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Degradation of endogenous steroids by microorganisms

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Introduction

There are many kinds of microorganisms in environment. Some of them can degrade steroid structures[1-7]. Such as *Rhodococcus erythropolis* can degrade testosterone to 4-androstene-3,17-dione and 1,4-androstadiene-3,17-dione[3-7]. This kind of degradation may change steroids profile during routine screening procedure.

The phosphate buffer (pH=6.9) is suitable for the microorganisms' living. Some experiments were in conformation of this result. The microorganisms breed in the new prepared phosphate buffer are enough to degrade a large number of steroids, even if it was prepared by MILLIPORE purified water and stored at 4°C for 3 months,.

The aim of this study is to investigate how the microorganisms in this phosphate buffer degraded the endogenous steroids.

Experimental

Incubation experiments

Androsterone, etiocholanolone, 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol, 5α-dihydrotestosterone, testosterone, androsterone glucuronide, etiocholanolone glucuronide, 5α-dihydrotestosterone glucuronide, and testosterone glucuronide were examined in this experiment. Two aliquots of each sample were prepared, one control (1000 ng of selected substrate) without phosphate buffer (pH=6.9), another 2 mL phosphate buffer (pH=6.9) spiked with 1000 ng of selected substrate, and incubated at 37°C for 16 hours. Considering over the possibility of temperature influence, the same experiments were repeated for 16 hours at 13 different temperatures from 4°C to 55°C (4, 10, 15, 20, 24, 27, 30, 34, 37, 40, 45, 50, 55°C).

Sample preparation and GC/MS analysis

The incubated solutions were adjusted to pH 9.6 with $NaHCO_3/Na_2CO_3$=1:1, 20%. The steroids were extracted with 4 ml of methyl t-butyl ether. The ether phase was separated and

evaporated to dryness with nitrogen. The residue was derivatized with 50µl of MSTFA/TMSI/ Ethanethiol (1000:3:2) at 70°C for 30 min and 1µl of the derivatized solution was injected into the GC/MS system. GC/MS(Agilent 7890A/5975C) conditions: GC-Injection port 280°C, interface:300°C, carrier gas: He, constant pressure: 100 kpa, column: HP-1, 17m×0.2mm×0.11µm, temperature program:180°C (3.3°C/min) 231°C (30°C/min) 310°C (2min), MS-Source temp 230°C, quad temp 150°C, SIM mode.

Result and Discussion

1. Androsterone, etiocholanolone, 5α-androstane-3α,17β-diol, 5β-androstane-3α,17β-diol, 5α-dihydrotestosterone and testosterone were degraded by the microorganisms. Every endogenous steroid was degraded to several degraded products. They had cross-transformed each other (Table 1).
2. 7 explicit degraded products (including androsterone (5), etiocholanolone(6), 5α-androstane-3,17-dione (10), 1,4-androstadiene-3,17-dione (12), 5α-dihydrotesto-sterone (13), 4-androstene-3,17-dione(14) and testosterone(15)) were confirmed.
   6 unknown products ((1), (2), (3), (4), (9), (11)) were observed without standards confirmation (Figure 1). The mass spectra of unknown products ((1), (3), (9)) were similar to that of 5α-androstane-3,17-dione (10), while another 3 unknown products ((2), (4), (11)) similar to 5α-dihydrotestosterone (13), but with different RT (Figure 2). Compared with the investigation which was described in a review of Mareck et al.[7], the unknown product (1) should be 5β-androstane-3,17-dione.
3. The conjugated endogenous steroids cannot be degraded to any other free steroids by the microorganisms in this phosphate buffer.
4. The activity of microorganisms was usually influenced by temperature, so the endogenous steroids were incubated for 16 hours at 13 different temperatures. The degradation ratio of etiocholanolone (6) was shown in Figure 3. The maximal degradation ratio appeared at 27°C, while the minimal degradation ratio was observed at 4°C and 55°C. All other endogenous steroids showed very similar behaviors of degradation with temperatures.

Conclusions

1. The conditions of urine sample were more complicated than this phosphate buffer. So if the degraded products were found in screening, these degradation make the steroids

profile change. The transportation of urine sample to the laboratory should be kept in good condition.

2. 55°C is a good temperature for hydrolysis procedure with less degradation.
3. By filtrating the phosphate buffer with 0.22 μm filter or boiling, degradation can be avoided because of the removal of microorganisms.

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Table 1: The relationship between endogenous steroids and their degraded products

Endogenous steroids	**Degraded products**
Androsterone (5)	(9)*; 5α-androstane-3,17-dione (10); 1,4-androstadiene-3,17-dione (12); 4-androstene-3,17-dione (14)
Etiocholanolone (6)	(1)*; (3)*
5α-androstane-3α,17β-diol (7)	Androsterone (5);(9)*; 5α-androstane-3,17-dione (10); (11)*; 5α-dihydrotestosterone (13); 4-androstene-3,17-dione (14)
5β-androstane-3α,17β-diol (8)	(1)*;(2)*;(3)*;(4)*; Etiocholanolone (6)
5α-dihydrotestosterone (13)	(9)*; 5α-androstane-3,17-dione (10); 4-androstene-3,17-dione (14); Testosterone (15)
Testosterone (15)	1,4-androstadiene-3,17-dione (12); 4-androstene-3,17-dione (14)

