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PROPERTIES OF A 4-ENE-3-KETOSTEROID-5α-REDUCTASE IN CELL EXTRACTS OF THE INTESTINAL ANAEROBE, *EUBACTERIUM* SP. STRAIN 144*

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Summary—When *Eubacterium* sp. 144 was grown in the presence of progesterone, extracts of these cells contained a 4-ene-3-ketosteroid-5α-reductase (5α-reductase). No evidence for the presence of a 5β-steroid-reductase or a 5α to 5β-steroid-isomerase was found. 5α-Reductase activity was dependent on reduced methyl viologen as the electron donor and this could be generated biologically by adding pyruvate or H_2 to cell extracts or chemically by adding sodium dithionite. NADH or NADPH with or without flavin nucleotides were not electron donors for 5α-reductase. Most of the 5α-reductase activity (60–65%) of crude extracts was located in the membrane fraction and the enzyme was solubilized by treatment with 1% Triton X-100. Optimum 5α-reductase activity occurred at pH 7.0–7.5 in potassium phosphate buffer but was stimulated by Tris–HCl buffer (pH 8.0–9.0). 5α-Reductase activity was highest at 10% (v/v) methanol and was progressively inhibited by higher methanol concentrations. Sulfhydryl reagents strongly inhibited 5α-reductase but the enzyme was not affected by other metabolic inhibitors. Extracts prepared from cells induced with 16-dehydroprogesterone and grown without hemin contained 5α-reductase and 16-dehydroprogesterone-reductase activities equivalent to those found in extracts of induced cells grown with hemin. This indicates that hemin is not required for the synthesis of active steroid double bond-reductases in strain 144.

INTRODUCTION

Eubacterium sp. strain 144 was isolated from rat feces by Bokkenheuser *et al.* [1] and shown to catalyze the 16α-dehydroxylation-reduction of 16α-hydroxysteroids yielding 17α-derivatives. Isolation of a bacterium capable of this biotransformation confirmed the originally postulated mechanism for the formation of 17α-steroids in humans [2] and the role of the intestinal flora in this reaction [3–5]. The 16α-dehydroxylation-reduction sequence involves the participation of two enzymes 16α-hydroxyprogesterone dehydroxylase (16α-dehydroxylase) which dehydrates 16α-hydroxyprogesterone giving 16-dehydroprogesterone [6, 7] and 16-dehydroprogesterone-reductase (16-DHPR) which reduces 16-dehydroprogesterone giving 17α-progesterone [8].

Studies with resting cells revealed that, in addition to 16α-dehydroxylase and 16-DHPR, strain 144 also catalyzed the reduction of the 4-5-ene double bond by an enzyme designated progesterone-reductase (PR) and reduction of the 3-keto group by 3α-hydroxysteroid dehydrogenase (3α-HSDH) [9]. These other activities were not discovered until experiments were done with cells grown in the presence of hemin. Such cells showed maximum 16-DHPR and PR activities when provided with a suitable electron donor (pyruvate or H_2). Hemin was proposed to act as a precursor for the formation of a cytochrome-containing electron transport system that mediated electron flow from pyruvate or H_2 to the steroid double bond-reductases. Evidence from this study [9] and more recent results [8] have also shown that the syntheses of 16-DHPR and PR are induced by appropriate steroids; that of 16-DHPR requires steroids containing a 16-ene-20-ketone while that of PR requires a 4-ene-3-ketone.

When strain 144 was grown in the presence of hemin and progesterone, resting cells catalyzed

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the H_2-dependent reduction of progesterone giving 5α-pregnadione, 5β-pregnadione and 3α-hydroxy-5β-pregnanolone based on GLC identification of the products [9]. These results suggested that strain 144 might possess both 5α- and 5β-steroid-reductase activities hence the designation of this reaction as PR. The present study was undertaken to develop an assay for PR in cell extracts of strain 144 and to determine some of the properties of PR. The results show that strain 144 contains a readily detectable 4-5-ene-5α-steroid-reductase (5α-reductase) but 5β-steroid-reductase activity was not found.

