Mode of action of steroid desmolase and reductases synthesized by *Clostridium* "*scindens*" (formerly *Clostridium* strain 19)

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Abstract A recently isolated hitherto unknown Clostridium from human feces, designated *Clostridium "scindens"* (formerly strain 19), synthesizes at least two enzymes active on the side-chain of the steroid molecule and two enzymes active on the hydroxyl groups of the 7-position of bile acids. Steroid desmolase, responsible for side-chain cleavage of corticoids, and 20α-hydroxysteroid dehydrogenase have not been detected in any other bacterial species of the resident colonic flora. Steroid desmolase is Eh-dependent (optimum ca. −130 mV), requires a hydroxy group at C-17, and preferably an α-ketol group in the side-chain; an α-hydroxy group at C-20 reduces and a β-hydroxy group at C-20 prevents side-chain cleavage. With suitable substrates, the yield of C-19 steroids is proportional to the bacterial multiplication rate. 20α-Hydroxysteroid dehydrogenase (20α-HSDH) is also Eh-dependent (optimum ca. −300 mV) and reduces the C-20 keto function to an α-hydroxy group, regardless of the presence or absence of a hydroxy group at C-17. 7α-Dehydroxylase metabolizes cholic and chenodeoxycholic acid, while 7β-hydroxysteroid dehydrogenase acts upon ursodeoxycholic acid. The latter two enzymes are not specific for *C. scindens*.—**Winter, J., G. N. Morris, S. O'Rourke-Locascio, V. D. Bokkenheuser, E. H. Mosbach, B. I. Cohen, and P. B. Hylemon.** Mode of action of steroid desmolase and reductases synthesized by *Clostridium "scindens"* (formerly *Clostridium* strain 19). *J. Lipid Res.* 1984. **25:** 1124–1131.

Supplementary key words *Clostridium* species • bile acids • corticoids

The mammalian sex hormones are made in the gonadal tissue by side-chain cleavage of cholesterol or C-21 steroids (1). The enzyme, desmolase, is also synthesized by certain free-living fungi, e.g., *Cylindrocarpon radicicola* (2). In 1971, Eriksson and Gustafsson (3) reported desmolase in feces from a normal human subject; it metabolized cortisol to a C-19 steroid (**Fig. 1**). Their work, together with investigations in our laboratories, indicated that the fecal desmolase is of bacterial origin, that the organisms are a part of the normal colonic flora, and that they occur in a concentration of 10^7–10^8/g feces (4). Recently, we isolated from human feces a clostridium with the ability to cleave the side-chain of cortisol and 17α-hydroxyprogesterone, but not of corticoids lacking a 17-hydroxy group (5). As the taxonomic position of this obligate anaerobic organism is unclear, it has provisionally been designated *Clostridium "scindens"* formerly strain 19 (5). The distinct possibility that this bacterial species belongs to the resident flora of the human colon suggests that desmolase plays a role in the normal catabolism of biliary 17α-hydroxy corticoids and prompted us to examine the conditions and mechanisms governing side-chain cleavage by *C. scindens*. In the course of these studies it became apparent that the organism synthesizes at least three additional enzymes with effect on the steroid molecule. These findings are described in the present report.

EXPERIMENTAL PROCEDURES

Materials

Media. The following anaerobic media were used in our experiments. Supplemented peptone broth (SPB) and SPB II were obtained from Becton, Dickinson and Co., Rutherford, NJ. Prereduced brain-heart infusion broth (PR) was from Scott Laboratories Inc., Fiskeville, RI. Eh of these media was −130 to −140 mV. Brain-heart infusion broth with cysteine (BHIC) was purchased from Baltimore Biological Laboratories, Cockeysville, MD. It was prepared from the dehydrated products and had the following composition per liter: dehydrated

Abbreviations: SPB, supplemented peptone broth; PR, pre-reduced brain-heart infusion broth; BHIC, brain-heart infusion broth with cysteine; BHIC-Ec, BHIC biologically reduced by *Escherichia coli;* TLC, thin-layer chromatography; HSDH, hydroxysteroid dehydrogenase.

