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The Air Oxidation of Catecholamines by Flavin Mononucleotide

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Polarographic studies of the aerobic oxidation of catecholamines under the influence of FMN in various concentrations, and spectrophotometric investigations of the formation of FMN–catecholamine complexes, have been carried out. By following the course of the oxidation reaction polarographically, the rate constants have been found. The oxidation of catecholamines by air was found to be disturbed through complex formation and to be facilitated by light illumination. The equilibrium constants for the formation of some catecholamine complexes with FMN have been calculated.

It has been established that such flavins as flavin mononucleotide (FMN) and flavin adenine dinucleotide can form charge transfer (CT) complexes with tryptophane, purines, and catechol.[1] These flavins act as catalysts for the redox reactions in flavin enzymes. The oxidation of ethylenediamine tetraacetate or reduced nicotinamide adenine dinucleotide (NADH) in the presence of flavins has been reported to be facilitated by light exposure.[2] This oxidation reaction is inhibited if the donor compounds coexist in the systems.[3] Such acceptors as methyl viologen do not inhibit the reaction. Menadiol diphosphate is oxidized by oxygen in the presence of riboflavin with light exposure.[4]

It was expected that such catecholamines as 3,4-dihydroxyphenylalanine (DOPA), 3-hydroxytyramine (dopamine), and adrenaline, which are susceptible to be oxidized by oxygen,[5] might form the CT complex as donors. No papers on the study of the flavin-sensitized photooxidation of catecholamines have yet been found, however. Both catecholamines and flavins are biochemicals utilized as medicines. Therefore, it is important to clarify the interaction between flavins and catecholamines. For this purpose, we studied the formation of CT complexes in FMN-catecholamine systems and the oxidation of catecholamines in the presence of FMN. In this paper we will present the results obtained by the polarographic and spectrophotometric methods.

Experimental

The determination of FMN was carried out by spectrophotometry at 450 nm. L-DOPA, dopamine hydrochloride, and L-adrenaline bitartrate were used as catecholamines. All the sample solutions were prepared in a 0.1 mol/l phosphate buffer (pH 7.0).

Anode polarograms were obtained by using a Yanaco platinum rotating electrode equipped with a Hokuto Denko linear scanning unit LS-lD and potentiostat HA101, and a Toa Electronics Ltd. X-Y recorder SYR-1A. The platinum rotating electrode used was 1 cm long, with a diameter of 0.1 cm, and had a rotation of 600 rpm. The sweep speed for recording polarograms was set at 3 V/min. The accuracy of the half-wave potentials obtained was estimated to be within ±0.01 V. The intensity of the illumination and the dissolved oxygen were measured by means of a Tokyo Koden illumination meter ANA-300 and an Anal Instrument Co DO meter respectively. The absorption spectra were determined with a Shimadzu UV-200 spectrophotometer.

Results

Polarography. In several 50 ml beakers, 40 ml portions of solutions containing FMN and catecholamines were prepared and shaken under the air at 30 °C with illumination (400—3000 lx). Polarograms were measured at desired intervals. Some results are shown in Fig. 1. In the case of adrenaline, the half-wave potential, $E_{1/2}$, of the anodic wave was found to shift from +0.14 to +0.16 V *vs.* SCE with an increase in the FMN concentration from 2.5 to 20 mmol/l. Towards the end of the oxidation process, the waveheight at each $E_{1/2}$ gradually reduced and finally disappeared overnight, but another anodic wave became predominant, its $E_{1/2}$ value being clearly determined to be at +0.28 V *vs.* SCE. The waveheight at the first $E_{1/2}$ changed with the lapse of time. It increased measurably during the first 30 min and then decreased. After that, the oxidation seemed to occur in the first order

