INCORPORATION OF MOLECULAR OXYGEN DURING THE BIOSYNTHESIS OF UBIQUINONE IN AN AEROBIC BACTERIUM, Pseudomonas desmolytica

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Summary

By using $^{18}O_2$, it was shown that two atoms from atomospheric oxygen are incorporated into a ubiquinone-9 molecule during biosynthesis by Pseudomonas desmolytica. Separate chemical experiments proved that both oxygen atoms in carbonyl groups of the ubiquinone molecule will exchange with water, while the two ^{18}O atoms incorporated do not, in 0.2N HCl at 85°C.
These experiments lead to the conclusion that the molecular oxygens are incorporated into two methoxy groups linked to C-5, 6 of the quinone ring. The importance of the cooperative relationship between respiratory system and oxygenation system in oxygen metabolism of bacteria is emphasized.

It is well known that ubiquinones occure in highly aerobic tissues and aerobic organisms (1), and is associated with electron transport and oxidative phosporylation. Folkers and collaborators proposed a scheme of ubiquinone formation in a photosynthetic anaerobe, Rhodospiriluum rubrum (2), which was recently supported by Whistance et al. for various facultative and aerobic bacteria including Pseudomonads (3).

On the other hand, the ubiquinone contents in facultative microorganisms such as Escherichia coli and Saccharomyces cerevisiae change markedly with the amount of oxygen supplied during their growth (1, 4). Furthermore, cell suspensions of anaerobically grown yeast will synthesize ubiquinone upon aeration (5). These observations suggest that ubiquinones not only are closely related to oxygen metabolism but also molecular oxygen is essential for their own bio-

synthesis. There has been no experimental evidence, however, for the incorporation of molecular oxygen during the biosynthesis of ubiquinone.

The present communication concerns the direct utilization of molecular oxygen as a substrate for ubiquinone biosynthesis in Pseudomonas desmolytica, and the position of incorporated oxygen atoms in the ubiquinone molecule.

Methods and Materials

$^{18}O_2$ and $H_2{}^{18}O$: $^{18}O_2$ was prepared by electrolysis of $H_2{}^{18}O$ (46% O-18, 0.13% O-17) purchased from Miles Laboratories, and mixed with ordinary O_2 gas before use. $H_2{}^{18}O$ used in the experiment of "oxygen-exchange reaction" was purchased from Bio Rad Laboratories.

Culture condition: Pseudomonas desmolytica IAM1508 was selected from a number of aerobic bacteria because it is rich in ubiquinone (6). The bacteria were grown in specially designed 7.3L closed-culture-flask containing 1.5L of medium of the following composition: glucose 2, casamino acid 0.5, yeast extract 0.5, $(NH_4)_2SO_4$ 0.1, K_2HPO_4 0.15, KH_2PO_4 0.05, and $MgSO_4 7H_2O$ 0.05 per cent respectively, under rotary shaking and at 30°C for 29 hrs. The $^{18}O_2$ enriched gas phase was composed of 25% of O_2 and 75% of N_2.

Extraction and purification of ubiquinone: 1.5g (dry wt.) of lyophilized cells were extracted three times with a mixture of ether and ethanol (3 : 1). The ubiquinone was separated from other lipids by acetone fractionation, and purified by alumina column chromatography (7). Finally, the ubiquinone was crystallized from ethanol at -10°C.

Mass analysis: The mass spectra were measured by using a Model RMU-6D mass spectrometer of Hitachi Co., Ltd. The ^{18}O contents in the ubiquinone were determined by scanning repeatedly the spectra of pyrylium ion (m/e 235) or benzylium ion (m/e 197), and the relative intensity of the peaks positioned at +1 ~ +6 to the base peak (m/e 235 or m/e 197) was calculated. CEC 21-620A mass spectrometer of the Consolidated Electrodynamics Corp. was used for analyzing $^{18}O_2$ in gas phase.

