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Dopamine, 6-hydroxydopamine, iron, and dioxygen – their mutual interactions and possible implication in the development of Parkinson's disease

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Abstract

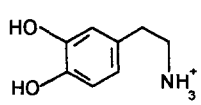
The reactions of dopamine (1-amino-2-(3,4-dihydroxyphenyl)-ethane, DA), 5-hydroxydopamine (5-OHDA), and 6-hydroxydopamine (6-OHDA), with molecular oxygen — with and without the addition of catalytic amounts of iron(III) and other metal ions — have been studied and the implication of these results with respect to the chemistry involved in the progress of Parkinson's disease is discussed. In the presence of O_2 DA reacts spontaneously without the necessity of metal-ion catalysis under the production of stoichiometric amounts of H_2O_2, to form initially pink dopaminochrome, which is not stable and reacts further (without the consumption of dioxygen) to form the insoluble polymeric material known as 'melanine'. DA reacts with iron(III) yielding an intermediate 1:1 complex, which decomposes releasing Fe(II) and the semiquinone, which reacts further under involvement of both Fe(III) and dioxygen. 6-OHDA reacts without showing the necessity of such an intermediate, and it is shown to be able to release iron as Fe(II) from ferritine. On the other hand, it is shown (in vitro) that Fe(II) reacts in a Fenton type reaction with DA and the present H_2O_2 producing 5-OHDA and especially 6-OHDA. Based on these mutual interacting reactions a mechanism for the initiation and progress of Parkinson's disease is suggested. The catalytic effects of some other transition-metal ions are presented and an explanation for the peculiarly toxic effects of manganese(II) is put forward. Finally, a possible reason for the effect that nicotine has in the mitigation of Parkinson's disease is discussed.

Keywords: Parkinson's disease; Dopamine; 6-Hydroxydopamine; Iron(III); Iron(II); Manganese(II); Fenton's reagent; Nicotine

1. Introduction

1.1. Chemical aspects

Dopamine is 1-amino-2-(3,4-dihydroxyphenyl)ethane, existing in neutral solution as the protonated form, **I**, and, where necessary in this paper, is



Dopamine, I

referred to as H_2LH^+, where the phenolic protons are written to the left of L. The protonation equilibria for the anion have been determined by Gergely and Kiss [1] and are listed in Eq. (1), Eq. (), below. (Note that here the dopamine anion is written as H_3L^+, since these are macro-constants and therefore cannot be assigned to specific protonations.)

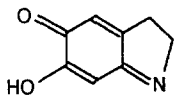
$$L^{2-} + H^+ \Leftrightarrow HL^- \log K_1^H = 13.1$$
$$HL^- + H^+ \Leftrightarrow H_2L \log K_2^H = 10.41 \quad (1)$$
$$H_2L + H^+ \Leftrightarrow H_3L^+ \log K_3^H = 8.82$$

When a neutral solution of dopamine, **I**, is exposed to air it, after a while, turns pink due to its oxidation (via several steps) to dopaminochrome, **II**, even when transition-metal ions are strictly excluded. The pink colour then slowly

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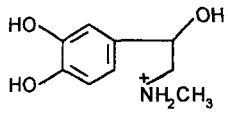
disappears to be replaced by a precipitate of the polymeric material known as melanin.



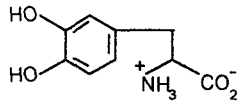
Dopaminochrome, II

The addition of acid inhibits the reaction unless metal ions such as Fe^{3+}, Cu^{2+}, or VO^{2+} are present.

However, the markedly different behaviour of the closely related — both chemically and biochemically — catecholamines adrenaline (1-(3,4-dihydroxyphenyl)-2-(methylamino)-ethanol), **III**, and dopa (3-(3,4-dihydroxyphenyl)alanine), **IV**, should be noted. In the rigorous absence of metal ions they are stable towards oxidation



Adrenaline, III



Dopa, IV

by dioxygen even in neutral solution [2,3].

In acid solution (pH < 4.5) the metal-ion catalysed oxidation of dopa proceeds smoothly to completion [4]. In the case of dopamine, however, although added metal ions initially start an oxidation process, this soon comes to an end because the metal ions are efficiently removed from the solution by the final product, melanin, or by a soluble precursor of it. The involvement of metal ions in this reaction is now being studied in greater detail.