MATERIALS AND METHODS

Cell growth and extract preparation

Eubacterium sp. strain 144 was grown as described by Watkins and Glass [8], except that progesterone, instead of 16-dehydroprogesterone, was added to the medium (final concentration of 10 μg/ml and 1% (v/v) methanol) to induce 5α-reductase synthesis. Cells were harvested using anaerobic precautions, suspended in 50 mM potassium phosphate buffer, pH 7.5 plus 1 mM dithiothreitol (DTT), and broken by passage through a French pressure cell [8]. The broken cells were centrifuged anaerobically at 10,000 ***g***, at 4°C, for 20 min to give crude cell extract. Unless indicated otherwise, crude extract was used for all 5α-reductase experiments. The membrane fraction was separated from the cytoplasm by centrifugation of crude extract at 105,000 ***g***, at 4°C, for 2 h. The supernatant was removed and the membrane pellet was suspended in a minimal volume of potassium phosphate buffer (above) containing 1% (v/v) Triton X-100. The membranes were incubated at 4°C for 30 min, recentrifuged (above), and the supernatant was removed. All protein-containing fractions were stored at 4°C under H_2 in serum bottles.

Enzyme assays

All assays were carried out under anaerobic conditions with continuous gassing by argon or H_2 [8]. Unless otherwise indicated, 5α-reductase activity was measured in reactions containing (final vol 2.0 ml) 100 mM potassium phosphate buffer, pH 7.5; 0.2 mM DTT; 2 mM methyl viologen (MV); 10 mM sodium dithionite; 0.35 mM 4-androstene-3,17-dione; 10% methanol (added with the steroid); and crude extract (1 mg/ml). With this assay, 5α-reductase activity was followed by measuring the consumption of the u.v.-absorbing steroid, 4-androstene-3,17-dione (below). However, for most of the experiments we used [4-^{14}C]4-androstene-3,17-dione (0.35 mM, 0.67 μCi/μmol final sp. act.) as the substrate so that the products formed (5α-androstane-3,17-dione and 3α-hydroxy-5α-androstan-17-one) could be detected (below). All components, except the electron donor and steroid, were preincubated for 5 min at 37°C before initiating the reaction with dithionite and steroid. Reactions were incubated for 5 min and 0.2 ml removed, added to screw cap tubes containing 0.5 ml dH_2O plus 3 ml diethylether, and the reaction stopped immediately by extracting the steroids. At the end of an experiment, the ether layers for all the samples were removed, each sample was extracted again (3 ml ether), and the ether extracts for each sample were combined. The extracts were evaporated to dryness, the residues dissolved in 0.5 ml methanol, and the samples were filtered with nylon Acrodiscs 13 (0.45 μm pore size, Gelman Sciences, Ann Arbor, MI). Under our assay conditions 1 U of 5α-reductase activity is equal to 1 nmol 4-androstene-3,17-dione reduced per 2 min and specific activity is equal to U/mg protein.

3α-HSDH activity was measured spectrophotometrically by following the change in absorbance at 340 nm. The assay contained (final vol of 1 ml) sodium CHES buffer, pH 9.5 (17 mM), $NADP^+$ (0.5 mM), crude extract (0.01 mg) and was started by adding sodium deoxycholate (1 mM).

3α-HSDH was removed from crude extract by passing about 50 mg of extract over a 1 × 4 cm column of Procion Red HE-3B (Pierce Chemical Co., Rockford, IL) equilibrated with anaerobic buffer (50 mM potassium phosphate, pH 7.5 plus 1 mM DTT). Unabsorbed material was eluted with the same buffer and collected in a serum bottle that was flushed with N_2.

Steroid analysis

Steroids were separated and quantitated by HPLC [10] except that a 4.6 × 150 mm Ultrasphere C18 (5 μ particle size) column (Beckman Instruments Inc., San Ramon, CA) was used. For assays using unlabeled 4-androstene-3,17-dione as the substrate, the steroid was detected at 254 nm [10] and quantitated by external standardization. For reactions with [4-^{14}C]4-androstene-3,17-dione, the steroids were

monitored with a radioisotope detector (Model 171, Beckman Instruments Inc., San Ramon, CA) and quantitated by area normalization.

