

Dopamine: Effect of Concentration and pH on the Electron Emission. Reaction Mechanisms

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Abstract. Dopamine (DA) in airfree, aqueous solution (pH 7.4; 37°C) is able to emit e^-_{aq} when electronically excited in its singlet state by monochromatic UV light ($\lambda=254$ nm; 4.85 eV/hv). The quantum yield $Q(e^-_{aq})$, was studied in dependence of DA concentration and pH of the media. The DA transients resulting from the electron emission form products, which also have the ability to emit e^-_{aq} , but with much lower yield. Since DA in the ground state can consume e^-_{aq} , it is classified as an electron mediator. Some biological consequences based on the e^-_{aq} emission are mentioned.

It has been recently shown that steroid hormones in polar media emit electrons (e^-_{aq} , 'solvated electrons') when excited in the singlet state (1-7). The yield of e^-_{aq} depends on the molecular structure, concentration, pH of the media and temperature. The effects of these important parameters, as well as the role of functional groups, such as -OH, -NH₂, -COOH etc., was found to play an essential role in the electron ejection process in relation to fluorescence (8).

It was also established that adrenaline in aqueous, airfree solution is likewise able to emit e^-_{aq} . (9). The hormone transients resulting from the electron emission process lead to the formation of metabolites with various biological properties, depending on the substances present in the given milieu. However, the hormone transients resulting from e^-_{aq} emission can also be regenerated by electron transfer from a potent electron donor (10, 11). Based on experimented data, it was also suggested that the emitted e^-_{aq} are also responsible, at least partly, for the hormone communication between hormones and with other systems in the organism (12). This ability is based on electron transfer processes *via* the brain.

In relationship to adrenaline, it is of biological and medical interest to investigate dopamine (DA) in the same

respect. DA (4-(2-aminoethyl) benzene-1,2-diol) is a neurotransmitter and neurohormone generated mainly in the substantia nigra and other areas in the brain. The death of a predominant proportion of dopamine-producing cells causes Parkinson's disease. In addition DA also increases the efficiency of anticancer drugs (13), influences sexual functions (14) and exerts neuroprotective action of sex steroids in Parkinson's disease (15).

DA can exist as a cation, $+H_3ND(OH)_2$ (D represents the DA skeleton), zwitterion, $+H_3ND(OH)O^-$, neutral form, $H_2ND(OH)_2$ and as an anion, $H_2ND(O^-)_2$ depending on the pK ($pK_1=8.86$, $pK_2=10.5$ and $pK_3>12$) (16,17). The aim of this study was to gain deeper insight into the involved reaction mechanisms with respect to e^-_{aq} emission and metabolite formation. The experiments embrace the determination of e^-_{aq} yield at various DA concentrations and pH of the airfree aqueous media. The DA degeneration was studied by an (HPLC) method.

Materials and Methods

All chemicals were of highest purity available. DA.HCl ($\geq 99\%$, Fluka-Aldrich, Vienna, Austria) and triple distilled water were used for preparation of the solution. The pH(=7.4) was adjusted with NaOH. The substrates, DA and chlorethanol, used as specific scavenger for e^-_{aq} , were dissolved in airfree water and immediately irradiated with UV light. A low-pressure mercury lamp (HNS OSRAM, 12 Watt) with incorporated Vycor-filter for removal of the 185 nm line was used for excitation of DA in its singlet state. The lamp was mounted in a special, double-wall irradiation vessel (18), providing an intensity of monochromatic light ($\lambda=254$ nm) of $I_0=1 \times 10^{18}$ hvml⁻¹min⁻¹ in 4 π geometry at 37°C. Monochloroacetic acid was used as actinometer (19). The irradiation apparatus was connected with a thermostat for keeping the desired temperature at a constant level during the irradiation procedure. The emitted electrons by DA were specifically scavenged by 1×10^{-2} mol/l chlorethanol, which does not absorb UV light of the wavelength used. The Cl⁻ ions contained *a priori* in the samples solution were considered in the evaluation of the results. The Cl⁻ yield obtained by UV-irradiation of DA.HCl samples in the absence of chlorethanol was subtracted from the total Cl⁻ yield of the samples UV-irradiated under the same conditions, but in the presence of electron scavenger. Hence, only the yield of emitted e^-_{aq} was determined.