1.2. Medical aspects

Some populations of melanised neurons of the brain have been shown to be selectively vulnerable [5] in that, in contrast to most other neurons of the lower brain stem [6], they show an increased rate of degeneration in both normal ageing and in the development of Parkinson's disease (PD). These dopaminergic neurons are also specifically sensitive to manganese, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA) and to the, as yet unidentified, factors [7] that are presumed to give rise to PD. In this respect the apparent interplay between 'pro-neurotoxins' such as MPTP and 6-OHDA that can be converted by monoamineoxidase-B (MAO-B) into neurotoxins is not yet fully understood. It has been suggested [8] that an alternative mechanism for the alleged antidegenerative action of the MAO-B inhibitor, deprenyl, could be inhibition of the formation of H_2O_2 and oxygen radicals by MAO. The destruction of pigmented brain stem nuclei, particularly those of the *Substantia nigra* (SN) appears to be the key to the pathogenesis and pharmacology of PD [9]. Although the cause of dopaminergic cell death remains unknown, excess iron within the SN has been implicated in the progression of the disease through its participation in the continuous formation of cytotoxic free radicals [10].

Based on the demonstration of this increase in 'free' iron in the SN of the brains of deceased patients who had suffered from PD [11], together with its accumulation on melanin particles in the remaining cells [12] it has recently been proposed [13] that iron-melanin interactions may be involved in the degeneration of dopaminergic neurons. A possible mechanism that leads to an increase in the level of iron not protected by being bound to ferritin is thus required. This decompartmentation of iron (or 'tissue iron overload') is known [14] to lead to a state of oxidative stress, and it is highly relevant that many cytotoxic compounds that readily promulgate redox reactions can release iron from storage, a well-documented example is that of paraquat toxicity of the lung which has been linked [15] to the ability of paraquat to completely release the iron from ferritin. Again, studies have demonstrated that, in rats, intraventricular pre-injection of desferral (a selective iron chelator) attenuates 6-OHDA induced lesions of nigrostriatal dopamine neurons [14]. This was made evident by the prevention of loss of striatal DA and the reduction of homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) (usually observed after injection with 6-OHDA).

1.3. Relevance of the in vitro studies

Seen in the light of the above observations, the following in vitro studies would seem to shed some light on the rale of iron and of 6-OHDA in the progression of PD.

Firstly, it is shown that uncomplexed iron(II) is able to react with H_2O_2 thus promoting a Fenton-like reaction that, via formation of the cytotoxic hydroxyl radical, converts dopamine to 5-OHDA and 6-OHDA.

Secondly, it was demonstrated by stopped-flow spectroscopy that whereas dopamine and 5-OHDA were oxidised by iron(III) via complex formation, the corresponding oxidation of 6-OHDA proceeded via an 'outer-sphere' interaction. This predicted, correctly, that *6-OHDA alone is capable of releasing iron from strongly co-ordinating ligands*, i.e., from ferritin.

Thirdly, kinetic studies of the reaction between dopamine and dioxygen, with and without iron(III) being present, showed that neither melanin nor iron-containing melanin played a part in this reaction and that although

iron(III) can *initiate* this reaction (even in acid media) *it is not a catalyst.* (The iron(III) is efficiently removed from the solution as an inert soluble complex and is eventually trapped within the melanin formed.) [16–18].

Finally, similar studies with manganese(II) showed that it does act as an efficient catalyst for the oxidation of dopamine, *because it is not trapped in the melanin* (or by a soluble precursor of the melanin).

Dopamine has, therefore, attracted much attention due to its postulated role in both Parkinson's and Alzheimer's diseases [19,20]. Especially important in this respect is the above mentioned role of the products of its oxidation and the rale of the iron-containing melanins found in the SN of deceased Parkinsonian patients [7,13,21–23]. This, together with our (E.H., R.F.J., and W.L.) interest in the role of co-ordination complexes in transition-metal ion catalysed autoxidation reactions, has stimulated the present study.

2. Materials and methods

2.1. Chemicals

The dopamines were obtained as the hydrochlorides from the Sigma Chemical Co. and used without further purification. (Replacement of the chloride ions by nitrate — by use of ion-exchange resins — for some runs was used to confirm that chloride did not, in general, influence the reactions being studied.) Whenever possible analytical grade reagents were used. Synthetic melanins were prepared by autoxidation according to Das et al. [24].

