Spontaneous Autoxidation of Dopamine

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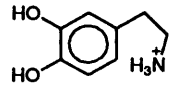
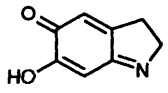
A detailed kinetic study has been carried out of the reaction of dopamine, 2-(3,4-dihydroxyphenyl)ethylamine, with dioxygen over the pH range 7–9 where it reacts spontaneously without the necessity of metal ion catalysis. The reaction was found to be accurately first-order in $[O_2]$ and in [dopamine] and first-order in $[H^+]^{-1}$ and, furthermore, stoichiometric amounts of H_2O_2 were shown to be produced. The other product of oxidation is, initially, the pink dopaminochrome which, however, is not stable and reacts further (without the consumption of dioxygen) to form the insoluble polymeric material known as 'melanine'. The rate-determining step is assumed to be hydrogen atom abstraction from the monodeprotonated species by O_2 (as with many other catecholamines, dopamine is stable towards oxidation in acidic media in the complete absence of metal ions) with a second-order rate constant of $k_1 = 0.47 \pm 0.05$ dm^3 mol^{-1} s^{-1} at 25 °C in a solution of ionic strength 0.1 mol^{-1} dm^{-3} (KCl).

Dopamine is 2-(3,4-dihydroxyphenyl)ethylamine, **I**, and is referred to as H_2LH^+ in this paper where the phenolic protons



I

are written to the left of L. When a neutral solution of dopamine is exposed to air, after a while it turns pink owing to oxidation to dopaminochrome, **II**, even in the absence of metal ions. Eventually, the pink colour disappears to be replaced by a precipitate of the polymeric material, melanine. Addition of a small amount of acid inhibits this oxidation, unless metal ions such as Fe^{3+}, Cu^{2+} or VO^{2+} are present.



II

However, the markedly different behaviour of the closely related, both chemically and biochemically, catecholamines, adrenaline [1-(3,4-dihydroxyphenyl)-2-(methylamino)ethanol] and DOPA [3-(3,4-dihydroxyphenyl)alanine], should be noted. In the absence of metal ions they are stable even in neutral solution. In acid solution (pH < 4–5) the catalysed oxidation of DOPA proceeds smoothly to completion.[1] In contrast, in the case of dopamine, although added metal ions initially start an oxidation process, this soon comes to an end as the metal ions are efficiently removed from the solution by the melanine (or a soluble, polymeric precursor). The involvement of metal ions in this reaction is being studied in greater detail.[2]

Dopamine has attracted much interest owing to its postulated role in Parkinson's and Alzheimer's diseases.[3,4] In particular, the role of the products of its oxidation and the role of iron-containing melanines found in the *substantia nigra* of the brains of deceased Parkinsonian patients have been discussed.[5–8] This, together with our interest in the role of coordination compounds as catalysts for autoxidation reactions, has stimulated the present study.

Experimental

Dopamine hydrochloride ($C_8H_{11}NO_2$·HCl) was supplied by Sigma Chemical Co. (St. Louis, MO) and used without further purification. The ionic strength was adjusted with potassium chloride (Merck, *pro analysi*) to be 0.100 mol dm^{-3} in chloride ion; the use of nitrate ion as background electrolyte gave identical results.

Iron(III) nitrate nonahydrate, manganese(II) sulfate monohydrate, copper(II) nitrate, oxovanadium(IV) sulfate, sodium dihydrogen phosphate and dipotassium hydrogen phosphate were from Merck and EPPS-buffer {3-[4-(2-hydroxyethyl)-1-piperazino]propanesulfonic acid} was obtained from Aldrich: all were of *pro analysi* quality. Catalase (from bovine liver) and superoxide dismutase (SOD; from bovine erythrocytes) came from Sigma.

Oxygen consumption was measured using a Clark-type electrode (EO 96, WTW) connected to a corresponding processor unit (Oxi 537, WTW). The output of the processor unit was connected to a Goerz-Metrawatt x-t-recorder. The pH was kept constant (within ±0.003 units) with an RTS 822 titrating unit (Radiometer, Copenhagen) coupled with a PHM 84 pH-meter and the amount of added base was recorded. The temperature was held constant within ±0.1 °C by a K2 Ultrathermostat (Lauda).