The products formed from 4-androstene-3,17-dione by strain 144 cell extracts were examined by mass spectral analysis on a Finnigan Incos 50 quadruple mass spectrometer interfaced with a Hewlett Packard 5840 gas chromatograph. Chromatographic separations were made on an Alltech 30 m × 0.32 mm (i.d.) RSL300 silica capillary column (0.3 μm polyphenylmethylsiloxane) (Alltech Associates, Inc., Deerfield, IL). Helium carrier gas had a linear velocity of ~40 cm/s. The column oven was first ramped from 70 to 170°C at 25°C/min, then from 170 to 300°C at 5°C/min and held at 300°C for 4 min. The capillary column was interfaced with a 2 m × 0.53 mm (i.d.) deactivated guard column. Samples were injected on the guard column at 70°C. Mass spectrometer conditions: ion source 175°C, transfer line 300°C, scan range 50–700 a.m.u./s, ionizing voltage 70 eV for electron ionization. The retention time for pure steroid standards were as follows: 3α-hydroxy-5β-androstan-17-one (21.8 min), 3α-hydroxy-5α-androstan-17-one (22.5 min), 5β-androstane-3,17-dione (22.9 min), 5α-androstane-3,17-dione (23.7 min), and 4-androstene-3,17-dione (25.2 min).

Protein

Protein concentration was determined by the bicinchoninic acid method of Smith *et al.* [11] and commercially prepared reagents (Pierce Chemical Co.). Bovine serum albumin was the standard.

Chemicals

4-Androstene-3,17-dione; 5α-androstane-3,17-dione; 5β-androstane-3,17-dione; 3α-hydroxy-5α-androstan-17-one; 3α-hydroxy-5β-androstan-17-one; 4-hydroxy-4-androstene-3,17-dione; 3β-hydroxy-4-androstene-17-one, and 5-androstene-3,17-dione were obtained from Steraloids Inc. (Wilton, NH). [4-^{14}C]4-androstene-3,17-dione (56 mCi/mmol) was from Amersham Corp. (Arlington Heights, IL). 4,16-Pregnadiene-3,17-dione (16-dehydroprogesterone), bovine serum albumin, *p*-chloromercuribenzoate (*p*-CMB), *o*-phenanthroline, α,α-dipyridyl, hemin, methyl viologen, 2(*N*-cyclohexylamino) ethane sulfonic acid (CHES) and *Clostridium pasteurianum* ferredoxin were from Sigma Chemical Co. (St Louis, MO).

RESULTS

Products

GC-MS analysis of the steroids formed when crude cell extract was incubated with 4-androstene-3,17-dione and MV plus a suitable electron donor (below) revealed 5α-androstane-3,17-dione and 3α-hydroxy-5α-androstan-17-one as the only products. The identities of these steroids were based on the GC retention times and mass spectra of known standards (data not shown). The same 5α-steroids were formed regardless of whether the reaction pH was 5.5 or 7.5. 5β-Steroids were not seen under any condition where PR activity was detectable. These results indicate the PR activity present was due to a 5α-reductase.

pH

With H_2 as the electron donor, 5α-reductase showed a pH optimum at 7.5 in potassium phosphate buffer (Fig. 1). 5α-Reductase was stimulated by Tris–HCl at pH values above 7.0 and showed maximum activity at pH 8–9.

Electron donors

A number of compounds were tested for their ability to act as electron donors for 5α-reductase (Table 1), but only H_2, pyruvate and dithionite were active. 5α-Reductase activity in the presence of other electron donors (NAD(P)H) was essentially the same as that of unsupplemented crude extract. In all three instances, MV was required as an electron carrier for the reaction. Ferredoxin from *Clostridium pasteurianum* was

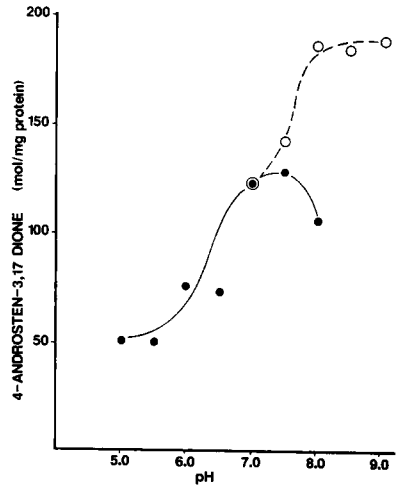

Fig. 1. Effect of pH on H_2-dependent 4-androstene-3,17-dione 5α-reductase activity. Assays were carried out with 100 mM potassium phosphate (●) or 100 mM Tris–HCl (○) buffers.