Iron(III) nitrate, manganese(II) sulfate, copper(II) nitrate, oxovanadium(IV) sulfate, sodium dihydrogen phosphate, and dipotassium hydrogen phosphate were supplied as the hydrated salts by Merck; all were of pro analysi quality. EPPS buffer (3-(4-(2-hydroxyethyl)-1-piperazino)-propanesulfonic acid) was from Aldrich. Catalase (from bovine liver) was obtained from Sigma.

2.2. Kinetic studies

Oxygen consumption was measured with a Clark-type electrode (EO96: WTW) connected to the corresponding processor unit (Oxi537: WTW) and the output recorded on a Goertz-Metrawatt x–t recorder. The pH was kept constant to within ± 0.01 pH units with an RTS822 titrating unit coupled to a PHM84 pH meter (both by Radiometer) enabling the rate of addition of base (or acid) to be monitored. The temperature was maintained to within ± 0.05°C by a K2 Ultrathermostat (Lauda). The reaction vessel, and its mode of operation, has been described previously [16]. Samples were occasionally withdrawn in order to record UV-Vis spectra on a Hitachi U-2000 spectrometer.

Anaerobic kinetic data and spectra were also obtained by use of both, a Tracor Northern TN-1710 diode-array and SX17.MV microvolume high-speed stopped flow spectrometer (both supplied by Applied Photophysics, Leatherhead, UK).

All kinetic studies were carried out at a constant ionic strength of 0.100 M by use of potassium chloride as background electrolyte. The iron concentration was chosen such that an effect could be made visible in an appropriate time. Extrapolations to physiological iron concentrations are nonetheless possible.

Iron release from ferritin was measured using the ferrous chelator ferrozine as a chromophore. The amount of Fe(II) released was evaluated spectophotometrically based on a molar absorption of $\epsilon_{562} = 27.9\ mM^{-1}\ cm^{-1}$ of the complex for a reaction mixture (1 ml final volume) containing 50 mM Hepes buffer (pH 7.0), 500 μM ferrozine and the other reagents (6-OHDA). The reactions were performed at 37°C against reference cuvettes containing all reactants except ferritin and initialised by the addition of 6-OHDA. The increase in absorption at 562 nm was continuously monitored using a standard spectrometer. Values accepted were the means of three replicate incubations.

2.3. Chromatography

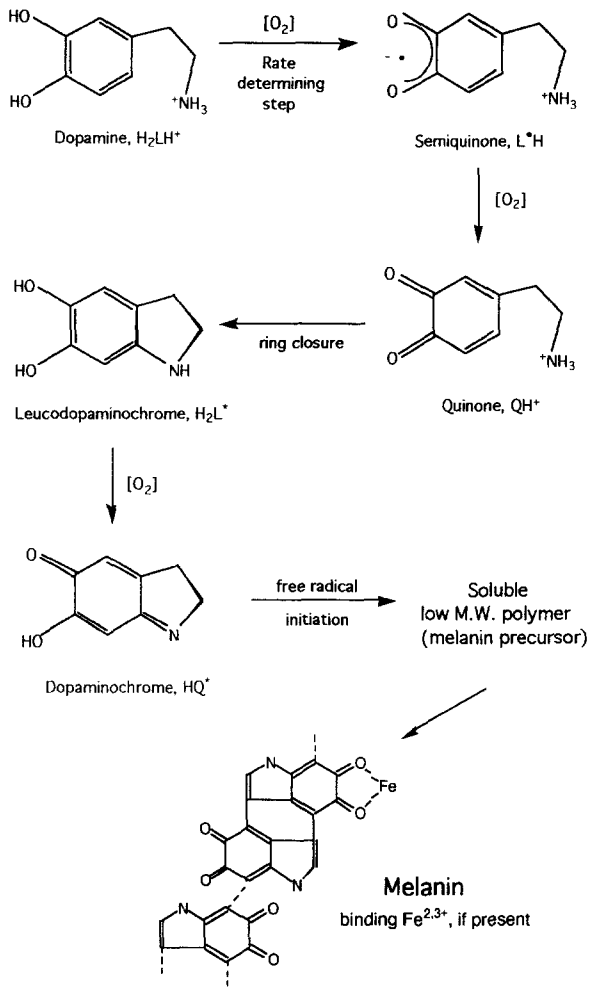
The reversed phase HPLC determinations were carried out using a Model 5000 solvent delivery module, a Coulochem 5100 A detector, and a 5010 analytical cell (all by ESA) connected to a CI-10 recording integrator (LDC/Milton); potential differences were +0.46 V and −0.22 V, respectively. Samples were introduced using a 100 μl injection loop and separations carried out using a 250 × 4.6 mm ID LC-18 column (5 μm average particle size). The mobile phase (pH 3) contained 20 mM potassium dihydrogen phosphate, 0.4 mM octane sulfonic acid, and 14% methanol (v/v). The flow rate of the mobile phase was 1.0 ml/min. (Prior to use, the mobile phase was filtered through a 0.22 mm membrane filter (Sartorius).) Peak identifications were established by admixture with authentic standards.