*structure unknown

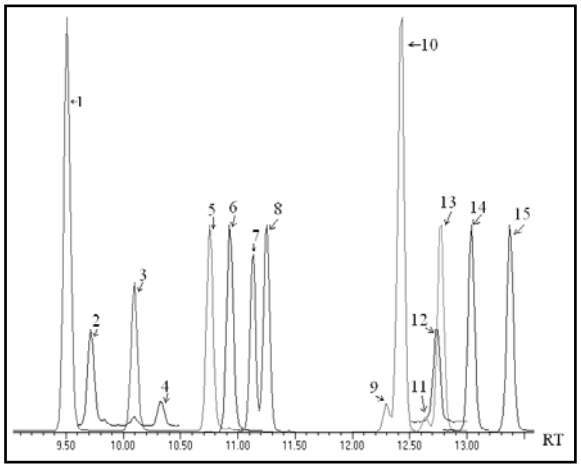

Figure 1. EIC of endogenous steroids and their degraded products

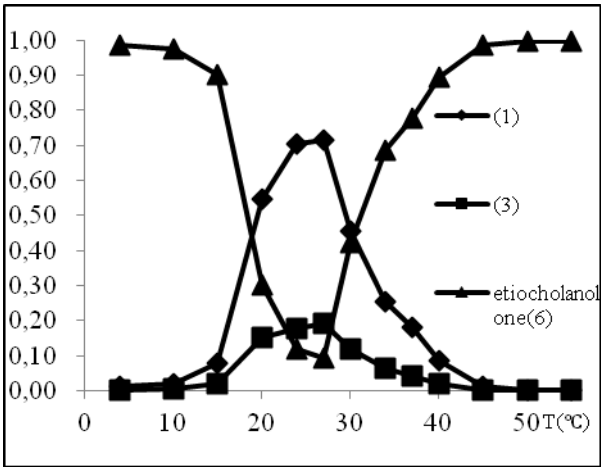

Figure 3. Degradation ratio of Etiocholanolone (6)

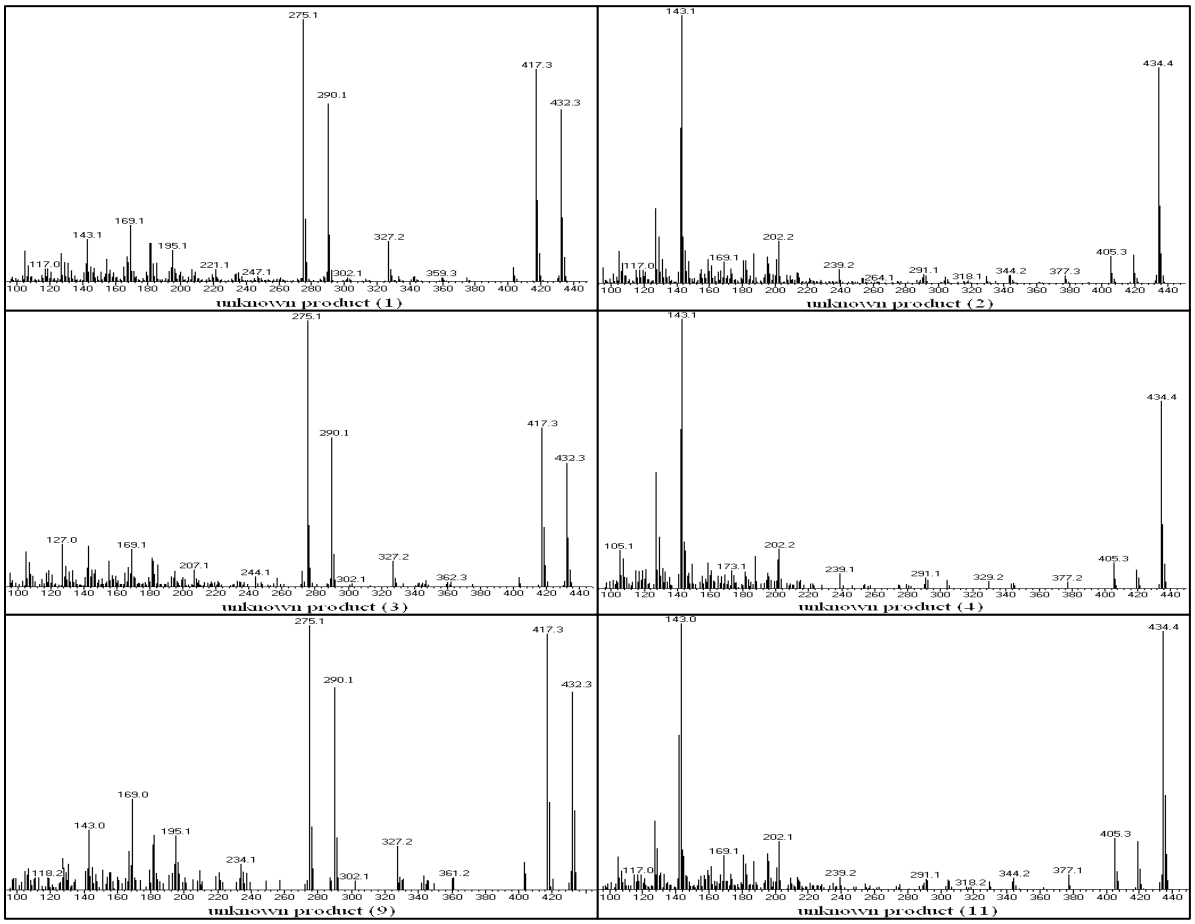

Figure 2. Mass spectra of 6 unknown degraded products as TMS derivatives