Table 1. Electron donors for 4-androstene-3,17-dione 5α-reductase activity

Electron donor	4-Androstene-3,17-dione (nmol/mg protein)
None	30
MV	37
H_2	40
H_2 + MV	186
Pyruvate	30
Pyruvate + MV	191
Dithionite	38
Dithionite + MV	159

Reactions contained crude extract (2.5 mg/ml), buffer (pH 7.5), and, where indicated, MV (0.5 mM), pyruvate (2 mM), or dithionite (2 mM). With pyruvate as the electron donor, CoA (0.1 mM) was also added. No 5α-reductase activity was detected with the following electron donors: NADH or NADPH (2 mM) each with or without FAD or FMN (0.2 mM). Reactions were incubated for 25 min.

tested in place of MV with dithionite as the electron donor but 5α-reductase activity was not found. All subsequent experiments were performed with dithionite and MV as the electron donating system.

Protein and methanol

5α-Reductase activity was proportional to crude extract protein at 0.5–1.5 mg/ml with a 5 min reaction (data not shown). At 1 mg protein/ml the reaction was linear for 8 min. These results were reproducible when the concentrations of dithionite and MV were 10 and 2 mM, respectively. At lower dithionite (2 mM) and MV (0.5 mM) concentrations, 5α-reductase activity tended to be erratic with respect to protein and was not detectable at low protein concentrations (0.5 mg/ml). Extract-free controls showed no chemical reduction of 4-androsten-3,17-dione by 10 mM dithionite.

Methanol, added to solubilize the steroid, inhibited 5α-reductase at concentrations above 10% (v/v) and activity was lost at 25% methanol (Fig. 2).

Subcellular distribution

Table 2 illustrates the distribution of 5α-reductase activity between the cytoplasmic and membrane fractions. Most of the 5α-reductase activity (60–65%) was found in the membrane fraction. The specific activity of the enzyme in the membranes was 22 times greater than that of the cytoplasm. Only a small amount of activity was present in the cytoplasm and about 20% of the total activity was unaccounted for following ultracentrifugation. The activity in the membrane fraction was measured using Triton X-100-solubilized material indicating 5α-reductase was not inactivated by the detergent

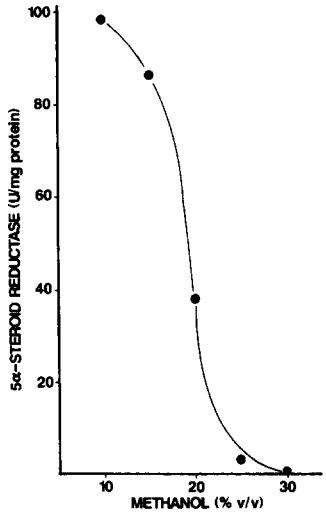

Fig. 2. Effect of methanol on dithionite-dependent 4-androstene-3,17-dione 5α-reductase.

at the concentration used. Furthermore, we were also able to detect good 5α-reductase activity in Triton X-100-solubilized membranes when H_2 served as the electron donor. This suggests that a portion of the hydrogenase present in strain 144 is associated with the cell membrane.

Inhibitors

The effects of several metabolic inhibitors on 5α-reductase activity were examined and the results are given in Table 3. With the exception of the sulfhydryl reagents ($HgCl_2$ and *p*-CMB) none of the compounds tested inhibited 5α-reductase. Several steroids were also tested for their ability to inhibit 5α-reductase, however, none had any effect. These included 3α-hydroxy-5α-androstan-17-one, 4-hydroxy-4-androstene-3,17-dione, 3β-hydroxy-4-androsten-17-one and 5-androstene-3,17-dione.

Hemin

Maximum steroid double bond reduction activities in resting cells of strain 144 depend partly on growth of the cells with hemin [9]. To determine whether hemin was required for

Table 2. Subcellular distribution of 4-androstene-3,17-dione 5α-reductase activity

Fraction	Total protein (mg)	Sp. act. (U/mg)	Total act. (U)	% Total act.
Crude extract	77	74	5698	—
Cytoplasm	65	11	715	13
Membrane	10	367	3670	64

Reactions contained crude extract (1 mg/ml), cytoplasmic fraction (1 mg/ml), or Triton X-100-solubilized membrane fraction (0.5 mg/ml) and were incubated for 5 min.

