

Semiquinone Radicals Generated from Catecholamines by Ultraviolet¹⁾ Irradiation²⁾

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A detection method of radicals during photolysis of catecholamines and 3,4-dihydroxyphenyl-L-alanine was established. Electron spin resonance spectra of the radicals derived from catecholamines under the photolytic condition showed hyperfine structures which were assigned to *o*-semiquinone anion radicals.

Keywords—epinephrine; norepinephrine; dopamine; catecholamines; photolysis; UV irradiation; electron spin resonance; semiquinone radical; 3,4-dihydroxyphenyl-L-alanine

Dopamine, norepinephrine and epinephrine in that order are biochemically synthesized from DOPA and named catecholamines after a common structure of catechol moiety and amino group of the side chain. They play important roles in the chemical transmission of the nervous system and the hormonal regulation of various organs. There are many studies on their chemical characteristics. They are easily oxidized chemically and enzymatically. The degradation mechanisms in these reactions starting with the oxidation of the catechol have been investigated.⁴⁾ However, the mechanism of photo-oxidation of catecholamines is not well-known, although biologically interesting. It was described that the oxidation of catecholamines into final products, melanins, was promoted by radiant energy.⁵⁾ A photochemical study of catechol⁶⁾ suggested that a primary intermediate of the photodegradation was an *o*-semiquinone radical. The occurrence of radicals from catecholamines and DOPA during the chemical oxidation was shown by ESR, although the spectra were not analysed.⁷⁾ On the other hand, the semiquinone radicals from catecholamines were considered to play a critical role in the course of photoaffinity labeling of catecholamine receptors.⁸⁾

In this paper, we report the generation of the semiquinone radicals from catecholamines and DOPA upon UV irradiation.

Experimental

Materials—L-Epinephrine, L-norepinephrine and dopamine·HCl were purchased from Sigma Chemical Co. DOPA was kindly supplied by Daiichi Seiyaku Co., Ltd.

Measurement of ESR and UV Spectra upon Photoirradiation—ESR spectra were measured with a JEOL PE-1 X band spectrometer equipped with 100 kHz field modulation. Signals of Mn²⁺ diffused in MgO and a paramagnetic center in a quartz cell were used as calibration standards for determination of ESR parameters. The coupling constants and the *g* factors are accurate to within ± 0.08 G and ± 0.0002 , respectively. An Ushio 500 W super high pressure mercury lamp (USH-500D) was used as a light source for photolysis. The

- 1) abbreviation—DOPA: 3,4-dihydroxyphenyl-L-alanine; ESR: electron spin resonance; UV: ultraviolet; tris: tris(hydroxymethyl)aminomethane; G: gauss.
- 2) This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture.
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ESR measurement was carried out by an *in situ* photolysis method, *i.e.*, the ESR spectra were taken during photoirradiation to a sample solution in a flat quartz cell (0.5 mm optical path length, 3 mm width, and 40 mm length) mounted in an ESR cavity. The solution of the sample dissolved in 0.03 M tris·HCl (pH 7.0) was allowed to flow slowly through the cell to avoid increasing temperature and depletion of the solute. The flow rate used was 0.3–3.7 ml/min and adjusted so as to attain the best record of ESR spectra. The solution flowed out of the cell was applied to a UV spectrometer, Cary Recording Spectrometer Model II.

Results

Degradation of epinephrine was confirmed from the UV absorption spectra taken just after the photolysis in the flat cell, as shown in Fig. 1. The spectral change became larger, as the flow rate slower. The same spectral change, although different in the velocity, was observed when another lamp such as a fluorescent lamp for health Toshiba FL-20E or a xenon lamp Hitachi-150 W was used. The UV absorption spectra of the irradiated solution in Fig. 1 were similar to those obtained by Walaas who suggested the existence of adrenochrome and melanin in a solution irradiated in the same manner.⁵⁾

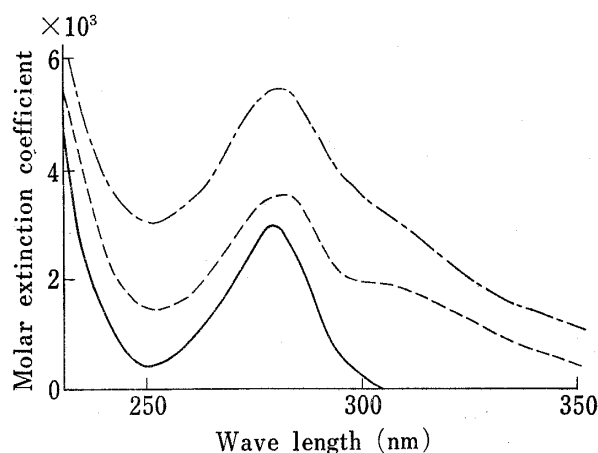


Fig. 1. UV Spectral Change of Epinephrine upon UV Irradiation

A solution of 2.5×10^{-4} M L-epinephrine (—) in 0.03 M tris·HCl (pH 7.0) flowed through the quartz cell upon the UV irradiation with a lamp USH-500D. The flow rate was 0.8 (—) or 0.3 (— · —) ml/min.