3. Results and discussion

3.1. The autoxidation of dopamine in the absence of metal ions

The course of the uncatalysed autoxidation of DA is shown in Scheme 1.

The kinetics of this reaction have been studied in some detail [16] and these can be summarised as follows: The consumption of oxygen is accurately first-order in oxygen for over three half-lives and is independent of ambient light. Protons are released during the reaction at an identical rate the oxygen consumption. The rate constants are



Scheme 1.

inversely proportional to $[H^+]$ and directly proportional to the total concentration of dopamine, $[L]_T$. The rate law is therefore:

$$-d[O_2]/dt = A[L]_T/[H^+][O_2] \quad (2)$$

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 [25]), i.e., one proton is released for every two oxygen molecules consumed. The production of peroxide was demonstrated by stoichiometric recovery of oxygen after addition of catalase to a reacted solution. The stoichiometry of the reaction is therefore:

$$H_2LH^+ + 2O_2 \rightarrow HQ^* + H^+ + 2H_2O_2 \quad (3)$$

The obtained rate law has been interpreted that the reaction being followed is the rate-determining abstraction of a hydrogen atom from the monodeprotonated dopamine, HLH, and that all subsequent steps shown in Eq. (4) are fast [26–28]. Since dopaminochrome is considered a final product of the oxygen-consuming and the proton-releasing reactions, its further reaction to melanin 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 polymerisation reaction. Allowing for the consumption of a second oxygen molecule by leucadopaminochrome, A can be interpreted as $2\ k_1\ K_m^{OH}$; K_m^{OH} being the *microconstant* for the deprotonation of the phenol site ($pK_m^{OH} = 8.90$ [1]). This yields a value k_1 of $0.47 \pm 0.05\ M^{-1}\ s^{-1}$ at 25°C at an ionic strength of 0.100 M (Cl^-). The variation with temperature establishes an apparent activation energy of 75 kJ mol^{-1}.

$$\begin{aligned} &H_2LH^+ \Leftrightarrow HLH + H^+ \\ &HLH + O_2 \rightarrow LH^{\cdot} + H^+ + O_2^- \\ &LH^{\cdot} + O_2 \rightarrow QH^+ + O_2^- \\ &HO_2 + O_2^- + H^+ \rightarrow H_2O_2 + O_2 \\ &QH^+ \rightarrow H_2L^* + H^+ \\ &H_2L^* + O_2 \rightarrow HQ^* + H_2O_2 \end{aligned} \quad (4)$$

3.2. Effect of metal ions on the reaction of dopamine with dioxygen

The effects of added metal ions on the autoxidation of dopamine are summarised in Table 1. It can be seen that Fe(III), and VO^{2+} have little, if any, influence on the rate of autoxidation, and this might well be because the metal ion becomes encapsulated by an inert, probably polymeric, but soluble, precursor of melanin. Neither melanin itself nor metal-ion-containing melanin has any effect on the rate at neutral pH. The unreactivity of Cu(II), on the other hand, might be due to the effect of chlorocomplexes on the Cu(II)/Cu(I) redox couple (chloride-free solutions have not been examined in this case).