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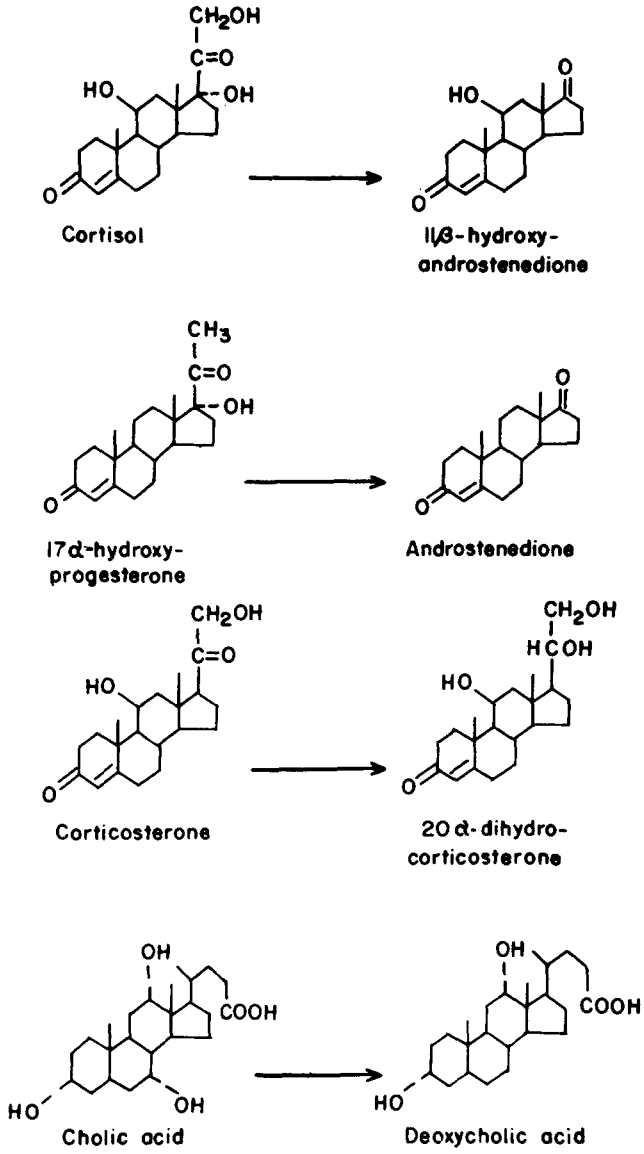

Fig. 1. Steroid metabolism by *C. scindens.*

brain-heart infusion broth, 37 g; cysteine-HCl, 0.5 g; $NaHCO_3$, 1 g; and 4 ml of 0.025% aqueous resazurine (J. T. Baker Chemical Co., Phillipsburg, NJ). The medium was distributed in 50-ml amounts, and sterilized at 121°C for 20 min. After cooling at room temperature it was biologically reduced (6) by the addition of 0.1 ml of a 24-hr culture of *Escherichia coli* (BHIC-Ec). *E. coli,* a fast growing facultative anaerobe, lowers the Eh of the medium to −200 mV or less within an hour or two and is without effect on the steroid molecule. Thus, it provides a highly suitable environment for the study of anaerobes with enzyme active on the steroid molecule.

Source of microorganism. The organism synthesizing desmolase was isolated in our laboratories and initially known as *Clostridium* strain 19 (5). As further studies revealed that it is a hitherto unknown species, the name *C. scindens* (to cut) has been proposed and a complete description of its characteristics is being prepared for The International Journal of Systematic Bacteriology. *E. coli, Proteus mirabilis,* and *Klebsiella pneumoniae* (7, 8) were also isolated in our laboratories. Pure cultures of these strains were incubated at 37°C in SPB for 24 hr immediately prior to use in metabolic experiments.

Steroids and bile acids used for conversion or as reference compounds (Fig. 1) in the chromatographic tests were purchased from Sigma Chemical Co. (St. Louis, MO) and required no further purification.

Eh and pH were measured on a Beckman Zeromatic II Instrument (Beckman Instruments, Inc., Fullerton, CA). Eh measurements were made with a platinum electrode. Readings were checked regularly with quinhydrone-saturated buffers at pH 4 and 7, and were within 10 mV of the theoretical values.