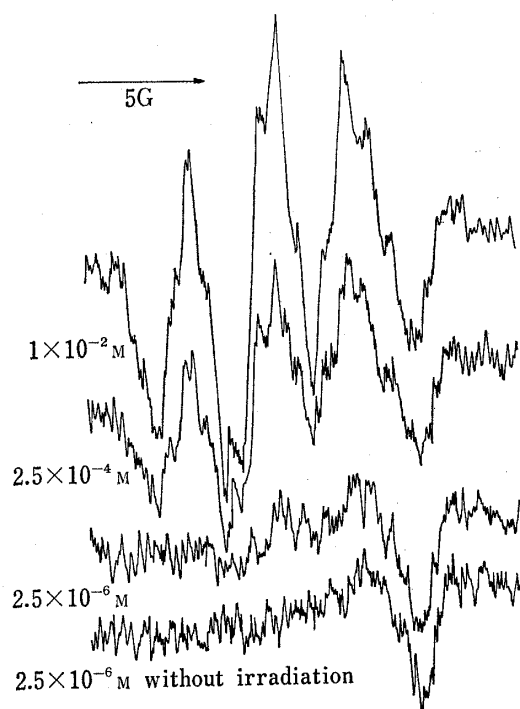


Fig. 2. First-Derivative ESR Spectra of the Radical from Solutions of Epinephrine at Various Concentrations

The spectra were measured by the *in situ* photolysis method described in the text. The magnetic field increased from left to right.

Under the photolytic condition, the ESR signals were detected at various concentrations of epinephrine. The concentration limit for detection of the signal was around 2.5×10^{-6} M epinephrine which was a usual concentration for biological experiments. No spectrum except for a background signal from the quartz cell was observed without the photoirradiation, as shown in Fig. 2.

At the concentration of 10 mM epinephrine, the signal was strong enough to attain a higher resolution by lowering a modulation width, as shown in Fig. 3. Deaeration by bubbling nitrogen gas through the solution did not give a significant change to the spectrum. The pH of the solution did not change after the photolysis. There has been found no report on the ESR spectrum of the radical from epinephrine itself except for a poorly resolved one

observed by Borg during chemical oxidation.^{7a)} ESR data of 4-alkyl-*o*-benzosemiquinone anion radicals generated by a chemical oxidation⁹⁾ were utilized as a reference. Further, we obtained a well-resolved ESR spectrum by the photoirradiation of 4-methylcatechol under the condition in Experimental. The ESR parameters for an *o*-semiquinone anion radical from 4-methylcatechol, $A(4\text{-Me})=4.83\text{G}$, $A(5\text{-H})=3.83\text{G}$, $A(6\text{-H})=1.03\text{G}$, $A(3\text{-H})$ = unresolved, and $g=2.0045$, were in good agreement with the previously reported values, $A(4\text{-Me})=5.1\text{G}$, $A(5\text{-H})=4.0\text{G}$, $A(6\text{-H})=1.0\text{G}$ and $A(3\text{-H})=0.25\text{G}$ for the radical in an alkaline solution.^{9b)} The radical must be anionic because pK_a values of semiquinones are known to be around 4.¹⁰⁾ Comparing with these data, the spectrum of the radical derived from

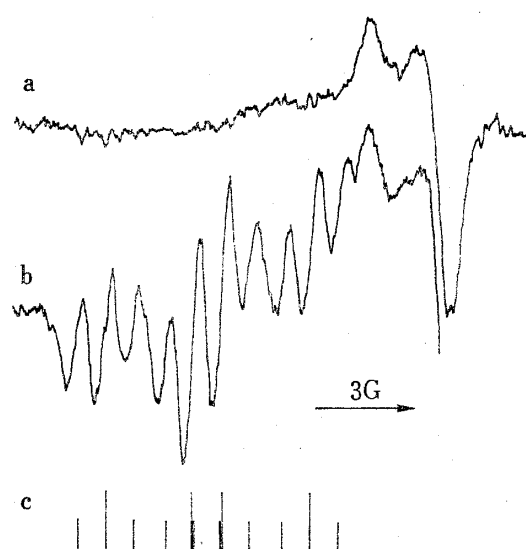


Fig. 3. A High Resolution Spectrum of the Semiquinone Radical from Epinephrine

- a : a background signal from the cell.
 b : An ESR spectrum observed during the photoirradiation of $1 \times 10^{-2}\text{M}$ epinephrine.
 c : The stick spectrum showing the line positions calculated from the ESR parameters shown in the text.

epinephrine was analysed to obtain the stick diagram shown in Fig. 3c. In the 4-alkyl-*o*-semiquinones, the order of the magnitudes of coupling constants for the ring protons has been shown to be independent of the kinds of 4-alkyl substituents. Thus, the most probable assignment for the hyperfine coupling constants was $A(5\text{-H})=3.58\text{G}$, $A(7\text{-H})=2.72\text{G}$, and $A(3\text{-H})=A(6\text{-H})=0.88\text{G}$ ($g=2.0047$). A proposed structure generated by the irradiation is shown in Chart 1.