In a thermostatted glass vessel equipped with a lid that held the pH and oxygen electrodes and inlet tubes (the lid was closely fitting, but could be slid up and down) 110 cm^3 of the slightly acidified dopamine solution were saturated with oxygen until a constant value of the oxygen concentration was reached (usually 10–15 min). Then the oxygen tube was removed, the lid lowered until it touched the surface of the solution and the reaction started by automatic addition of base (the desired pH was established in less than 2 min). The solution was stirred (necessary for the correct functioning of the oxygen electrode) by use of a circular teflon non-vortexing magnetic stirrer supplied by Watman Lab-Sales. Occasionally a sample was withdrawn in order to record a UV–VIS spectrum (Hitachi U-2000 spectrometer). Additional spectra, together with the kinetics of formation of dopaminochrome, were obtained with a Tractor Northern diode array high-speed-spectrophotometer, supplied by Applied Photophysics Ltd. (London). These stopped-flow data were recorded by mixing slightly acidified dopamine solutions with alkaline phosphate buffer, both saturated with oxygen.

Table 1 Typical observed rate constants for the consumption of oxygen [$k^{obs}(O_2)$] and for the addition of base [$k^{obs}(OH^-)$], the amount of base added [$n(OH^-)$] and A values calculated according to eqn. (5)

T/°C	pH	$[L]_T$/mmol dm^{-3}	$k^{obs}(O_2)/10^{-3}$ s^{-1}	$k^{obs}(OH^-)/10^{-3}$ s^{-1}	$n(OH^-)$/μmole	$A/10^{-9}$ s^{-1}
7.2	8.60	5.05	0.323			0.183
	8.21	10.0	0.220			0.155
	8.40	10.0	0.383			0.175
	8.65	10.0	0.791			0.203
	8.81	10.0	1.37			0.246
	8.60	20.0	1.37			0.198
						0.19 ± 0.03
15.0	8.22	5.05	0.210			0.286
	8.00	10.0	0.248			0.282
	8.18	10.0	0.354			0.268
	8.40	10.0	0.672			0.307
	8.61	10.0	1.23			0.345
	8.20	20.0	0.698			0.251
						0.29 ± 0.03
25.0	8.31	3.07	0.668	0.664	55.8	1.24
	8.00	5.00	0.499	0.434	67.6	1.15
	7.65	9.98	0.495	0.418	57.8	1.28
	8.01	10.0	1.06	1.04	53.6	1.20
	8.10	9.97	1.27	1.08	71.5	1.16
	8.20	9.97	1.84	1.62	66.1	1.33
	7.80	20.1	1.47	1.24	67.6	1.33
	7.23	30.0	0.556	0.445	63.7	1.28
	7.80	30.0	1.80	1.91	70.2	1.17
	7.25	40.0	0.791	0.848	59.7	1.29
	7.41	9.84	0.338			1.45[a]
	7.80	10.0	0.484	0.495	67.0	0.87[b]
	8.00	10.1	0.802			0.90[c]
	7.20	20.0	1.23			1.69[d]
	7.20	20.0	1.22			1.68[e]
	7.67	12.9	0.956			1.82[f]
	8.40	5.00	1.37			1.26[g]
						1.27 ± 0.09
37.2	7.23	5.06	0.275			3.81
	7.64	5.06	0.675			3.51
	7.22	10.0	0.549			3.77
	8.11	10.0	3.95			3.52
	7.14	20.0	0.794			3.30
	7.65	20.0	2.71			3.48
						3.5 ± 0.2

[a] 0.0503 mol dm^{-3} KH_2PO_4. [b] 2.11 mol dm^{-3} methanol. [c] 4.70 mol dm^{-3} methanol. [d] 1.0 mmol dm^{-3} Fe^{III}. [e] 2.0 mmol dm^{-3} Fe^{III}. [f] 0.0400 mol dm^{-3} EPPS-buffer. [g] *Ca.* 1000 units SOD added.