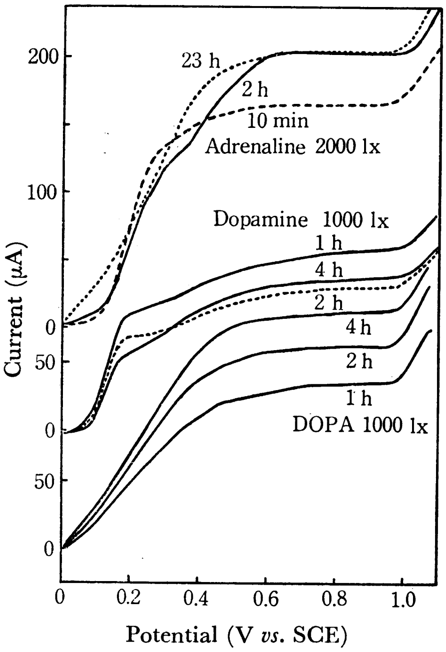


Fig. 1. Polarograms of catecholamine–FMN systems.
Catecholamine: 1 mmol/l
FMN: 20 mmol/l (Adrenaline)
5 mmol/l (Dopamine or DOPA)
pH 7.0, 30 °C

Table 1. Equilibrium constants and velocity constants

	Equilibrium Constant	Velocity Constant (h^{-1})			
	(l/mol)	400 lx	1000 lx	2000 lx	3000lx
Adrenaline	19±4	0.12	0.18	0.25	0.24
DOPA	11±4	—	—	—	—
Dopamine	89±10	—	0.14	0.068	—

with respect to adrenaline. The reaction velocity was found to be independent of the FMN concentration and to be influenced by illumination. The velocity constants were obtained from the slopes in Fig. 2; they have been summarized in Table 1.

The reaction velocity in the case of dopamine was smaller than that in adrenaline and was also independent of the FMN concentration. The value of the first $E_{1/2}$ of dopamine fell on +0.12 V *vs.* SCE when the FMN concentration varied from 2.5 to 20 mmol/l. The second $E_{1/2}$ was apparent in the region between 0.38 and 0.43 V, but the exact value could not be obtained because the waveheight at the first $E_{1/2}$ still remained after 22 h. The velocity constant obtained from the slope in Fig. 2 is recorded in Table 1.

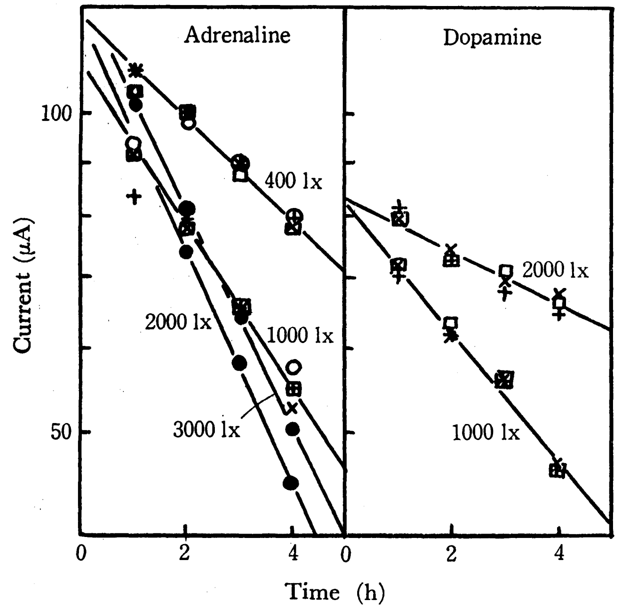


Fig. 2. Air oxidation of catecholamines.
Adrenaline or dopamine: 1 mmol/l, FMN: +; 2.5, ■; 5, ×; 10, ○; 20 mmol/l ●; average in each concentration of 2.5—10 mmol/l.

In the case of DOPA, $E_{1/2}$ was found to be centered in the region of 0.2—0.3 V. However, the definite position of $E_{1/2}$ and the waveheight at $E_{1/2}$ could not be obtained. The waveheight at about 0.6 V was found to increase considerably with the lapse of time (Fig. 1).