Table 3. Effects of inhibitors on 4-androstene-3,17-dione 5α-reductase activity

Inhibitor	Sp.act. (U/mg)
Control (+DTT)	98
EDTA	108
NaN_3	110
NaCN	108
Acriflavin	108
o-Phenanthroline	120
α,α-Dipyridyl	98
Control (−DTT)	82
$HgCl_2$	0
p-CMB	0
O_2	72

Crude extracts were prepared with (+) or without (−) DTT. Sulfhydryl inhibitors (0.5 mM) and O_2 (air) were tested with DTT-free extracts and assays. With O_2, extracts were exposed to air with gentle shaking for 10 min, before being assayed under anaerobic conditions. All other inhibitors (1 mM, except EDTA, 4 mM) were tested against DTT-containing extracts and assays. Inhibitors were incubated with enzyme for 10 min at 37°C before initiating the reactions.

activity of the steroid double bond-reductases in cell extracts, assays were carried out using extracts prepared from cells grown in the presence and absence of hemin. As shown in Table 4, the specific activities of 16-DHPR and 5α-reductase in crude cell extracts were the same regardless of whether the cells were grown with or without hemin.

3α-HSDH

Resting cells of strain 144 catalyze the reduction of the 3-keto group to give 3α-hydroxysteroids and an $NADP^+$-linked 3α-HSDH activity was detected in cell extracts that were believed to account for this reaction [9]. In the present study, 3α-HSDH was detected at a sp. act. of 1–1.7 μmol NADPH/min/mg protein in crude extract. To confirm that the reactions catalyzed by 5α-reductase and 3α-HSDH were performed by separate enzymes, crude extract was passed over a short column of Procion Red HE-3B which selectively binds $NADP^+$-linked hydroxysteroid dehydrogenases [12]. Spectrophotometric enzyme assays of the treated extracts indicated that 90% of the total $NADP^+$-linked 3α-HSDH activity was removed by this procedure. Figure 3 shows that such extracts retained 5α-reductase activity but did not catalyze reduction of the 3-keto group.

The amount of 3α-hydroxy-5α-androstan-17-one generally detected in our standard assay with [4-^{14}C]4-androstene-3,17-dione as the substrate was low partly because of the short reaction time but also because the reactions were not supplemented with $NADP^+$. Table 5 shows the formation of 3α-hydroxy-5α-androstan-17-one by crude cell extract was stimulated by adding $NADP^+$ and that dialyzed

Table 4. Dithionite-dependent 16-dehydroprogesterone-reductase and 4-androstene-3,17-dione 5α-reductase activities of *Eubacterium* sp. 144 grown with and without hemin

Enzyme	Sp.act (U/mg) (+)Hemin	(−)Hemin
16-DHPR	116	124
5α-Reductase	109	114

Crude extracts were prepared from cells grown in media with or without hemin. 16-Dehydroprogesterone (10 μg/ml) was added to the media to induce the synthesis of both 16-DHPR and 5α-reductase. 16-DHPR activity was measured as described previously [8].

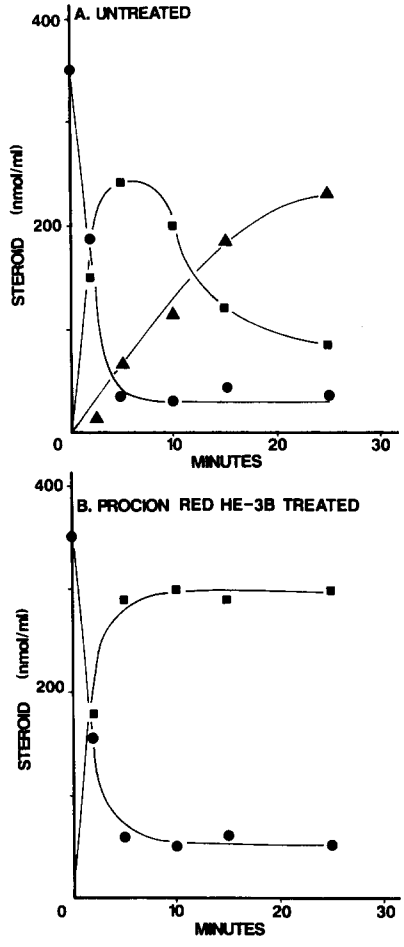

Fig. 3. Effect of Procion Red HE-3B treatment on dithionite-dependent 4-androstene-3,17-dione 5α-reductase and 3α-hydroxysteroid dehydrogenase activities in cell extracts. 4-Androstene-3,17-dione (●), 5α-androstane-3,17-dione (■) and 3α-hydroxy-5α-androstan-17-one (▲).