Attempts to estimate hydrogen peroxide by Marklund's modification[9] of Bonet–Maury's Ti^{IV} reagent[10] failed owing to the general absorption of melanine.

Results and Discussion

Protonation Constants of Dopamine.—The protonation equilibria for dopamine are listed in reactions (1)–(3) together

$$L^2 + H^+ \rightleftharpoons HL^- \quad \log K_1^H = 13.1 \quad (1)$$

$$HL^- + H^+ \rightleftharpoons H_2L \quad \log K_2^H = 10.41 \quad (2)$$

$$H_2L + H^+ \rightleftharpoons H_3L^+ \quad \log K_3^H = 8.82 \quad (3)$$

with the values of the protonation constants reported by Kiss and Gergely[11] (note that dopamine is written here as H_3L^+ as these are macroconstants and therefore cannot be assigned). The microconstant, K_M^{OH}, for the protonation of HLH (*i.e.* the addition of the second phenolic proton whilst the amino group is protonated) was also determined by these authors (log $K_m^{OH} = 8.87$).

Kinetics.—The consumption of oxygen is first-order over three half lives and is independent of ambient light. Phosphate buffer leaves the rate unchanged and this enabled the investigation of the reaction in a stopped-flow spectrometer. Borax buffer could not be used because it inhibits the reaction by forming a compound with the catechol function of dopamine; the EPPS-buffer is also unsuitable because it strongly accelerates the reaction. Typical first-order rate constants, k^{obs}, are collected in Table 1 and data for 25 °C are shown in Fig. 1. The reaction of base is also first-order and the rate constants obtained are identical to those calculated from the oxygen measurements (Table 1).

As demonstrated in Fig. 2, the rate constants are inversely proportional to $[H^+]$ and directly proportional to the total concentration of dopamine, $[L]_T$. The rate law is thus given by

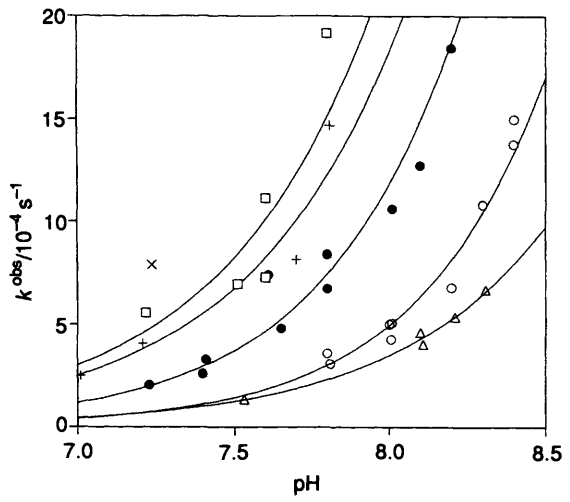


Fig. 1 Typical first-order rate constants for the autoxidation of dopamine. Experimental conditions: T = 298 K, I = 0.100 mol dm^{-3}, $[L]_T$ = 0.003 (△), 0.005 (○), 0.01 (●), 0.02 (+), 0.03 (□) and 0.04 (×) mol dm^{-3}.

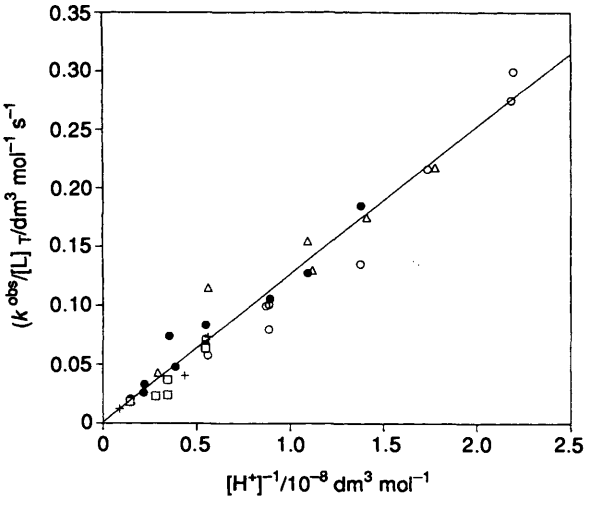


Fig. 2 $k^{obs}/[L]_T$ *v.* $1/[H^+]$. The slope of the regression line gives A = $1.27.10^{-9}$ s^{-1}. Symbols are the same as in Fig. 1.