Spectrophotometry. The absorbances of the FMN–catecholamine systems at 505 nm were measured in a way similar to that described in a previous paper.[6] Dissolved oxygen from the sample solutions was removed by bubbling in nitrogen gas. The results plotted according to Benesi–Hildebrand's equation are shown in

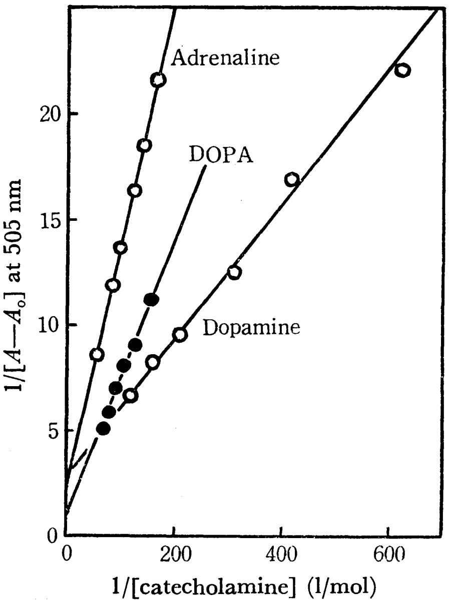


Fig. 3. Benesi-Hildebrand's plots of FMN–catecholamine systems.
FMN: 8.0×10^{-4} mol/l Oxygen free, pH 7.0, 25 °C

Fig. 3. The equilibrium constants calculated from the results in Fig. 3. are summarized in Table 1.

In a 100-ml beaker a 20 ml solution containing 3.2 mmol/l DOPA with or without 8.0×10^{-2} mmol/l FMN was prepared and stirred on a waterbath at 25 °C with illumination. The absorbance change at 340 nm was determined[7] and found to increase linearly with the lapse of time within an hour. The initial velocities (v_0's) were calculated from the absorbance change (340 nm)-time curves. The v_0's of DOPA oxidation were compared with different strengths of illumination (Figs. 4 and 5). DOPA oxidation was found to be facilitated in the presence of FMN and with stronger illumination. The plots of $1/v_0$–1/DOPA concentration fall on a straight line. Its oxidation was very slow without light exposure, even when FMN was present in the solution. In another experiment, when nitrogen gas was bubbled through a solution, the absorbance at 450 nm diminished to 82% after illuminating for 16

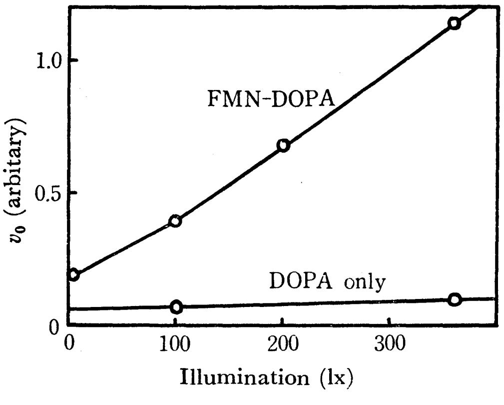


Fig. 4. Influence of illumination to the initial velocity of DOPA oxidation reaction.
FMN: 8.0×10^{-5} mol/l, DOPA: 3.2×10^{-3} mol/l, Oxygen: 2.7×10^{-4} mol/l, pH 7.0, 25 °C.

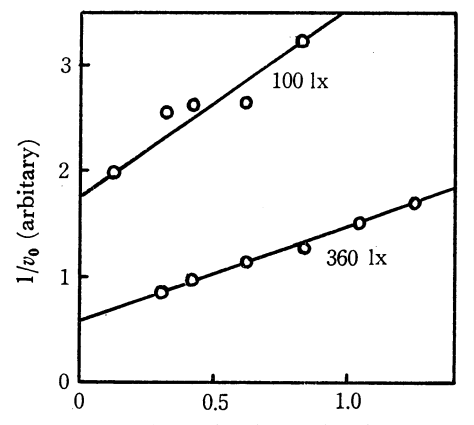


Fig. 5. Plots of 1/DOPA *vs.* $1/v_0$. FMN: 8.0×10^{-5} mol/l, Oxygen: $2,7 \times 10^{-4}$ mol/l, pH 7.0, 25 °C.

h at 1300 lx. Similar results were obtained when dopamine and adrenaline were used in place of DOPA. The ratios of the absorbance change of dopamine and adrenaline against DOPA were 0.4 and 1.2 respectively at 360 lx.