Solvents for thin-layer chromatography (TLC) were reagent grade. Methanol (technical grade) was used for the precipitation of proteins from the converting media.

Methods

Conversion experiments. Steroids and bile acids were dissolved in methanol and added to the sterilized medium at a concentration of 20 μg of steroid per ml, unless otherwise indicated; the concentration of methanol (v/v) per culture was 0.5%. The medium was then seeded with 0.25 ml of a pure bacterial culture and incubated at 37°C for 2–7 days.

Kinetic experiments were performed with SPB or PR seeded with 0.1 ml of an 18-hr-old culture of *C. scindens.* Cortisol was added immediately or after stipulated periods of incubation at 37°C. Samples were collected at regular intervals. Growth of *C. scindens* was followed by measuring the optical density of the culture at 430 nm on a Gilford spectrophotometer, model 250 (Gilford Instruments, Oberlin, OH). The metabolism of the substrate was followed as stated below.

Separation of substrates and metabolites. After incubation the products were extracted and identified as previously described (9, 10). Briefly, the steroids were extracted by vortexing 4 ml of culture with 5 ml of methylene chloride for 30–60 sec and then recovering the organic phase. Bile acids were isolated by adjusting the pH to 2 with 1 N HCl followed by extraction with ethyl acetate. The organic phase was dried over sodium sulfate and evaporated under nitrogen gas at 40–50°C. The residue was redissolved in 50 μl of acetone and spotted on Bakerflex silica gel (1B2F; J. T. Baker). The following solvent systems were used for TLC: solvent A, isooctane–ethyl acetate–acetic acid 5:25:0.2 (v/v/v) for separation of cortisol, cortisone, corticosterone, deoxycorticosterone, 11-deoxycortisol, cortol, 21-deoxycortisone, tetra-

hydrocortisol, and their metabolites; solvent B, ethyl acetate–hexane 3:7 (v/v) for separation of 17α-hydroxyprogesterone and its metabolites; and solvent C, chloroform–methanol–acetic acid 40:21:1 (v/v/v) for separation of bile acids and their corresponding metabolites.

The steroid spots were located either under ultraviolet light (254 nm) or by spraying the TLC plates with a mixture of sulfuric acid–ethanol 1:1 (v/v) as previously described (11). The bile acid spots were located by spraying with a mixture of 2% parahydroxybenzaldehyde in methanol and 50% sulfuric acid in methanol (10:1, v/v). The metabolites were quantitated by gas–liquid chromatography (GLC) performed on a Hewlett Packard 5880A Instrument (Hewlett Packard and Co., Palo Alto, CA) equipped with flame ionization detector under the following conditions: fused silica capillary column packed with OV 101; carrier gas helium; injector and detector temperature 230°C. Oven temperature was 230°C for bile acids and programmed 180°–230°C with an increase of 3°C per minute for the steroids. Steroids were derivatized to the methoxyme–trimethylsilyl products and purified on a Lipidex column (Packard Instrument Co., Downers Grove, IL) prior to GLC (12). The bile acids were chromatographed as methyl esters of the trimethylsilyl derivative (13).

Gas–liquid chromatography–mass spectrometry was carried out on Hewlett Packard 5992 B instrument equipped with a jet separator. The conditions for the analysis were as follows: injection temperature 250°C, programmed oven temperature 180°–230°C with an increase of 3°C/min; electron impact 70 eV; column packed with 2% OV-101 on 100–120 WHP (Hewlett Packard); source pressure 10^{-6} torr.

RESULTS

Structures of substrates and their corresponding metabolites are shown in Fig. 1.