In acid solution, iron(III) (and several other metal ions) *initiates* the reaction of dopamine with dioxygen, but is not a true catalyst because reaction ceases when 1 mol of

Table 1
The effect of metal ions (100 μM) on the autoxidation of DA ($[L]_T$ = 10 mM, pH = 7.60)

Metal	k_{obs} ($10^{-4}\ s^{-1}$)	$k_{obs}^{M}/k_{obs}^{nonM}$
none	7.2	1.0
Fe(II)	5.6	0.8
Fe(III)	6.7	0.9
Zn(II)	6.6	0.9
Cu(II)	8.6	1.2
VO^{2+}	10.6	1.5
Ni(II)	17.2	2.4
Mn(II)	29.1 (3.7 *)	4.0
Co(II)	46.2 **	6.4

* Half-order rate constant (units of $10^{-5}\ M^{1/2}\ s^{-1}$).
** Possible half-order contribution.

oxygen is consumed per mol of iron. The reason for and the nature of the reaction is being investigated at present (E.H., R.F.J., and W.L.) but it demonstrates that Halliwell's often quoted generalisation [29] that transition-metal ions invariably catalyse the autoxidation of catecholamines is not valid.

Regarding the disputed role of iron-neuromelanin interactions [9,14,30], it is interesting to note that melanin, whether iron-containing or not, does not catalyse the autoxidation reaction, as shown in Fig. 1. A small deviation in the presence of iron-containing melanin at prolonged time shows even a slight inhibition of the reaction. This observation might deserve further study and might be due to properties of the synthetic melanin. However, this does not affect the conclusion that neither melanin nor iron-containing melanin catalyses the autoxidation of DA.

In view of the sensitivity of the dopaminergic neurons to manganese [31,32], it is of particular interest to note that manganese(II), alone of the metal ions studied, acts as a true catalyst [33] The kinetics of this reaction are complicated, involving the initial oxidation of the complexed manganese to Mn(III), although a small amount of regenerated Mn(II) is responsible for sustaining the chain reaction. Furthermore, the manganese is not removed from solution by incorporation into the neuromelanin (as is the case with iron) [34].

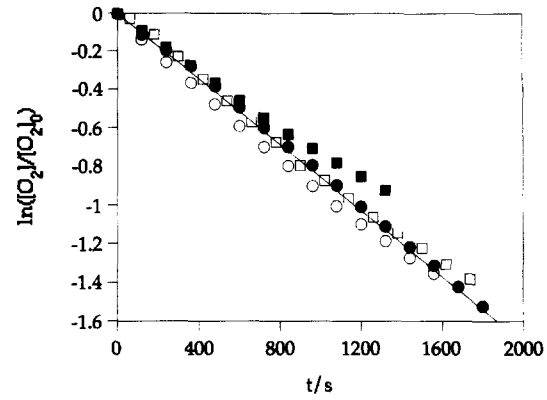


Fig. 1. Semilogarithmic concentration/time diagram for the autoxidation of DA. 10 mM DA alone (○), and synthetic melanin (□), iron(III) (●) and iron(III)-containing melanin (■) added; pH is 7.6 in 0.100 M KCl.

Finally, the remarkable activity of nickel(II) in catalyzing DA autoxidation should be noted (Table 1), as this is a highly unusual role for this element.

3.3. The hydroxylation of dopamine by Fenton's reagent

A mixture of iron(II) with hydrogen peroxide is known as Fenton's Reagent and gains its reactivity from the