Table 5. Effect of dialysis on 4-androstene-3,17-dione 5α-reductase and 3α-hydroxysteroid dehydrogenase activities in crude extract

Extract	NADP+	Products (nmol/ml) 5α-androstene-3,17-dione	Products (nmol/ml) 3α-hydroxy-5α-androstan-17-one
Undialyzed	–	117	35
Undialyzed	+	43	91
Dialyzed	–	123	0
Dialyzed	+	42	95

Crude extract was dialyzed overnight against 2 l of anaerobic 50 mM potassium phosphate buffer, pH 7.5 containing 1 mM DTT while gassing with N_2. Reactions contained dialyzed or undialyzed cell extract (1 mg/ml), NADP+ (1 mM), and [4-^{14}C]4-androstene-3,17-dione. Reactions were incubated for 5 min.

extract required NADP+ for 3α-hydroxysteroid production.

DISCUSSION

The results of this work show that *Eubacterium* sp. strain 144 contains a 5α-steroid-reductase which is dependent on a source of low redox potential electrons for activity. Previously, Glass and Burley [9] reported that resting cells of strain 144 converted progesterone to 5β-pregnadione, 5β-pregnanolone and 5α-pregnadione when incubated with H_2 (PR activity). We were unable to detect any 5β-steroid-reductase activity in cell extracts of strain 144, even with reduced pyridine nucleotides with or without flavin nucleotides as electron donors, although 5α-steroid-reductase activity was readily seen. In light of these results, we have repeated the resting cell experiments as described previously [9] at a pH of 5.5 and 7.5 using either H_2 or pyruvate as electron donors. GC-MS analysis of the products derived from progesterone showed that only 5α-steroids (5α-pregnadione and 5α-pregnanolone) were produced by strain 144 under any of the conditions tested (T. L. Glass, unpublished results). Reexamination of the original GLC data used previously [9] to identify the products of progesterone reduction formed by strain 144 suggests the 5β-steroids were misidentified. The compound identified as 5β-pregnadione was probably 5α-pregnanolone. The nature of the compound thought to be 5β-pregnanolone is not certain. Based on the present study and earlier results [1, 9, 10], the steroid transformations catalyzed by strain 144 are given in Fig. 4 and the PR activity reported before [9] should be designated as 5α-steroid-reductase. This interpretation is consistent with the observation that rat fecal suspensions transform 16α-hydroxyprogesterone ultimately to 5α,17α-pregnanolone [5] and the fact that strain 144 was originally isolated from rat feces [1].

5α-Reductase required the same electron donors (pyruvate, dithionite, or H_2) as did 16-DHPR [8] for activity. These compounds can generate reduced MV which is necessary for 5α-reductase activity. As with 16-DHPR, the physiological electron donor for 5α-reductase is not known. However, since strain 144 5α-reductase can use reduced MV, this enzyme may be useful for the production of 5α-steroids by the electrochemical techniques of Simon *et al.* [14].

The similarity in electron donor requirements for 5α-reductase and 16-DHPR again supports the notion that a common electron transport mechanism couples pyruvate and H_2 oxidation to steroid double bond reduction in strain 144. This pathway is thought to involve a cyto-