Metabolism of 17α-hydroxysteroids

Media. Within 2 days cortisol (20 μg/ml) in SPB, SPB II, or PR was metabolized to a C-19 steroid by *C. scindens* (**Table 1**). Eh at the end of the incubation was −130 mV. The metabolite absorbed light at 254 nm, indicating that the 4-ene-3-keto function was intact. After TLC, it developed a purple color after spraying with sulfuric acid–ethanol. The R_f, relative retention time (RRT), and the mass spectrum matched those of 11β-hydroxy-4-androstene-3,17-dione. When the metabolic experiment was performed in the more reduced medium (BHIC-Ec, Eh = −300 mV) we often, but not always, noted the presence of two metabolites. One of these was identified as 11β-hydroxy-4-androstene-3,17-dione. The other compound was more polar than cortisol and absorbed light at 254 nm; on spraying with sulfuric acid–ethanol, it developed a brown rather than the purple color, typical for the C-19 steroids. Chromatographic data and the mass spectrum matched those of the C-20-reduced metabolite, namely 11β, 17α,20α,21-tetrahydroxy-4-pregnen-3-one.

As could be expected, in SPB and PR (Eh, −130 mV) *C. scindens* cleaved the side-chain of 17α-hydroxyprogesterone yielding 4-androstene-3,17-dione and, in the more anaerobic BHIC-Ec (Eh, −300 mV), it reduced the substrate to 17α,20α-dihydroxy-4-pregnen-3-one.

Concentration of substrates. Regardless of the concentration of substrate between 20 μg/ml and 300 μg/ml, cortisol was quantitatively metabolized to 11β-hydroxyandrostenedione in SPB, SPB II, and PR. In the more reduced BHIC-Ec, on the other hand, the products were substrate-dependent: low concentrations of cortisol (20–100 μg/ml) favored side-chain cleavage while higher concentrations (150–300 μg/ml) promoted reduction of the 20-keto group (**Fig. 2**).

17α-Hydroxyprogesterone was a less suitable substrate for the *C. scindens* enzymes. In concentrations from 10–50 μg/ml, 50% was converted to androstenedione in SPB and PR and 50% remained unmetabolized. In BHIC-Ec the keto group at C-20 was reduced. Higher substrate concentrations reduced yields without changing products.

Kinetics. In the first series of experiments, eight vials of PR supplemented with cortisol (200 μg/ml) were seeded with *C. scindens,* incubated, and sampled every 3 hr. Side-chain cleavage began within a few hours of incubation and was quantitative within 15 hr (**Fig. 3**). Small amounts of 20α-dihydrocortisol accumulated in the early phases of the incubation were quickly metabolized to 11β-hydroxyandrostenedione. On the assumption that 20α-dihydrocortisol might interfere with the desmolase activity, we isolated the compound and used it as substrate for *C. scindens.* In concentrations up to 20 μg/ml it was metabolized to 11β-hydroxyandrostenedione, but in concentrations of 100 μg/ml only 20% was converted. The presence of 20–100 μg/ml of 20α-dihydrocortisol in the medium did not interfere with the side-chain cleavage of cortisol (100 μg/ml).

In subsequent experiments we investigated the correlation between bacterial multiplication and desmolase activity. Cortisol (100 μg/ml) was added to cultures of *C. scindens* in SPB before incubation or after 4, 6, 8, 10, 12, and 16 hr of incubation. Samples were taken at 2-hr intervals and examined for bacterial growth and steroid metabolism. As in the above described kinetic experiments, 20α-dihydrocortisol was formed in the early phases of the incubation, only to disappear a few hours later. The most striking feature was that the intensity of side-chain cleavage increased to a maximum

TABLE 1. Conversion of 17-hydroxysteroids by *Clostridium scindens*

Substrate	Metabolite	Media	R_f[a]	RRT	Maximum Conversion
					μg/ml
Cortisol	—	—	0.38	2.19	—
	11β-hydroxy-4-androstene-3,17-dione	SPB; PR	0.60	1.18	300
	11β,17α,20α,21-tetrahydroxy-4-pregnen-3-one	BHIC-Ec	0.09	2.50	300
Tetrahydrocortisol	—	—	0.26	1.42	—
	3α,11β-dihydroxy-5β-androstan-17-one	SPB; PR	0.47	0.95	300
	20α-cortol	BHIC-Ec	0.07	2.80	300
Cortisone	—	—	0.43	1.94	—
	4-androstene-3,11,17-trione	SPB; PR	0.57	0.72	300
	17α,20α,21-trihydroxy-4-pregnene-3,11-dione	BHIC-Ec	0.10	ND[c]	—
11-Deoxycortisol (substance S)	—	—	0.62	1.61	—
	4-androstene-3,17-dione	SPB; PR	0.68	1.83	300
	17α,20α,21-trihydroxy-4-pregnen-3-one	BHIC-Ec	0.21	1.79	300
21-Deoxycortisone	—	—	0.65	1.45	—
	4-androstene-3,11,17-trione	SPB; PR	0.57	0.72	20
	17α,20α-dihydroxy-4-pregnene-3,11-dione	BHIC-Ec	0.36	1.84	20
17α-Hydroxyprogesterone	—	—	0.68	1.27	—
	—	—	0.20[b]	—	—
	4-androstene-3,17-dione	SPB; PR	0.29[b]	0.85	25
	17α,20α-dihydroxy-4-pregnen-3-one	BHIC-Ec	0.50	1.17	25