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Key Words: Dopamine, electron emission, biological consequences.

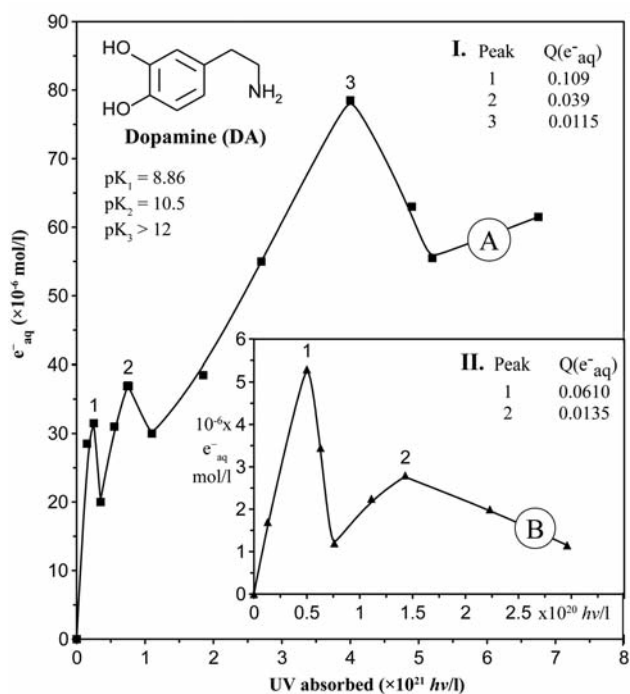
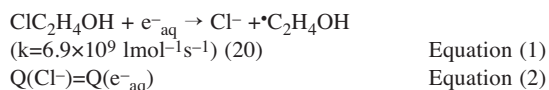


Figure 1. Emission of e^-_{aq} from: (A) 5×10^{-5} mol/l DA and (B) 5×10^{-4} mol/l DA in airfree aqueous solution (pH=7.5) at 37°C as a function of absorbed monochromatic ($\lambda=254$ nm) UV light. (I) Inset: $Q(e^-_{aq})$ values at the peaks of curve (A); (II) Inset: $Q(e^-_{aq})$ values at the peaks of curve (B). The data are corrected for the Cl^- ions included in the DA-HCL molecule.



The produced Cl^- ions were determined spectrophotometrically (21). HPLC analysis was also performed in order to follow the DA photodegradation, as well as the regeneration process, using Hewlett-Packard, model 1046/1050 with computer on line; column: Hypersil ODS 5 μm , 4x125 mm; injection sample 20 μl , solvent mixture: 45% water, adjusted to pH=2.8 by phosphoric acid, 50% methanol and 5% acetonitrile. The flow rate was: 1 ml/min.

It should be also mentioned that DA is extremely sensitive to oxygen. HPLC analysis showed that during the dissolution of DA samples in aerated water (pH=7.4), oxidation is essential. The oxidation yield depends on substrate concentration, temperature and duration of the handling. Based on this fact, in the present work, the DA samples were dissolved directly in the irradiation vessel in water saturated with argon (15-20 min) at pH=7.4. The UV irradiation ensued at 37°C.

Results and Discussion

The electron emission process was first studied using 1×10^{-5} to 1×10^{-4} mol/l DA in dependence of the absorbed UV dose at pH \approx 7.4 and 37°C. The Cl^- ions originating from hydrochloric acid contained in the DA.HCl sample was taken

Table I. Initial quantum yields, $Q_i(e^-_{aq})$, in dependence of the DA concentration.