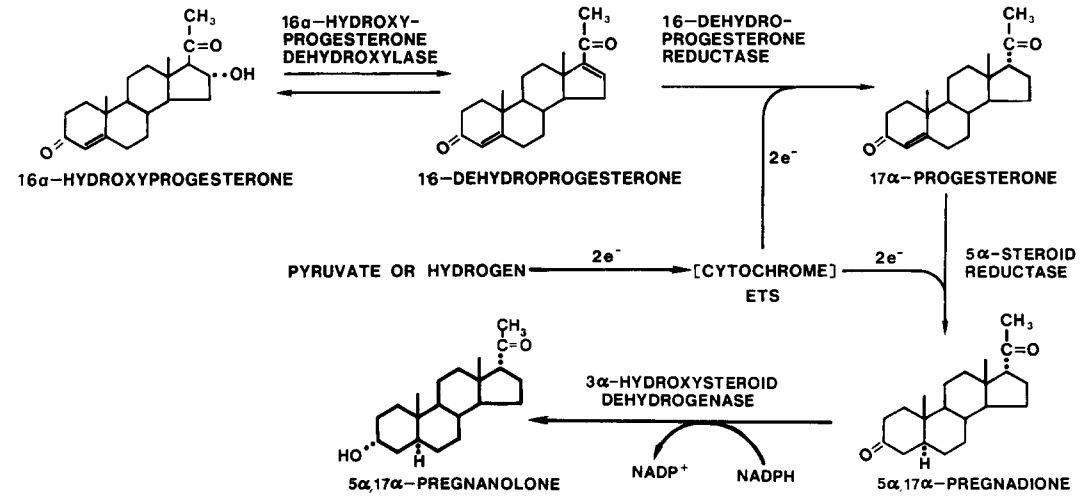

Fig. 4. Biotransformation of 16α-hydroxyprogesterone by *Eubacterium* sp. strain 144.

chrome because strain 144 must be grown with hemin to obtain resting cells with 16-DHPR and 5α-reductase activities [9]. However, this observation did not exclude the possibility that the steroid double bond-reductases themselves might contain heme as a prosthetic group. Our results show the activities of 16-DHPR and 5α-reductase in extracts of cells grown without hemin are equivalent to those in extracts of cells grown with hemin. This indicates that hemin is not a prosthetic group for the steroid double bond-reductases, but functions at a step prior to these enzymes.

A number of features were found that distinguish 5α-reductase from 16-DHPR [8]. The presence of Tris–HCl in the assay stimulated 5α-reductase activity, but was inhibitory to 16-DHPR. Both enzymes showed optimum activity at 10% (v/v) methanol, but 25% (v/v) methanol completely inhibited 5α-reductase while only partially inhibiting 16-DHPR. Most of the 5α-reductase activity in crude extracts was found to be present in the membrane fraction and the enzyme could be solubilized by Triton X-100. In contrast, only 20% of the crude extract 16-DHPR activity was present in the membrane fraction and this activity was lost after Triton X-100 treatment. Finally, previous evidence [8], indicates the genes encoding 16-DHPR and 5α-reductase are differentially regulated. 16-Dehydropregnanolone can induce the synthesis of 16-DHPR, but not 5α-reductase. In the present study, cell extracts from progesterone-induced cells contained 5α-reductase activity but not 16-DHPR activity.

Aside from their requirements for low redox potential electrons for activity, the only similarities found between 16-DHPR [8] and 5α-reductase were in their sensitivities to various metabolic inhibitors. Both enzymes were resistant to most of the inhibitors tested, but $HgCl_2$ and *p*-CMB inhibited both 16-DHPR and 5α-reductases.

The presence of 5α-steroid-reductase activity among anaerobic bacteria appears to be uncommon compared to 5β-steroid-reductase [15]. *Clostridium putrificum* was reported to catalyze 5α-steroid reduction [16] and, more recently, *Clostridium* sp. strain J1 was isolated from human feces and found to form 5α-steroids [17]. However, further studies on the properties of 5α-reductase from these bacteria have not been reported. Two aerobic bacteria, *Nocardia* (now *Rhodococcus*) *corallina* [18] and *Mycobacterium smegmatis* [19, 20] contain 5α-steroid-reductases that have been characterized. The enzyme from *R. corallina* is cytoplasmic and is most active with NADPH as the electron donor. That from *M. smegmatis* was found in both the cytoplasmic and membrane fractions of cell extracts. Sodium dithionite was required as the electron donor, but activity was not dependent on MV. Indirect evidence (e.g. inhibition by acriflavin) suggested 5α-reductase from *M. smegmatis* was a flavoprotein. 5α-Steroid-reductase from strain 144 is distinguished from the 5α-reductases of *R. corallina* and *M. smegmatis* by its requirement for reduced MV as the electron donor, its preferential membrane location (compared to *R. corallina*), and its resistance to acriflavin (compared to *M. smegmatis*).

The physiological significance of the steroid double bond-reductases to strain 144 is not known. However, the association of these enzymes with the cell membrane, their requirements for low redox potential electrons (especially from H_2 with its availability in the gut), and the possible involvement of an electron transport mechanism for 16-DHPR and 5α-reductase activities poses the question of whether these enzymes may not be coupled to energy generation.

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