[a] Solvent system A, except as noted.
[b] Solvent system B.
[c] Not determined. Trace amount seen only occasionally on TLC.

during the log phase of the bacterial growth (**Fig. 4**), i.e., the time of the most rapid bacterial multiplication. Cortisol added in the early stationary phase, when the generation time is longer, was metabolized at a slower rate and to a lesser degree.

Side-chain cleavage of 17α-hydroxyprogesterone was incomplete and slower than that of cortisol (**Fig. 5**).

Substrate specificity. In Table 1 we have listed seven 17α-hydroxysteroids (20 μg/ml) which in SPB were converted to C-19 steroids by *C. scindens.* Of these, tetrahydrocortisol, cortisone, and 11-deoxycortisol followed the conversion pattern of cortisol, i.e., they were metabolized rapidly, quantitatively, and in high concen-

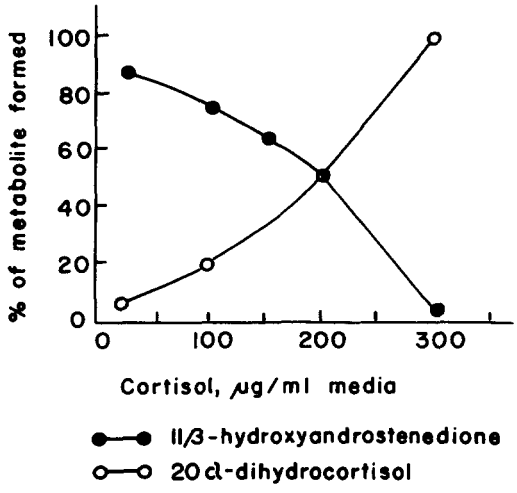

Fig. 2. Effect of concentration of cortisol on formation of metabolites in BHIC-Ec media; incubation time, 48 hr.

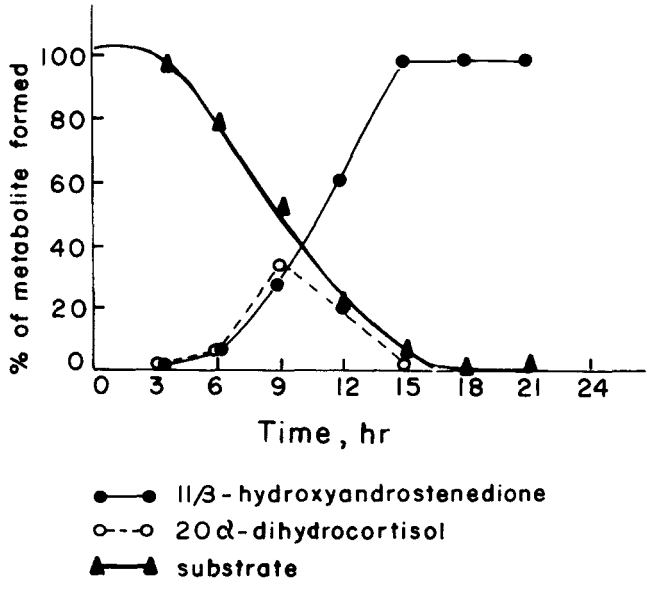

Fig. 3. Velocity of formation of 11β-hydroxyandrostenedione from cortisol by *C. scindens;* cortisol, 200 μg/ml media (PR).