eqn. (4), and k^{obs} can be expressed by eqn. (5) in which A is a constant.

$$-d[O_2]/dt = k^{obs}[O_2] \quad (4)$$

$$k^{obs} = A[L]_T/[H^+] \quad (5)$$

Some calculated values of A are given in Table 1 with a mean value of (1.27 ± 0.09) × 10^{-9} s^{-1} at 25 °C and at an ionic strength of 0.100 mol dm^{-3} (Cl^-). The variation of A with temperature establishes an apparent activation energy of 75 kJ mol^{-1}.

The addition of methanol, which is known to act as a radical trap, results in a 30% decrease in k^{obs}. The addition of SOD has no effect on the rate, suggesting that the superoxide ion is not a chain carrier in this reaction. Catalase, when added after the reaction has proceeded for two half lives, recovered about 50% of the oxygen consumed up to this point thus confirming peroxide to be the final product of oxygen reduction.

Fig. 3 illustrates the route by which dopamine is converted to dopaminochrome. The amount of added base (calculated from the intercept of the first-order plot) is independent of pH and dopamine concentration and for initially saturated oxygen solutions was found to be 0.061 ± 0.008 mmol. This is approximately half the initial amount of oxygen (0.132 mmol),[12] *i.e.* one proton is released for every two oxygen molecules consumed. Thus the overall reaction can be written as reaction (6), dopaminochrome acting as an acid in this pH range.

$$H_2LH^+ + 2O_2 \longrightarrow Q^{*-} + H^+ + 2H_2O_2 \quad (6)$$

The expression for k^{obs} above, [eqn. (5)], strongly suggests that the reaction being followed is the rate-determining abstraction of a hydrogen atom from the monodeprotonated dopamine, HLH, [reaction (7)], and that all subsequent steps

$$HLH + O_2 \xrightarrow{k_1} L^{\cdot}H + HO_2^{\cdot} \quad (7)$$

depicted in Fig. 3 are fast. In other words, after deprotonation of one of the hydroxy groups of dopamine, the molecule reacts in the rate-determining step with oxygen to give the semiquinone radical and superoxide ion. (Note that the alternative of electron transfer from HLH to O_2 is kinetically indistinguishable from hydrogen atom abstraction, since the resulting superoxide anion in the vicinity of the very strongly acidic semiquinone would be protonated rapidly.) The subsequent reaction of the semiquinone with another molecule of oxygen yielding the *ortho*-quinone is fast[13] and therefore is the most likely step to involve the second oxygen molecule. Although the disproportionation of superoxide anions is extremely slow, the reaction of the anion with the conjugated acid is very fast, fast enough to compensate for the very low concentration of the protonated form[13] (log $K_a \approx 4.8$). This finding is in accordance with the observations[14] of Misra and Fridovich for the autoxidation of adrenaline, where it was found that two different chains are acting: one based on the semiquinones acting as chain carriers and the other based on superoxide radicals, the latter predominant at higher pH. They discriminated between the two pathways by the amount of inhibition by superoxide dismutase. However, they demonstrated that below pH 9 the reaction proceeds exclusively *via* the semiquinone path. Also the cyclization of dopaminoquinone is known to be fast compared with the initial electron transfer in the pH range employed.[15] Since dopaminochrome is considered a final product of the oxygen-consuming and the proton-releasing reactions, its further reaction to melanine must involve neither protons nor oxygen. However, a contribution of a catalytic amount of hydroxyl radicals from the cleavage of an extremely small amount of the hydrogen peroxide produced is a possible candidate for initiating this polymerization reaction.