Discussion

It seemed that the complex formation might disturb the oxidation, as the velocity constant in the FMN–dopamine system was smaller than that in the FMN–adrenaline system. The illumination was found to enhance the air oxidation reaction in the case of adrenaline.

The reaction by which FMN oxidized NADH to form NAD, which has been reported by Radda and Calvin,[2] is a downhill reaction, because the redox potentials of NADH/NAD and $FMNH_2$/FMN are −0.32 and −0.22 V *vs.* NHE, respectively. The redox potential of adrenaline is known to be +0.80 V *vs.* NHE.[8] It is not probable that adrenaline or other catecholamines can be oxidized directly by FMN. Moore and Song[4] have reported that the energy transfer occurred from a photoexcited FMN to oxygen to attain a singlet state and that, in turn, the oxygen in the singlet state oxidized menadiol diphosphate. This oxidation reaction seems similar as that in the case of catecholamines.

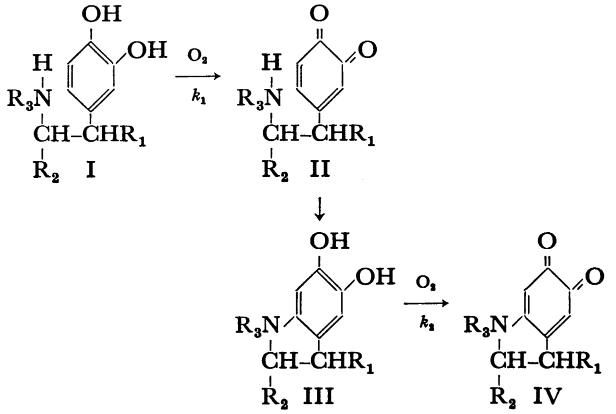
It has been recently reported that a preequilibrium complex must react directly with oxygen in the process of lumiflavin reduction by *N*-methyl-1,4-dihydronicotinamide.[9] A similar reaction was observed in the FMN–DOPA reaction in Fig. 5, which showed the following Michaelis–Menten-type reaction:

$$\begin{tikzcd} F + D \arrow[r, shift left] & FD \arrow[l, shift left] \arrow[r, shift left] \arrow[d, "O_2"] & FH_2 + \text{Product} \arrow[l, shift left] \arrow[d, "O_2"] \\ & \text{Product} + F + H_2O_2 & F + H_2 \end{tikzcd} \tag{1}$$

F: FMN D: Catecholamine

The fact that the reaction proceeded without illumination (Fig. 4) might be explained as a result of the oxidation of a trace amount of FH_2 produced in the equilibrium. The reduced flavin is known to be easily oxidized by oxygen.[10] It is plausible that the light reaction and the dark reaction proceed together.

It is known that catecholamine-oxidized can form a cyclic compound, III,[5] which may accumulate through the reaction if the velocity constant, k_1, is larger than k_2. This assumption is considered to be justified, because the reaction proceeds by the first order with respect to catecholamines. The half-wave potential of leucoadrenochrome is known to be +0.44 V *vs.* NHE at pH 7.[8] This is almost in accord with the second half-wave potential in adrenaline (+0.28 V *vs.* SCE) if some interaction between FMN and adrenaline is considered. Therefore, the second half-wave potentials in adrenaline and dopamine solutions can be assigned to III. Then the fact of the waveheight increase Fig. 1 seems reasonable because III is oxidized faster than I at the anode to limiting currents of the III and I of adrenaline, as shown in Fig. 1, are 205 and 165 μA respectively.



	R_1	R_2	R_3
Adrenaline	OH	H	CH_3
DOPA	H	COOH	H
Dopamine	H	H	H

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