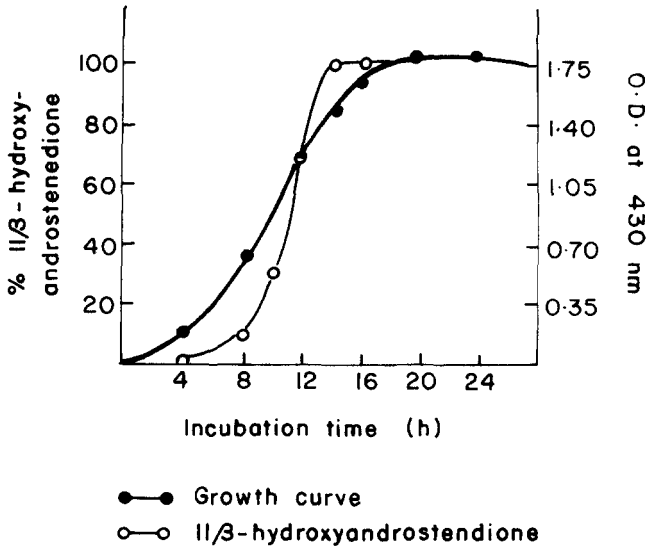

Fig. 4. Desmolase activity as a function of bacterial multiplication; (O–O–O), 11β-hydroxyandrostenedione; (●–●–●), growth curve.

trations. In contrast, 21-deoxycortisone and 20α-cortol were metabolized slowly, incompletely, and in low concentrations, as was 17α-hydroxyprogesterone. In conversion experiments where SPB was replaced with BHIC-Ec, the results varied. Interestingly, 20β-cortol as well as 17α-hydroxypregnenolone was the double bond at C-5 were not metabolized by strain 19.

Metabolism of 17-deoxycorticoids

Substrate specificity was studied using deoxycorticosterone, corticosterone, and 11-deoxycortisol in SPB, SPB II, PR, and BHIC-Ec (**Table 2**). The only enzymatic alteration observed was the reduction of the C-20 keto group to a 20α-hydroxy function. This conversion was independent of substrate, media, incubation time, or concentration. The reduction was slower than the side-chain cleavage and required, under our experimental conditions, 48 hr incubation for completion (Fig. 5). Maximal 20-keto reduction (200 μg/ml) was attained in SPB and PR with substrates having an α-ketol structure at C-20–C-21 (deoxycorticosterone, corticosterone, and 11-dehydrocorticosterone). Strangely enough, at the lower Eh existing in BHIC-Ec, the very same substrates were metabolized in 10 times lower yields. Substrates without a hydroxy group at C-21 (progesterone and 16α-hydroxyprogesterone) were not affected by the 20α-HSDH (Table 2).

Metabolism of bile acids

SPB, SPB II, and PR were supplemented with cholic, chenodeoxycholic, deoxycholic, and ursodeoxycholic acids (20 μg/ml), seeded with *C. scindens*, and incubated for 3 days at 37°C. Cholic acid (7α) in concentrations up to 350 μg/ml was rapidly 7α-dehydroxylated (Fig. 5, **Table 3**). Chenodeoxycholic acid (7α) was also 7α-

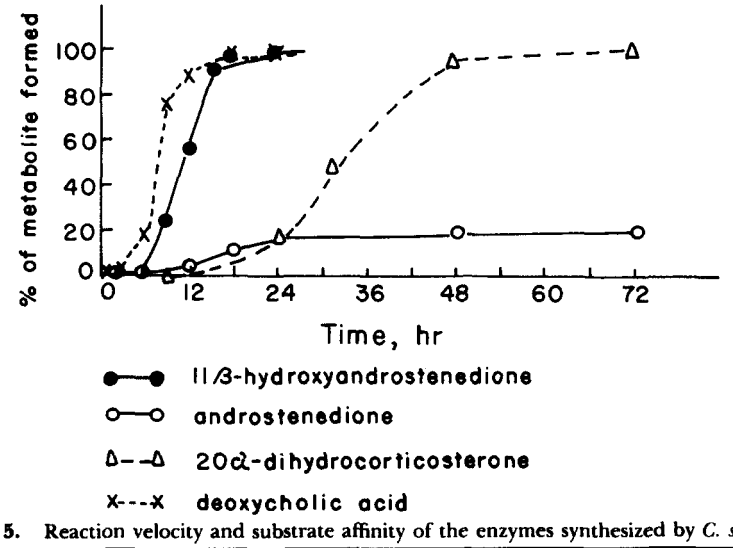

Fig. 5. Reaction velocity and substrate affinity of the enzymes synthesized by *C. scindens*.