From reaction (7) and allowing for the further (fast) consumption of an O_2 molecule, the rate of disappearance of O_2 is given by eqn. (8). Over the pH range 7.0–8.5 dopamine is

$$-d[O_2]/dt = 2k_1[HLH][O_2] \quad (8)$$

mainly protonated, so that the total concentration of dopamine, $[L]_T$, is given by eqn. (9) and hence making use of the

$$[L]_T \approx [H_2LH^+] \quad (9)$$

microconstant for the deprotonation of the phenolic hydroxy group, K_m^{OH}, eqn. (8) becomes eqn. (10). The constant A in

$$-d[O_2]/dt = 2k_1K_m^{OH}[L]_T[O_2]/[H^+] \quad (10)$$

eqn. (5) above is therefore $2k_1K_m^{OH}$ and, using the value for the microconstant given above, this yields a value k_1 of 0.47 ± 0.05 dm^3 mol^{-1} s^{-1} at 25 °C.

Spectra.—Typical spectra recorded during the course of the reaction are shown in Figs. 4 and 5 in the absence and presence

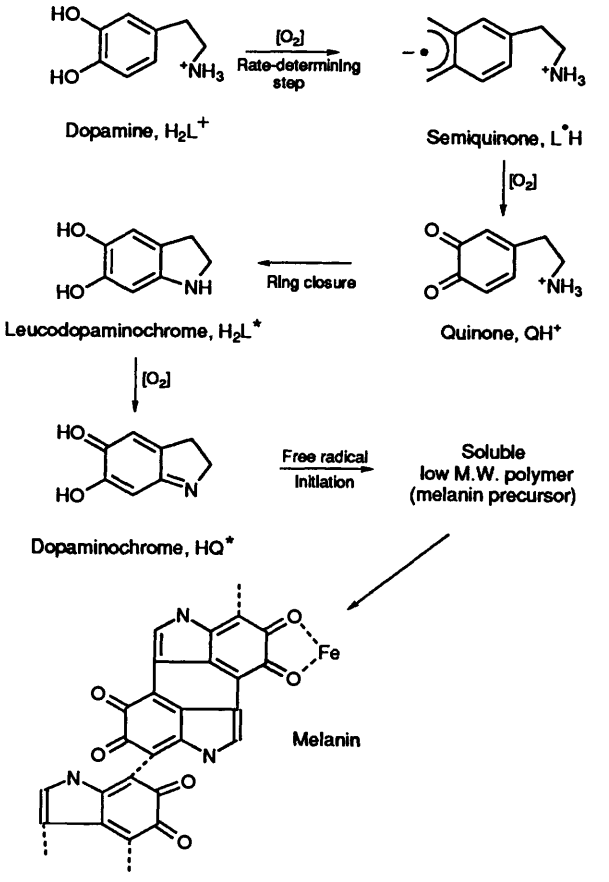


Fig. 3 The course of the autoxidation of dopamine. The presence of all the species shown, excepting melanine and its soluble precursor, have been established spectrophotometrically. The precursor must be a low molecular weight polymer capable of encapsulating a metal ion and melanine itself is generally accepted to be a highly cross-linked polymer containing phenolic, semiquinone and quinone groups capable of binding metals ions to the surface (or within the polymer if the metal ions are present during the polymerization).

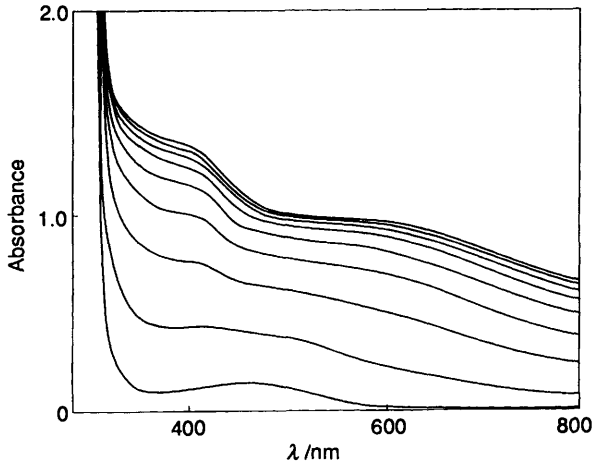


Fig. 4 Time-dependent UV–VIS spectra during the autoxidation of dopamine. Spectra were recorded every 300 s, the first (bottom) one 200 s after the start of the reaction. $[DAM]_T = 1.00 \times 10^{-2}$ mol dm^{-3}, $[O_2]_0 = 1.32 \times 10^{-3}$ mol dm^{-3}, pH 7.65, $I = 0.100$ mol dm^{-3}, $T = 298$ K.