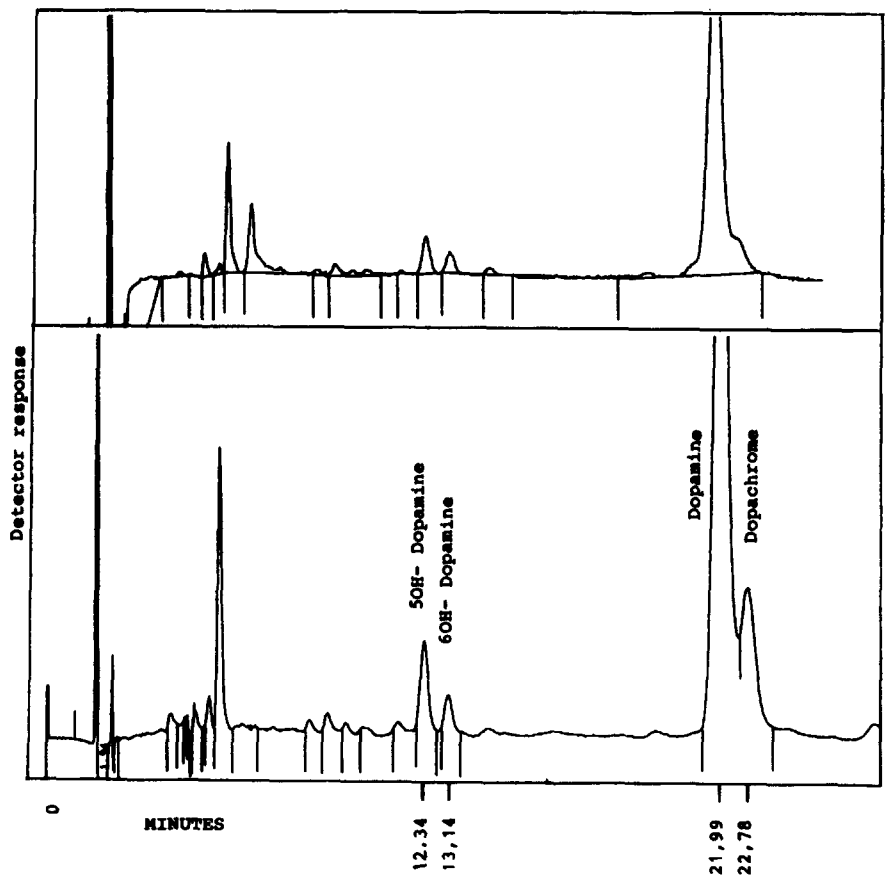


Fig. 2. Time course of the formation of 5-OHDA and 6-OHDA as effect of iron(II) (1 mM) in a mixture of DA (1 mM) and H_2O_2 (1 mM) at 298 K after 5 (upper trace) and 20 (lower trace) min, respectively. pH is 7.5 in physiological NaCl-solution.

formation of hydroxyl radicals Eq. (5). Portions of iron(II) and hydrogen peroxide were

$$Fe^{2+} + H_2O_2 \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+} \quad (5)$$

added to samples of dopamine and the resulting mixture analysed by HPLC (as described above) and a successful separation and identification of DA, 5-OHDA, and 6-OHDA achieved, for which a typical chromatogram is shown in Fig. 2.

These findings are in agreement with the those of Cohen et al. [35,36], namely that 5-OHDA and 6-OHDA are formed. However, these workers were not able to distinguish between these species; they used electrochemical detection, applying a potential of +0.8 V and retention times of about 1–2 min after injection. We found under similar conditions, but applying the coulometric method of detection, a good separation between 5-OHDA and 6-OHDA at higher potentials of about 3 V in a reduction mode. Using an analogous reversed-phase column, and ion-pair chromatography, we found much higher retention times of about 10–30 min, with a 1 ml/min flow rate. In the region of Cohen's measurements we found only decomposition products of the catecholamines. Furthermore, we did not use ascorbate buffer in the eluate as did Cohen et al. because it is electrochemically active and therefore prevents the identification of the HPLC peaks of interest (by strong overlapping).

3.4. Electron transfer between Iron(III) and the dopamines

The reaction between iron(III) and the dopamines (DA, 5-OHDA, and 6-OHDA) has been followed by stopped-flow techniques. Low pH had to be used to ensure 1:1 complex formation and thus simulate low DA concentrations. Typical spectra for the reaction with DA and 6-OHDA are shown in Figs. 3 and 4, respectively (5-OHDA reacts analogously to DA; under these conditions, we find an additional, yet unidentified intermediate [37], whose existence, however, does not interfere with our conclusions below).

These spectra show clearly that the initial, rapid, formation of an iron(III)-DA complex is followed by relatively slow decomposition (by means of internal electron transfer) to yield iron(II) and the dopamine semiquinone.

Fig. 4, however, illustrates the completely different behaviour of 6-OHDA. There is, from the spectra obtained, no evidence for the intermediate formation of an iron(III)-6-OHDA complex, suggesting the strong possibility that in this case electron transfer proceeds via an 'outer-sphere' reaction. It was therefore to be expected that, unlike the other dopamines, 6-OHDA would be able to remove iron from sources such as ferritin by means of prior reduction of the iron(III) to iron(II). This has been confirmed by Monteiro and Winterbourn [38] and Fig. 5 illustrates the time and concentration dependent release of iron from ferritin by use of 6-OHDA.