Symbol	Metabolite	Enzyme	Substrate	Concentration of substrate (μg/ml media)
●–●–●	11β-Hydroxyandrostenedione	Desmolase	Cortisol	200
O–O–O	Androstenedione	Desmolase	17α-Hydroxyprogesterone	20
Δ–Δ–Δ	20α-Dihydrocortisol	20α-HSDH	Corticosterone	200
X–X–X	Deoxycholic acid	7α-Dehydroxylase	Cholic acid	200

TABLE 2. Conversion of 17-deoxysteroids by *C. scindens*

Substrate	Metabolite	Media	R_f[a]	RRT	Maximum conversion (μg/ml)
Deoxycorticosterone	—	—	0.55	1.74	—
	20α-21-dihydroxy-4-pregnen-3-one	SPB; PR	0.29	1.71	200
		BHIC-Ec (variable)	0.29	1.71	20
Corticosterone	—	—	0.39	1.90	—
	11β,20α,21-trihydroxy-4-pregnen-3-one	SPB; PR	0.14	2.37	200
		BHIC-Ec	No conversion		—
11-Dehydrocorticosterone (compound A)	—	—	0.37	1.76	—
	20α,21-dihydroxy-4-pregnene-3,11-dione	SPB; PR	0.19	2.00	20
		BHIC-Ec			—
Progesterone	No conversion	SPB; PR; BHIC-Ec			
16α-Hydroxyprogesterone	No conversion	SPB; PR; BHIC-Ec			

[a] Solvent system A.

dehydroxylated but slower and only in concentrations up to 20 μg/ml. Ursodeoxycholic acid (7β) was not dehydroxylated at C-7.

Experiments with ursodeoxycholic acid in BHIC-Ec revealed that *C. scindens* synthesized a 7β-HSDH. Since *E. coli* synthesizes a 7α-HSDH, using cholic acid as substrate, we tested *C. scindens* for 7α-HSDH activity in BHIC bio-reduced with *Proteus mirabilis* and *Klebsiella pneumoniae* (6). No such activity was observed.

DISCUSSION

Steroid-metabolizing enzymes in *C. scindens*

Most intestinal bacteria metabolizing steroids belong to *Clostridia* or *Eubacteria* and limit their synthesis to one or a few enzymes (14, 15). It is remarkable, therefore, to observe an organism which elaborates at least four steroid enzymes. Desmolase catalyzes the oxidative side-chain cleavage and has not been found in any other organism belonging to the resident flora of the human gut. The 20α-HSDH is unique, since other known bacterial species of intestinal origin reduce the keto group at C-20 to a β-hydroxy group (16). 7α-Dehydroxylase and 7β-HSDH are also synthesized by a number of other intestinal organisms (17–19).

Substrate specificity of *C. scindens* desmolase

The enzyme has specific substrate requirements such as an α-hydroxy group at C-17 and preferably an α-ketol group at C-20–C-21. Any steroid with a side-chain possessing a methyl group at C-21 or a 20α-hydroxy group lowers the yield of C-19 metabolites. Steroids with a 20β-hydroxy group or a double bond at C-5 are not suitable substrates for the bacterial desmolase. A hydroxyl group, ketone, or hydrogen at C-11 and the presence or absence of a conjugated double bond in ring A are without effect on the enzymatic reaction.

