colonies lacked 17β-HSD activity before purification. Nevertheless no pure 17β-HSD mutants which did not exhibit the Amy⁻ (Arg⁻)-phenotype could be isolated: Either an additional Amy⁻ (Arg⁻)-phenotype or a wild type-like 17β-HSD activity was obtained during the following purification. Spontaneous loss of 20β-HSD was not detected. Also after mutagenization, stable 17β- and 20β-HSD mutants were only obtained among the Amy⁻ and auxotrophic isolates. Treatment of the wild type with AO and EBr resulted generally in a total loss of 17β-HSD among the Amy⁻ (Arg⁻)-mutants. Ca. 90% of EMS- and 30% of MNNG-induced Amy⁻ (Arg⁻)-mutants lacked 17β-HSD, too. Total loss of 20β-HSD, however, was observed only in two mutants (not listed in Table 2). Mutants listed in Table 2 were selected from the isolates of Table 1 with respect to two common features:

1. 17β- and 20β-HSD phenotypes were stable within a minimum of one year. During this period the markers were reexamined every third month. Maximum deviations of ca. 5% concerning absolute enzyme activity were observed during this period and identical results were obtained in the case of mutants lacking any activity.

2. The lag/log-transition after inoculating the cells to fresh complete medium was the same as that of the wild type (see Fig. 1 A and B). Generation times of ca. 80 min were obtained in all cases. Mutant log-phases were terminated ca. 8 h after inoculation.

Of special interest were mutants with an altered response to the inducers of 17β- and 20β-HSD (see Table 2):

17β-HSD mutants could be isolated which had lost the ability to be induced by estradiol (HY 64), by progesterone (HY 50) or by both steroids (HY 45). 20β-HSD mutants were obtained which lacked inducibility by hydrocortisone (HY 45, 66), by progesterone (HY 42) or by both steroids (HY 41, 48). In four cases no basal level of 17β-HSD (HY 34, 41) or 20β-HSD (HY 24, 25) was detected. Common to all mutant strains was the fact that the basal level of 20β-HSD was reduced (3 to 40 times). Furthermore the induced levels were reduced in all but one 20β-HSD