ON SIDE CHAIN CLEAVAGE OF 17α-HYDROXYPROGESTERONE BY TESTICULAR ENZYME

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Side chain cleavage of 17α-hydroxyprogesterone by rat testicular enzyme has been studied in this laboratory on the substrate specificity (Shikita and Tamaoki, 1965b), the requirement of NADPH, and the intracellular distribution of the enzyme (Shikita and Tamaoki, 1965a). Also , it was previously demonstrated that 17α-hydroxy-progesterone was the intermediate in the course of androgen formation from progesterone in the in vitro system employed (Shikita et al. 1964).

In this paper, the requirement of molecular oxygen for the side chain cleavage is reported and the origin of oxygen at 17-position of androst-4-ene-3,17-dione which was the direct product in this enzymic reaction was clarified by using one of stable isotopes of oxygen and a high resolution mass spectrometer for the analysis.

Materials and Methods

Throughout the experiment, the testicular tissue of adult rats of the Wistar strain, aged 2 months was used. The microsmal fraction of the tissue (105,000 x g precipitate) was used for the study of oxygen requirement. For the stable isotope work , as the soluble fraction (105,000 x g supernatant fluid) contained the activating

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principle for 17α-hydroxylase and pregnene-C_{17}-C_{20} lyase which located to the microsomal fraction, the mixture of the above two fractions or the supernatant fluid at 10,000 x g was employed .

Stable isotope of oxygen gas $^{18}O_2$ (91 atom % enrichment) was obtained from the Weizmann Institute of Science, Rehovoth, Israel, radioactive steroids were purchased from New England Nuclear Corp. (Boston , Mass.), and other steroid preparations were commercially obtained.

The detailed procedures of incubation, isolation and identification of the steroid products were previously reported (Shikita and Tamaoki, 1965a, 1965b).

Mass spectrometric analysis of the steroids obtained after the incubation was performed by one of the authors (H.S.) using Hitachi-Perkin-Elmer RMU-6D Mass spectrometer with and without double focus inlet. The mass spectra obtained were specifically analysed on the molecular ion peak and its isotope peaks region. When one atom of ^{16}O in a steroid molecule was replaced by ^{18}O, the increased height of the isotope peak shifted by two mass from the molecular ion peak M^+ is expected in the spectrum, in comparison to the corresponding peak of the natural standard steroid. This principle was already applied for the study of biosynthetic pathways on deutrium - labeled compounds (Popjak et al, 1961, 1962) and recently on ^{18}O labeled substance (Ryhage and Samuelsson, 1965).

Results and Discussion

Oxygen requirement for the side chain cleavage of 17α-hydroxyprogesterone

17α-Hydroxyprogesterone-4-^{14}C was incubated with rat testicular microsomes under oxygen enriched and anaerobic conditions. It was found that consumption of the substrate was strongly inhibited and correspondingly the production of androgens was reduced under oxygen poor atomosphere (Table 1). The result suggested that the lyase required molecular oxygen and was consistent with the result obtained with the testicular enzyme

Table I. Oxygen requirement for the side chain cleavage by rat testicular microsomes

	Gas Phase	
	Oxygen	Nitrogen
Recovered Substrate	4	56
Androstenedione	43	12
Testosterone	21	14
Cleavage*	64	26

The figures in the table indicate the yields of radioactivity in the fractions to the radioactivity initially added as the substrate or 17α-hydroxyprogesterone-4-^{14}C (36.8×10^3 dpm, 5 μg).

* Cleavage was expressed as the total of androstenedione and testosterone produced.

preparation of guinea pig (Lynn and Brown, 1958).

Incubation of steroids under $^{18}O_2$ atomosphere and mass-spectrometric analysis of the products

To elucidate the problem of whether the oxygen of 17-ketone of androstenedione produced by the side chain cleavage of 17α-hydroxyprogesterone originates from the required molecular oxygen or from 17α-hydroxyl group of the substrate, the following experiments were conducted.

