The Possible Role of Flavin on the Hydroxylation of Hydrocarbon by Bacterial Enzyme System

Sir:

A number of monooxygenases have been well established to be flavoproteins.1-4) Kusunose et al.5) observed some instances in which enzyme fractions obtained from Pseudomonas oleovorans have greater activity for octane oxidation when flavin adenine dinucleotide (FAD) is added. On the other hand, Heydeman and Azoulay6) described that an aldehyde dehydrogenase requiring Fe++ or Ca++ and flavin such as FAD or riboflavin is involved in paraffin hydrocarbon oxidation by extracts of Pseudomonas aeruginosa. Recently, evidences have been presented that bacterial hydrocarbon hydroxylation system consists of three proteins: hydrocarbon hydroxylase, non-heme iron protein (rubredoxin), and NADH-rubredoxin reductase.7,8) The present investigation was undertaken to clarify the possible role of flavin in hydrocarbon hydroxylation.

The organism utilizing hydrocarbons was kindly supplied by Prof. K. Yamada of Tokyo University, and recently it was identified as Pseudomonas denitrificans.* Growth of the organism on a mineral medium containing hexane as a sole carbon source and preparation of the three protein components (hydrocarbon hydroxylase, NADH-rubredoxin reductase, and rubredoxin) necessary for hydrocarbon hydroxylation were described in the preceding paper.8) Highly purified ferredoxin-NADP reductase was prepared from spinach leaves according to the method of Shin et al.9) Hydrocarbon hydroxylation activity was assayed by the procedure of Gholson et al.10) using decane-1-14C. Fatty acid omega-oxidation activity was assayed by the procedure of Kusunose et al.11) using lauric acid-1,14C. Enzymatic reduction of rubredoxin by NADH was measured by the decrease in absorbance at 497 mμ. Decane-1,14C and lauric acid-1,14C were purchased from the Radiochemical Centre, Amersham, England. 2-Amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine was a gift of Dr. A. Ichiyama of Kyoto University.

Fig. 1 shows the requirement of FAD for the maximal activity of decane hydroxylation by the system containing hydrocarbon hydroxylase, rubredoxin, and NADH-rubredoxin reductase. Flavin mononucleotide (FMN) or riboflavin could partially substitute for FAD.

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2) K. Yano, M. Morimoto and K. Arima, This Journal, 30, 91 (1966).
Each reaction mixture (1 ml) contained 100 μmoles of Tris-HCl buffer (pH 7.4), 0.25 μmole of NADH, 30 μmoles of decane-1-14C (1.4 × 10^5 c.p.m.), hydrocarbon hydroxylase (Fraction I-A, 0.4 mg of protein), NADH-rubredoxin reductase (Fraction II-A, 0.7 mg), and rubredoxin (Fraction III-A, 0.4 mg). FAD was added as indicated. After the incubation at 30°C for 10 min, the reaction products were separated and determined by the method of Gholson et al.10) For preparation of Fractions I-A, II-A and III-A, see the preceding paper.8)

Judging from these studies, it may be suggested that flavin such as FAD or FMN participates only as a cofactor of the NADH-rubredoxin reductase in hydrocarbon hydroxylation, which may be presented as follows. NADH → flavoprotein → rubredoxin → oxygen (NADH-rubredoxin reductase)
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Masamichi KUSUNOSE
Kosuke ICHIHARA
Emi KUSUNOSE
Junichiro NOZAKA
Junjiro MATSUMOTO

Toneyama Institute for Tuberculosis Research,
Osaka City University Medical School and
Research Laboratory of Biochemistry,
Toneyama Hospital, National Sanatorium
Toyonaka, Osaka.

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FIG. 3. Enzymatic Reduction of Rubredoxin by Spinach Ferredoxin-NADP Reductase.

Reaction mixture contained rubredoxin (2 mg of protein) and spinach ferredoxin-NADP reductase (1.5 µg) in 0.8 ml of 0.02 M Tris-HCl buffer (pH 7.4). The reduction of rubredoxin was initiated by the addition of NADPH (50 mµmoles).