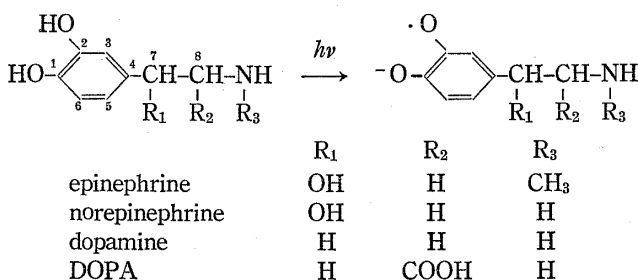


Chart 1. Generation of Semiquinone Anion Radicals from Catecholamines and DOPA by UV Irradiation

The ESR spectrum observed from norepinephrine was identical to that from epinephrine shown in Fig. 3b.

Fig. 4 shows the spectrum observed during the photoirradiation to an aqueous solution of dopamine. It was again ascribed to the semiquinone radical anion of dopamine in the same manner as the spectrum derived from epinephrine. The ESR parameters were $A(5\text{-H})=3.58\text{G}$, $A(7\text{-H})=3.03\text{G}$ (two protons), $A(6\text{-H})=0.96\text{G}$, $A(3\text{-H})=0.48\text{G}$, and $g=2.0044$.

The three catecholamines mentioned above gave only one ESR spectrum, respectively, upon the photoirradiation and their spectral intensity was almost independent on the flow rate of the solution. On the other hand, the photoirradiation of DOPA resulted in the spectra showing the presence of more than one species of radicals when the flow rate was slow. This complication could be the result of a secondary reaction such as photodecomposition of reaction products. The faster flow (the rate 3.7 ml/min corresponds to the residence time of 0.5 sec) was used to get the spectrum of one species shown in Fig. 5. This spectrum could not be that of the semiquinone radical anion of DOPA itself which should resemble the spectrum of Fig. 4 and should show the hyperfine splitting due to the two equivalent protons on C_7 . However, the g factor ($g=2.0045$) and total spread (10.7G) of the spectrum suggested that the

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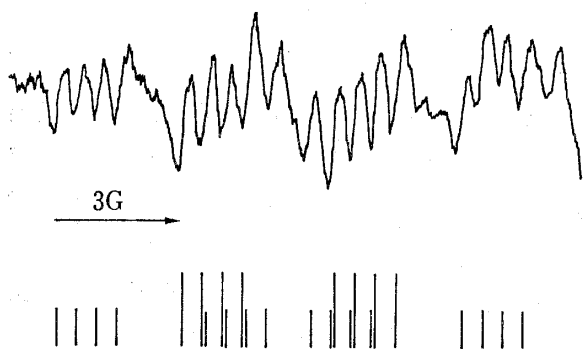


Fig. 4. An ESR Spectrum of the Semiquinone Radical Produced by Photolysis of $1 \times 10^{-2}M$ Dopamine and Its Stick Spectrum

A line in the highest field region is a background signal.

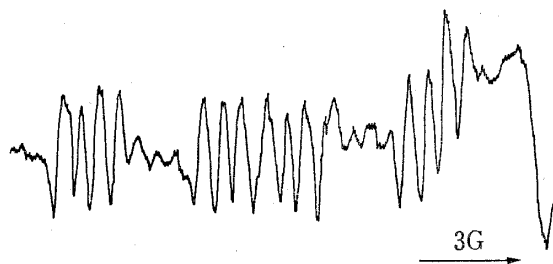


Fig. 5. An ESR Spectrum Observed during Photolysis of $1 \times 10^{-2}M$ DOPA

The background signal is the same as Fig. 3a.

basic structure of the radical must be a semiquinone type. The reason for the exceptional behavior of DOPA was not clear.

Discussion

In this experiment, a detection method is established for semiquinone radicals produced by photolyses of aqueous solutions of catecholamines at even $2.5 \times 10^{-6}M$ concentration.

It is easy to take ESR spectra of semiquinones from simple catechol and 4-alkyl-catechols by drastic chemical oxidation.⁹⁾ The chemical oxidation of catecholamines and DOPA, however, gives rise to cyclization of the aminoethyl side chain and further complicated oxidation.⁴⁾ The photo-oxidation proposed would be an elegant procedure to generate radicals from not only catecholamines, but also many photolabile compounds of biological interest.

Pigments, melanins, are produced through DOPA from tyrosine in a body.¹¹⁾ It is well-known that the pigment formation is induced by sunlight or UV irradiation using the same kind of lamp as the Toshiba FL-20E lamp.¹²⁾ In such a case, a transient occurrence of the radicals from DOPA and catecholamines might be highly probable.

Recently we successfully introduced a method of photoaffinity labeling to a β -adrenergic receptor of rat taenia cecum.⁸⁾ Now that characterization of the radicals has been done, the photoaffinity labeling using catecholamines themselves will be one of real techniques to molecular biological elucidation of catecholamine binding sites on proteins and adrenergic receptors in animal tissues.¹³⁾

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