DA (mol/l)	$Q_i(e^-_{aq})$	
	Peak 1	Peak 2
1×10^{-5}	0.2250	0.0874
2.5×10^{-5}	0.1475	0.0590
5×10^{-5}	0.1090	0.0390
7.5×10^{-5}	0.0738	0.0210
1×10^{-4}	0.061	0.0135

Table II. $Q(e^-_{aq})$ values obtained in series A: DA samples were dissolved in aerated water (pH=7.4; 37°C) and afterwards saturated with argon before UV irradiation. Series B: DA samples were dissolved and irradiated in airfree water (pH=7.4; 37°C).

DA (mol/l)	$Q_i(e^-_{aq})$	
	Series A	Series B
1×10^{-5}	0.087	0.225
5×10^{-5}	0.038	0.109
7.5×10^{-5}	0.021	0.074

into consideration. Mean values of e^-_{aq} resulting from several experimental determinations using 5×10^{-5} and 1×10^{-4} mol/l DA are shown in Figure 1. The obtained curves have more than one maximum. The calculated quantum yields of e^-_{aq} , $Q(e^-_{aq})$, at the maxima are given in the inset in Figure 1.

It is clear that for the first maximum in each case, the e^-_{aq} correspond to the electron emission from the substrate. The e^-_{aq} yields of the subsequent maxima originate from the DA photolytic products. Comparing the $Q(e^-_{aq})$ values of both experimental series (Figure 1, A and B), it is obvious that in series (A) the Q values are much higher than the following ones, which correspond to the e^-_{aq} emitted by the photolytic products of DA. The same effect is also observed using 1×10^{-5} and 7.5×10^{-5} mol/l DA (see Table I).

The obtained $Q_i(e^-_{aq})$ data demonstrate that the DA transients resulting from e^-_{aq} emission can lead to formation of products also able to emit e^-_{aq} , when excited in the singlet state. For a better illustration of the effect, the data of Table I are presented in Figure 2. This effect was also observed in the case of other hormones. It can be explained by the formation of 'associates' (unstable hormone complexes) at hormone concentrations $>10^{-9}$ mol/l (11). Since the DA molecules in the associate are mainly in the ground state, they consume some of the emitted e^-_{aq} , therefore, the yield of e^-_{aq} is decreasing with increasing DA concentration.

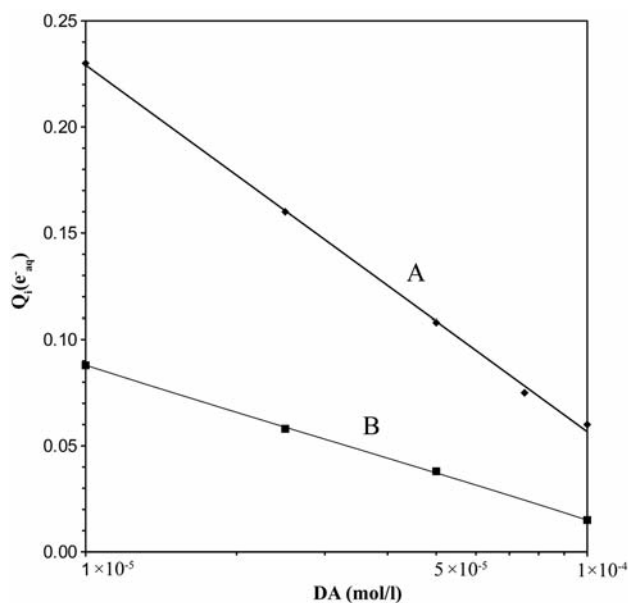
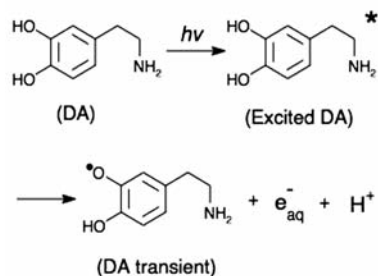


Figure 2. Initial quantum yield of emitted electrons, $Q_i(e^-_{aq})$, as a function of DA concentration; (A) first peak, (B) second peak from curves representing the e^-_{aq} in dependence of the absorbed UV quanta (see Figure 1).