TABLE 3. Conversion of bile acids by *C. scindens*

Substrate	Metabolite	Media	R_f[a]	RRT[b]
Cholic acid	—	—	0.07	2.95
	Deoxycholic acid	SPB; PR	0.33	2.69
Chenodeoxycholic acid	—	—	0.23	2.82
	Lithocholic acid	SPB; PR	0.64	2.27
Ursodeoxycholic acid	—	—	0.40	3.35
	7-Keto-lithocholic acid	BHIC-Ec	0.50	3.28 (3.77)
	No conversion	SPB; PR	—	—
Deoxycholic acid	No conversion	SPB; PR; BHIC-Ec		

[a] Solvent system C.
[b] Isothermal at 230°C.

C. scindens desmolase differs from desmolase synthesized by the free-living fungus *Cylindrocarpon radicicola*. The latter can utilize 17-deoxy steroids as substrates and is independent of the presence or absence of a hydroxyl group at C-21 (2). Furthermore, the fungal desmolase does not metabolize cortisol, the preferred substrate for the bacterial desmolase. Recent studies (20) showed that progesterone is a suitable substrate for the enzyme synthesized by *Aspergillius aureogulgens* while 17α-hydroxyprogesterone was not.

Although cortisol was used as substrate in the search of an organism synthesizing steroid desmolase, it is not the natural target for the enzyme, since it does not undergo enterohepatic circulation in humans. The natural substrate in humans could be the biliary excreted 17α-hydroxypregnanolone, but it is noteworthy that it is not the optimal substrate for the enzyme.

Mechanism of C-17–C-20 desmolase

Our kinetic experiments showed that the desmolase activity was closely associated with the multiplication phase of the bacteria (Fig. 4). Conversion of cortisol to C-19 steroids was optimal when the substrate was added before or during the logarithmic growth phase, while very little or no conversion occurred when it was added during the stationary phase.

C. scindens desmolase, in contrast to a testicular extract, is incapable of cleaving the side-chain of progesterone or any other 17-deoxysteroid. Work by Nakajin and Hall (21) implied that the first step in side-chain removal by a testicular extract is a 17α-hydroxylation. Neither in these studies nor in previous observations have we observed bacterial 17-hydroxylation. Synthesis of 17α-hydroxylase by anaerobic bacteria, although unlikely, cannot entirely be excluded because the ezymatic activity probably cannot be expressed at the low Eh prevailing in the conversion media and in the gut (6, 8, 9).

The molecular mechanism of side-chain cleavage by *C. scindens* is presently unknown but, if cleavage by fungal enzymes (1, 2, 22, 23) and gonadal tissue (1, 24) is a guide, it involves NAD(P)H and free oxygen (25). This is further supported by the fact that the side-chain cleavage by *C. scindens* desmolase does not take place in BHIC-Ec (Eh = −300 mV) where most oxygen is consumed by *E. coli* in the very early stages of incubation. Alternatively, it is conceivable that the anaerobic organism may perform the carbon-carbon cleavage by a hitherto unknown mechanism. Elucidation of the precise mode of action of different desmolases, including the importance of the anaerobic environment, must await the isolation and purification of the specific enzymes.

Specificity of 20α-HSDH

Deoxycorticosterone, corticosterone, and 11-dehydrocorticosterone were reduced to 20α-hydroxy compounds by *C. scindens*, while progesterone and 16α-hydroxyprogesterone were not suitable substrates for the enzyme. This clearly suggests that C-21 hydroxy steroids are the preferred substrates. It might be argued that the 16α-hydroxy group in 16α-hydroxyprogesterone provides a steric barrier for the enzyme, but this is refuted by the inability of the enzyme to reduce the C-20 keto function of progesterone. The nature of the substituents at C-11 (R = −H, −OH; = 0) did not interfere with the activity of the 20α-HSDH.

Enzymes active upon bile acids

7α-Dehydroxylase metabolized both cholic acid and chenodeoxycholic acid but our results indicate that a hydroxy function at C-12 was advantageous to the enzyme.

We demonstrated clearly that *C. scindens* synthesized a 7β-HSDH. Whether or not it also manufactures a 7α-HSDH is a moot point. We did not detect evidence of its existence; it could have been masked by the presence of a 7α-dehydroxylase if the latter enzyme has a higher reaction velocity than 7α-HSDH. This might be further accentuated by the low Eh of the culture, which unquestionably favors the dehydroxylation rather than the oxidative 7α-dehydrogenation.

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