Progesterone and 17α-hydroxyprogesterone were separately incubated under $^{18}O_2$ enriched atomosphere with rat testicular enzyme preparation in the presence of NADPH for four hours. After the incubation , the products were extracted with methylene-dichloride, then with ethanol, and the pooled steroid fraction was separated by thin layer chromatography. Finally the purified steroid preparations were mass-spectrometrically analysed always along with the respective authentic preparation as the control.

When progesterone was incubated under $^{18}O_2$ as gas phase, 17α-hydroxyprogesterone

which was produced by hydroxylation at 17α-position of progesterone was found to contain the ^{18}O derived from air phase in its molecule, or , showed the incorporation of one atom of ^{18}O per molecule of the steroid, while progesterone and 17α-hydroxyprogesterone which were incubated as the substrates under $^{18}O_2$ and recovered indicated no incorporation or exchange of ^{18}O. This suggested that the ^{18}O which incorporated into the molecule of 17α-hydroxyprogesterone existed in the form of 17α-hydroxyl group, but not as 3- or 20-ketones, as steroid hydroxylases generally did (Hayano, 1962).

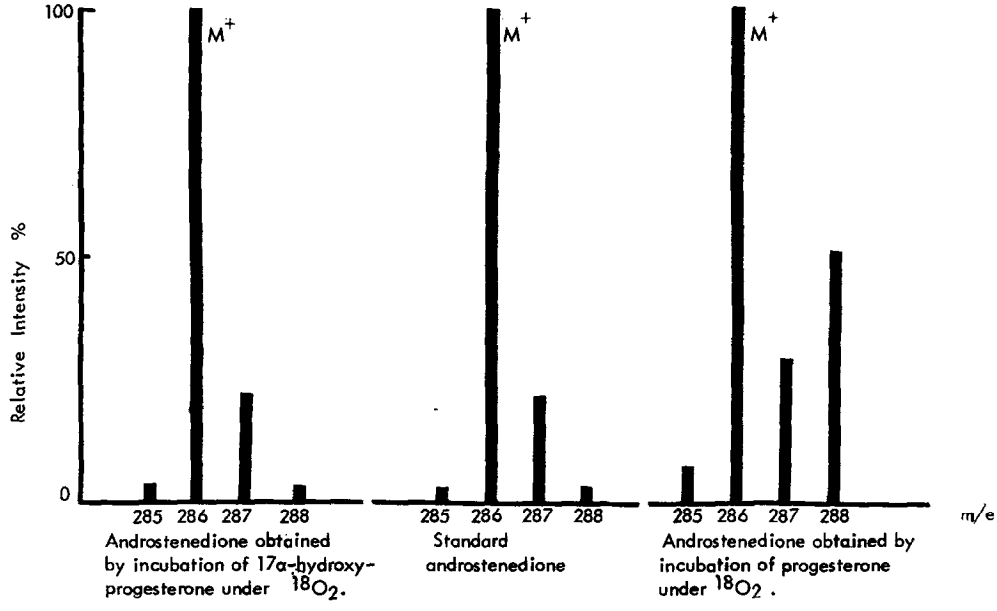

Fig. 1. Mass spectra of androstenedione
(Molecular ion peak and its isotope peaks)

Then, the direct product of the side chain cleavage or androstenedione fractions obtained from the incubation experiment of progesterone and 17α-hydroxyprogesterone separately as the substrates under the isotopic oxygen gas were examined with mass spectrometer. As shown in Figure 1, the spectrum of the androstenedione which was obtained from the incubation with 17α-hydroxyprogesterone as the substrate was found

identical to the one of natural androstenedione preparation, or , in other words, the molecular oxygen which was required for the side chain cleavage did not incorporated into the molecule of androstenedione, when the 17α-position of progesterone was already occupied by ^{16}OH group.

On the other hand, the androstenedione fraction obtained by the incubation of progesterone as the substrate under the isotopic oxygen gas clearly indicated that ^{18}O did incorporate into the molecule of androstenedione, most probably through 17α-^{18}OH-progesterone. It was also confirmed that the oxygen atom at 3- and 17-position of androstenedione did not exchange gaseous oxygen.

