Spectrophotometric Studies on Lysine Monooxygenase, a Flavoprotein *  
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The spectral changes of the enzyme-bound FAD were investigated in the anaerobic and aerobic reactions of lysine monooxygenase, a flavoprotein. Under anaerobic conditions, the enzyme FAD was fully reduced with lysine via a transient intermediate having a broad absorption band in the long wavelength region. This anaerobic species was presumably involved in the dehydrogenation of lysine leading to the formation of an α-keto acid. During the steady state of the aerobic reaction, a separate spectral species with a long wavelength absorption was observed. Both lysine and oxygen were required for its appearance. Kinetic evidence supports the intermediacy of this species in the oxygenation of lysine to an acid amide.

A number of oxygenases have recently been identified to be flavoproteins. Our metal analyses with several flavoprotein oxygenases failed to detect any metal in significant quantity 1, suggesting that flavin is not merely an electron carrier, but it serves as the site of oxygen activation. Lysine monooxygenase is an example of such flavoprotein oxygenases. The enzyme crystallized from a pseudomonad contains two moles of FAD per mole of enzyme and shows an absorption spectrum with maxima at 385 and 460 nm 2. One atom of molecular oxygen is incorporated into lysine, and acid amide is formed concomitant with decarboxylation (Eq. 1). Under anaerobic conditions the enzyme-bound FAD is fully reduced with lysine, and α-keto acid is produced in an amount equivalent to the reduced enzyme (Eq. 2). When the fully reduced enzyme thus obtained is reoxidized by the admission of oxygen, lysine is also converted to α-keto acid, not acid amide. These results indicate that the dehydrogenation of lysine and the full reduction of FAD are not involved in the oxygenation of lysine.

\[
\text{R} \text{CH-NH}_2 + \text{O}_2 \rightarrow \text{R} \text{C}=\text{NH} + \text{CO}_2 + \text{H}_2\text{O}^* \quad (1)
\]

\[
\text{R} \text{CH-NH}_2 + \text{FAD} \rightarrow \text{R} \text{C}=\text{NH} + \text{FADH}_2 + \text{H}_2\text{O} \quad (2)
\]

In order to elucidate the catalytic reaction mechanism of the enzyme, the spectral change of the enzyme FAD was investigated during the anaerobic and aerobic reactions. A preliminary report has appeared elsewhere 4. Under anaerobic conditions, the enzyme was reacted with lysine and the absorption change at various wavelengths was followed by the stopped-flow technique (Fig. 1). At the flow-stop, the spectrum of the enzyme was identical with that of the oxidized enzyme except for a slight change of the 385 nm absorption peak. Then a spectral species appeared transiently which showed a broad absorption band in the long wavelength region. The rate constant for its rapid formation was dependent on the concentration of lysine.

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giving a saturation curve. The $K_m$ for lysine was 20 mM and the maximal rate constant of 75 s$^{-1}$ was obtained by extrapolating to the infinite concentration of lysine. Subsequent FAD reduction to the fully reduced enzyme involved an extremely slow step.

In the presence of oxygen, another spectral species with a long wavelength absorption was observed during the steady state of the reaction (Fig. 2). This species was distinct from the oxidized or reduced enzyme or the mixture of both and also distinguished from the anaerobic species since the 460 nm absorption weak was blue-shifted by approximately 5 nm. The appearance of this species required the presence of both lysine and oxygen. When lysine was exhausted, it was converted back to the oxidized enzyme. The rate constant for its formation as determined by the stopped-flow technique, was found to be dependent on the concentration of lysine and oxygen, respectively, each giving a curve of Michaelis-Menten type. $K_m$ values for lysine and oxygen were 0.60 and 0.53 mM, respectively, and the maximal rate constant was 141 s$^{-1}$. As compared with $K_m$ (0.59 mM for lysine and 0.55 mM for $O_2$) and $V_{max}$ (88 s$^{-1}$) for the overall oxygen consumption, these values are consistent with an explanation that this aerobic species is involved as an intermediate in the overall aerobic reaction.

The requirement of both lysine and oxygen suggests the relevance of this new spectral species to the oxygenated enzyme-substrate complex as was reported with hemoprotein or non-heme iron protein oxygenases$^5$-$^8$. However, for its definite characterization, further investigations will be required to elucidate the chemical events how flavin, lysine and oxygen react together to form such a ternary complex. The anaerobic species with a long wavelength absorption may be involved only in the anaerobic full reduction of the enzyme FAD leading to the formation of an α-keto acid.

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