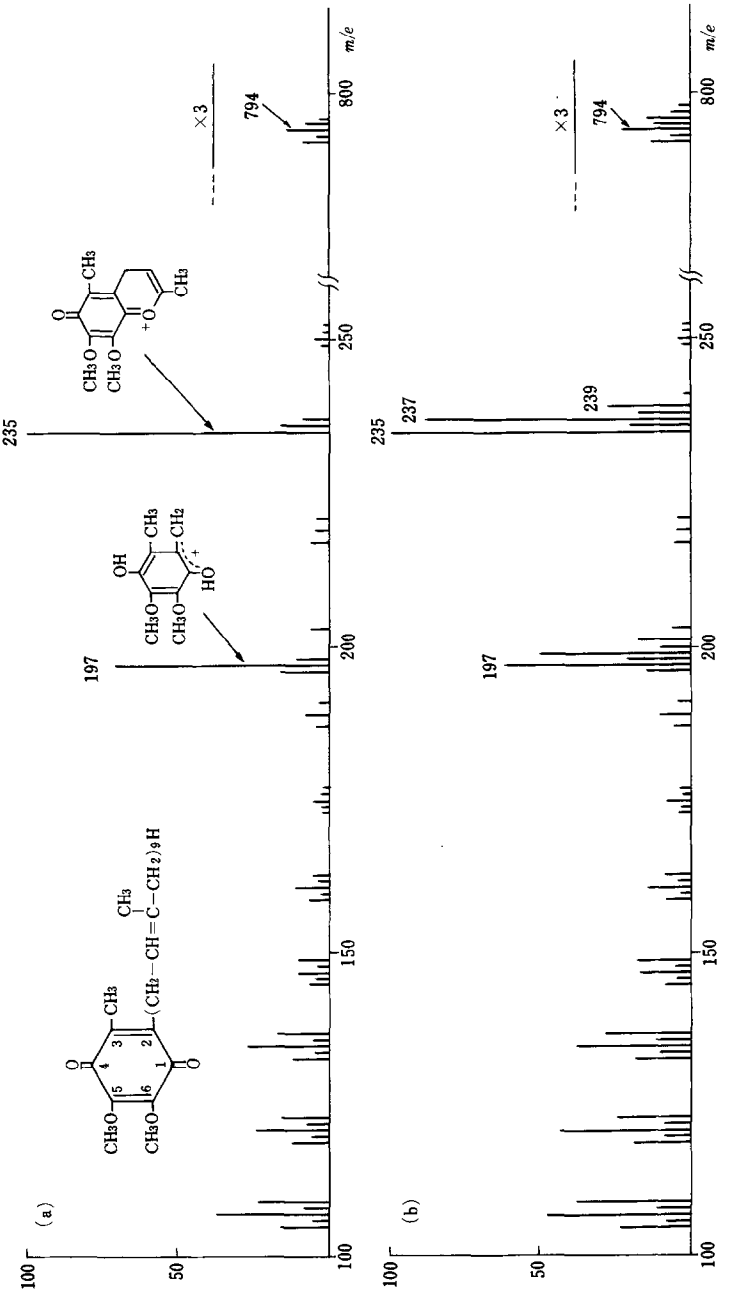

Fig 1 Mass spectra of the ubiquinone from *Ps. desmolytica* of ordinary shaken culture (a), and closed culture under nitrogen–oxygen gas mixture containing 32.3% oxygen-18 (b).

Oxygen-exchange reaction: The reaction mixture consisted of 0.5 mg of ubiquinone-9 (or ubiquinone-9-^{18}0), 0.18 ml $H_2{}^{18}0$(or $H_2{}^{16}0$), 0.02 ml 2N-HCl and 2 ml absolute ethanol. Microtubes (150 mm x 12 mm) containing the above reaction mixture were cooled with liquid nitrogen, and sealed under reduced pressure. After five-hours' incubation at 85°C, the ampoules were opened, and the contents were evaporated to dryness at low temperature. The ubiquinone was crystallized from absolute alcohol and subjected to mass spectrometry.

Results and Discussions

In the mass spectra of ubiquinones, there is a small parent peak, and two dominant peaks positioned at m/e 197 and m/e 235, and assigned as benzylium ion and pyrylium ion, respectively. Those fragments contain the four oxygen atoms of the ring moiety of the original ubiquinone (8, 9). The ubiquinone of P. desmolytica from ordinary shake-culture revealed the same characteristic fragmental pattern in the mass spectrum as previously reported (8, 10). This type of the quinone is assigned as ubiquinone-9 (UQ_9) from the molecular peak m/e 794 (Fig. 1a).