Furthermore, in order to confirm the origin of the oxygen at 17-position of androstenedione, the 17α-^{18}OH-progesterone which had been isolated after the incubation of progesterone under $^{18}O_2$ atomosphere, and confirmed mass-spectrometrically was again incubated with the testicular enzyme, this time, under natural oxygen or $^{16}O_2$ phase. The androstenedione thus produced indicated that the isotope peak (288) shifted from the

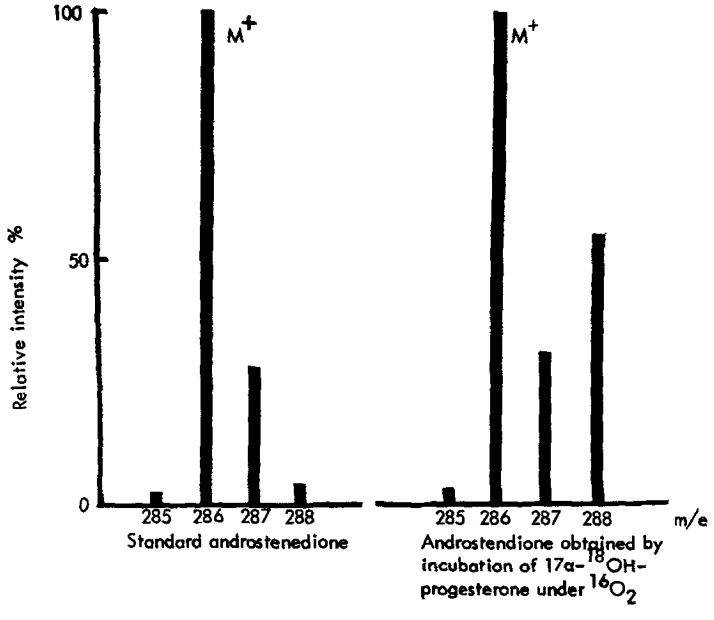

Fig. 2. Mass spectra of anarostenedione

(Molecular ion peak and its isotope peaks)

molecular ion peak by two mass was remarkably higher than the corresponding peak obtained from the standard preparation, (Fig. 2), indicating the significant incorporation of ^{18}O at 17-position of the produced steroid. This fact suggested that the oxygen which is present as 17-ketone of androstenedione originated from the oxygen of the 17α-hydroxyl group of 17α-hydroxyprogesterone, but not from the oxygen in gas phase.

Further quantitative analysis of the products and the studies on the mechanism of the cleavage are now under progress, and are to be published elsewhere shortly.

Summary Molecular oxygen was required for the side chain cleavage of 17α-hydroxyprogesterone by rat testicular enzyme. The oxygen in the form of 17α-hydroxyl group of 17α-hydroxyprogesterone was transferred to 17-ketone of androstenedione during the course of the cleavage.

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References

Hayano, M. Oxygenases ed. by O. Hayaishi, p. 181, Academic Press, N.Y. (1965).
Lynn, W.S. and Brown, R.H. J. Biol. Chem. 232, 1015 (1958).
Popják, G., Goodman, D.S., Cornforth, J.W., Conforth, R.H. and Ryhage, R. J. Biol. Chem. 236, 1934 (1961).
Popják, G., Cornforth, J.W., Cornforth, R.H., Ryhage, R. and Goodman, D.S. J. Biol. Chem. 237, 56 (1962).
Ryhage, R. and Samuelsson, B. Biochem. Biophys. Res. Communs. 19, 279 (1965).
Shikita, M., Kakizaki, H. and Tamaoki, B. Steroids, 4, 521 (1964).
Shikita, M. and Tamaoki, B. Endocrinology, 76, 563 (1965a).
Shikita, M. and Tamaoki, B. Biochemistry (Washington), 4, 1189 (1965b)