of iron(III), respectively. It can be seen that (*i*) general absorption increases owing to the formation of colloidal melanine, (*ii*) a peak at 480 nm attributable to dopaminochrome appears in the initial stages of the reaction, but then disappears, (*iii*) peaks at *ca.* 405 and 620 nm appear in the final stages of the reaction. However, note that the former is suppressed in the

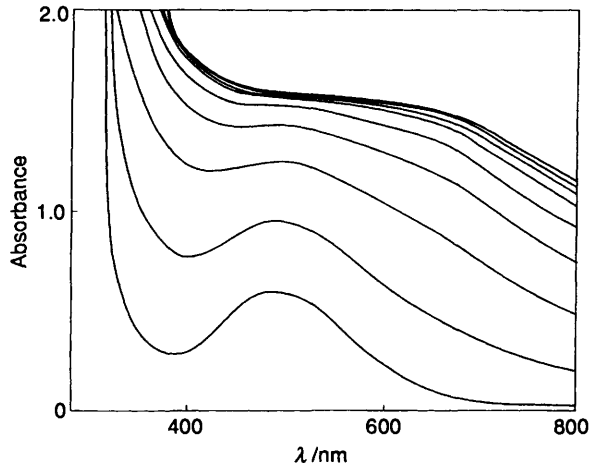


Fig. 5 Time-dependent UV–VIS spectra during the autoxidation of dopamine in the presence of 1.0×10^{-4} mol dm^{-3} iron(III). Experimental details are given in Fig. 4.

presence of iron(III) ions, whereas the latter is unchanged. Also it can be seen that the peak at 495 nm, due to $Fe(LH)_2^+$, disappears during the reaction. Since dopamine is in large excess and its complex is a good indicator for iron(III), it can be concluded that iron is removed from solution, certainly by inclusion into the melanine (adsorption of iron at the surface of melanine can only account for 5% of the total iron present under our conditions).[16]

It may be of interest that UV-light ($\lambda < 300$ nm) suppresses the polymerization reaction leading to melanine. Furthermore, iron(III) acts as a quencher for this suppression in the wavelength range 300–350 nm. Both of these effects are no doubt related to the free radical character of the melanine-forming reactions.

Conclusions

Dopamine reacts spontaneously with dioxygen at neutral to alkaline pH *via* hydrogen abstraction which is rate determining. The subsequent reactions leading to dopaminochrome are comparatively fast and could not therefore be investigated. The degradation of the latter to the polymeric melanine requires neither dioxygen nor protons, but might well require free-radical initiation, perhaps by a catalytic amount of $OH^{\bullet}$. Hydrogen peroxide is a main product of the reaction, even in the presence of iron.

Metal ions such as Fe^{III}, Cu^{II} or VO^{2+} have little if any influence on the rate of autoxidation, but can initiate the reaction in acid solution. However, the exceptional behaviour of Mn^{II} should be noted: preliminary results suggest that manganese is catalytically active and that the reaction proceeds *via* two pathways: one being first-order, the other half-order in $[O_2]$. This is interesting in the light of the special sensitivity of neurons involved in the course of Parkinsonism to manganese.[17,18] The fact that manganese is, unlike iron, not removed by the melanine requires further consideration. These findings indicate that Halliwells generalization[19] that transition metal ions generally catalyse autoxidation reactions of the catecholamines is not sustainable. Further work on this and the stoichiometric reaction of iron(III), dopamine and dioxygen at slightly acid pH is in progress at our institutes.

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