Simultaneously with the generation of e^-_{aq} , a decrease of the pH in the irradiated solution can also be observed (Figure 3). This fact gives a hint to the reaction mechanisms. The decrease of pH, as well as the yield of ejected e^-_{aq} , strongly depends on the DA concentration in airfree media. The electron emission from electronically excited, e.g. neutral DA form, is taken as an example, showing the simultaneously formation of H^+ ions (equation 3).



Equation (3)

Two series of experiments concerning the already mentioned high sensitivity of DA to oxygen were performed. A series: DA sample was dissolved in aerated water (pH=7.4), but UV irradiation at 37°C was performed after saturation of the solution with argon. B series: The samples were dissolved and irradiated in airfree water under the same conditions. The obtained mean $Q(e^-_{aq})$ values of

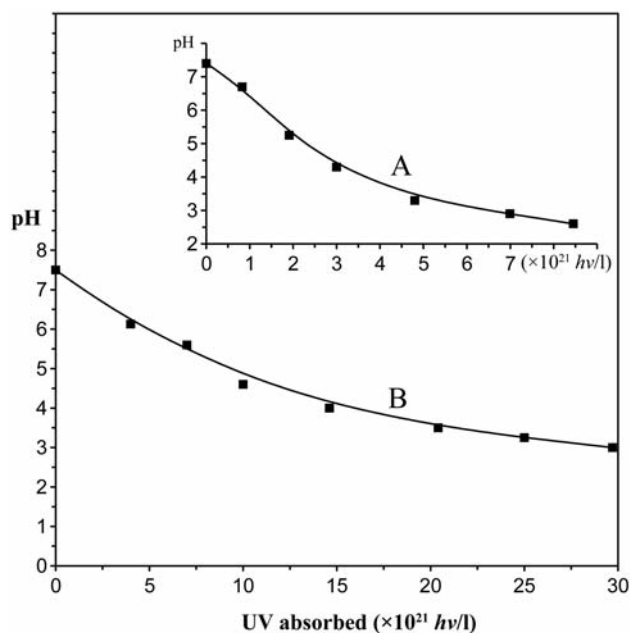


Figure 3. pH change as a function of absorbed UV quanta of monochromatic light ($\lambda=254$ nm) for: (A) 1×10^{-5} mol/l DA and (B) 7.5×10^{-5} mol/l DA; in both cases in the presence of 1×10^{-2} mol/l chlorethanol in airfree aqueous solution at 37°C.

both series are given in Table II for comparison. The oxidation effect of DA is strongly reflected in the obtained $Q(e^-_{aq})$ yields.

In this context it might be of interest to mention that the $Q(e^-_{aq})$ yield of e^-_{aq} emitted from 5×10^{-5} mol/l DA increases from 0.1090 at 37°C to 0.1260 at 45°C. This temperature effect has also previously been observed for other hormones. From a photophysical point of view, it is known that the electron emission process from excited molecules in singlet state competes with other temperature-dependent processes, such as with fluorescence, intersystem-crossing *etc.* (8).

The pH dependence of the e^-_{aq} emission process from DA was also investigated, using 1×10^{-4} mol/l DA at pH of 6.5, 9.1 and 11.5. The obtained e^-_{aq} yields are presented in Figure 4 as a function of absorbed UV dose (hv/l). Obviously, with the increase of pH, the yield of ejected e^-_{aq} is also very strongly increased. This observation is in concordance with the corresponding pK_1 to pK_3 values mentioned before.

The results demonstrate the fact that with dissociation of the $-OH$ and $-NH_2$ groups, less energy is needed for the e^-_{aq} emission, and is in accord with previous results (8).