The mass spectrum of pure crystalline ubiquinone obtained from the cells of P. desmolytica grown in the presence of $^{18}0_2$ clearly showed new additional peaks at +2, +4 to m/e 235 or m/e 197; this is considered as the result of the incorporation of $^{18}0$ into the ubiquinone (Fig. 1b). The number of $^{18}0$ atoms incorporated was calculated from the relative intensity at region m/e 235 (7, 8 -dimethoxy-2, 5-dimethyl-6-cyclohexadienone-1-pyrylium ion). This value corresponds well with the interpretation that two oxygen atoms incorporate from molecular oxygen into a ubiquinone molecule (Table 1).

A ubiquinone molecule contains four oxygen atoms; two nuclear carbonyl oxygens at C-1, 4 and two methoxy oxygens linked to C-5, 6 of quinone ring. We examined the oxygen atoms derived from molecular oxygen in the isolated $^{18}0$-containing ubiquinone by means of "oxygen-exchange method". Many investigations of oxygen exchange in organic compounds with solvent show that carbonyl groups

Table I Relative peak height of the pyrylium ions of isolated ubiquinone from *Ps. desmolytica*

M/E	ordinary UQ_9	^{18}O-incorporated UQ_9		theoretical value*
		apparent	corrected	
235	100	100	100	100
+2	5.3	93.0	87.7	95.4
+4	0.7	33.4	28.1	22.7
+6	0.4	2.0	0	0

*: This value is based on the interpretation of twe oxygen-atoms' incorporation, and calculated as follow:

M: M+2: $M+4=(100-32.3)^2$: $32.3\times(100-32.3)\times 2$: 32.3^2

Table II Relative peak height of the pseudomonad's $UQ_9-^{18}O$ after chemical reaction

M/E	before reaction	after reaction
235	100	100
+2	87.7	91.3
+4	28.1	27.2

exchange with water rather easily, while hydroxyl or ether groups do not (11). The chemical exchange of oxygen atom between ubiquinone and water was clearly observed when $UQ_9-^{16}O$ was incubated with $H_2{}^{18}O$ in 0.2N HCl acid solution at 85°C. However, $UQ_9-^{18}O$ biosynthesized by the bacterium showed no decrease in ^{18}O contents after incubated with $H_2{}^{16}O$ under the same condition (Table II). Therefore, it can be concluded that $UQ_9-^{18}O$ contains the labeled oxygens in the two methoxy groups but not in the carbonyl groups. The carbonyl oxygen at C-1 is believed to be introduced directly from the hydroxyl group of p-hydroxybenzoic acid, a precursor of ubiquinone.

It is proved here that two methoxy oxygen atoms at C-5, 6 of the quinone ring of ubiquinone-9 are derived from molecular oxygen. The quinoid oxygen atoms of menaquinone-9 were recently reported not to be derived from molecular oxygen in Mycobacterium phlei (12). The above findings can also explain the gradual restoration of respiration in facultative organisms and the lower ubiquinone contents of cells grown at low oxygen tension. For example, culture of E. coli and other facultative bacteria has the tendency to reduce ubiquinone contents when grow at low O_2 tension but menaquinone contents increase (13), and respiration

of Saccharomyces cerevisiae can be only gradually restored to its maximum rate when changed from anaerobic condition. It may be expected that ubiquinone biosynthesis is one of the important rate-limiting factors in oxygen adaptation process in these organisms. In heme biosynthesis 0_2 fixation does not take place, though the process obligately needs oxygen not merely as electron acceptor but as biochemical reagent (14, 15). It is worthy of attention that a cooperative relationship exists between respiration where 0_2 serves as electron acceptor and the oxygenation systems in which 0_2 serves as a building materials.

A different system which introduces oxygen atoms into ubiquinone precursor must be existed in a photosynthetic anaerobe, R. rubrum, for cell suspensions of this organism under illumination convert p-hydroxybenxoic acid to ubiquinone anaerobically, perhaps through another "oxygen donor" (16). We are now determining whether ubiquinone biosynthesis in R. rubrum grown aerobically in darkness utilizes molecular oxygen.

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