Summing up it can be stated that as a consequence of the e^-_{aq} emission, a decrease of the pH in the medium occurs depending on the DA concentration (see Figure 3). As a result, the concentration ratio of DA cation, zwitterion and

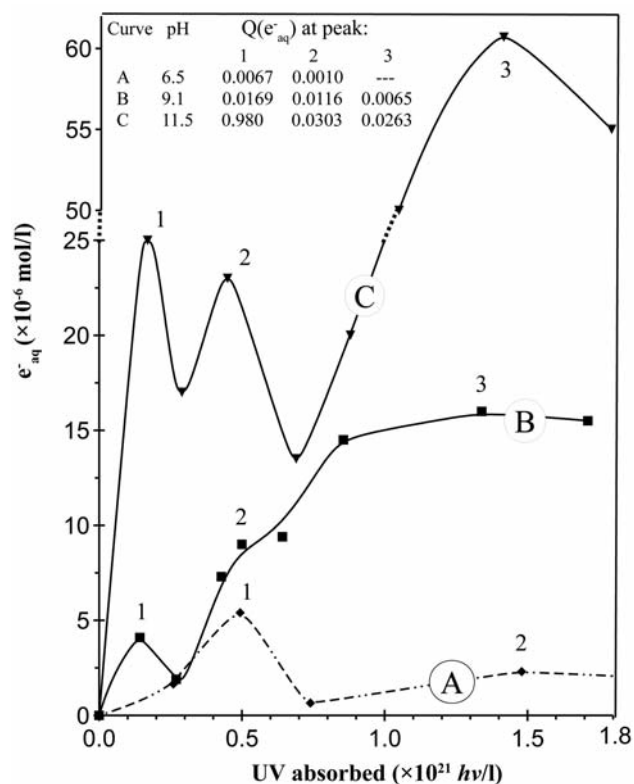


Figure 4. Yields of emitted e^-_{aq} (mol/l) from 1×10^{-4} mol/l DA at 37°C in airfree aqueous solution at: (A) pH=6.5, (B) pH=9.1 and (C) pH=11.5 as a function of absorbed UV dose of monochromatic UV light, $\lambda=254$ nm. (I) Inset: $Q(e^-_{aq})$ values at the maxima of curves (A) to (C). Remark: corrected for e^-_{aq} contribution of OH^- anions.

neutral form is continuously changed. This dynamic state is naturally reflected in the e^-_{aq} yield. Therefore, the involved reaction mechanisms are rather complicated. The complexity of the mechanisms is increased further by the formation of products also able to emit e^-_{aq} (Figure 1, maxima of the curves).

The biological consequences of the studies could be founded on the fact that DA is generated mainly in the nervous tissue by hydroxylation followed by decarboxylation of tyrosine, passing several reaction steps. It is a precursor of noradrenaline and adrenaline in the biosynthesis of these neurotransmitters. Having in mind the hypothesis suggested for hormone communication by electron transfer with other biological systems *via* the brain (12), it is supposed that the same might be also valid for DA. This would also explain the broad spectrum of DA interaction with other hormones, cytostatics *etc.* (13-15). Hence, according to these premises, it is conceivable that DA can communicate by electron transfer, without the necessity of forming complexes with one of the five receptors.

Conclusion

The obtained results can be summarized in the following highlights: (i) Airfree aqueous DA at physiological pH and temperature can eject e^-_{aq} when excited in the singlet state. (ii) This property strongly depends on substrate concentration (formation of associates), pH (effect of pK values) and temperature. (iii) The transients resulting from the e^-_{aq} emission lead to products which are also able to emit e^-_{aq} by excitation. (iv) The results demonstrate that DA, like other previously investigated hormones (1-5), can be classified as an electron mediator (emitting and consuming electrons).

Acknowledgements

The Authors are deeply indebted to the Austrian Science Fund (FWF) for the financial support which enabled the project: "Free Radical Action on Sexual Hormones in Respect to Cancer" to be carried out; Contract no.P21138-B11. Thanks are also expressed to Mrs. Dr. R.M. Quint for valuable help in HPLC analysis.

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Received August 2, 2011

Revised October 4, 2011

Accepted October 6, 2011