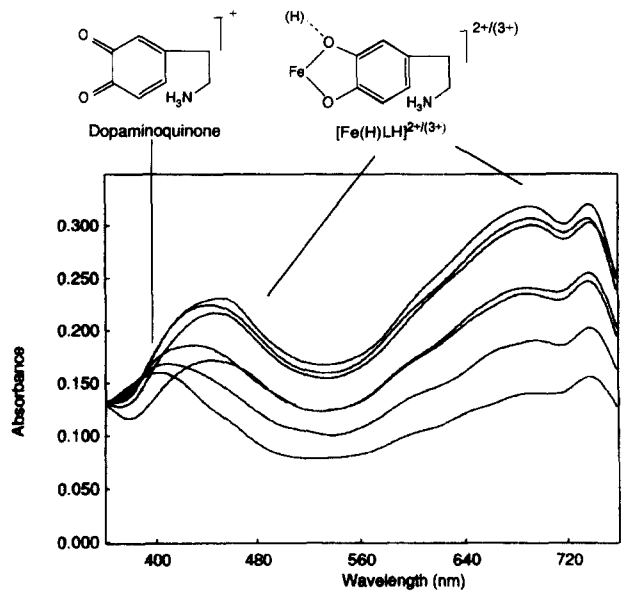


Fig. 3. Time dependent spectra of the reaction of DA (3.0 mM) with iron(III) (0.25 mM); pH 2.49 in 0.1 M KCl. Spectra taken after 0.625, 1.25, 2.50, 5.0, 18.75, 31.25 and 50 s, respectively. Rate of electron transfer: $2.25 \cdot 10^{-2}$ s^{-1}.

Typical spectra recorded during the course of the autoxidation reaction at neutral pH are shown in Fig. 6. It can be seen that (i) general absorption increases due to the formation of colloidal melanin, (ii) a peak at 480 nm attributable to dopaminochrome appears in the initial stages of the reaction but then disappears, (iii) peaks at about 405 and 620 nm appear in the final stages of the reaction.

However, in the presence of iron(III), as shown in Fig.

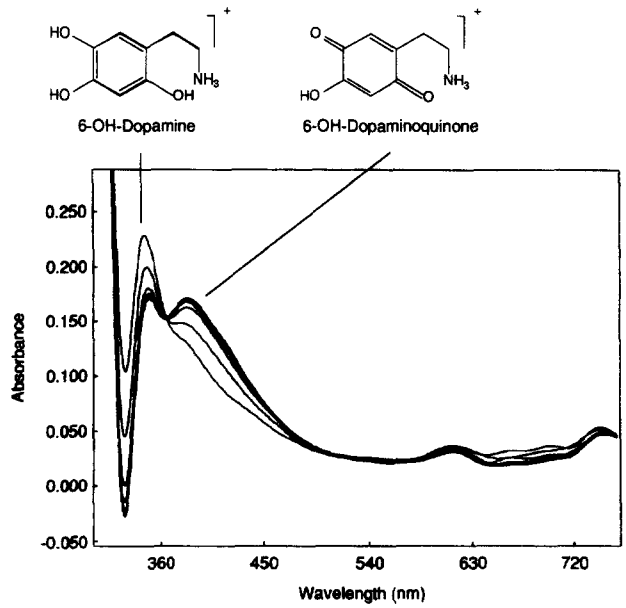


Fig. 4. Time dependent spectra of the reaction of 6-OHDA (3.0 mM) with iron(III) (0.25 mM); pH 2.49 in 0.1 M KCl. Spectra taken after 0.25, 0.625, 1.25, 2.50, 5.0 and 7.50 s, respectively. Rate of electron transfer: 600 $M^{-1}s^{-1}$.

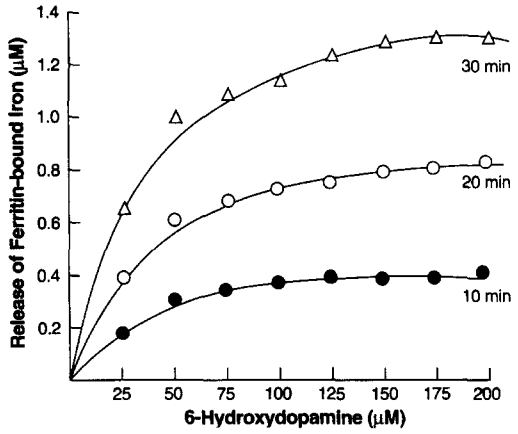


Fig. 5. Reductant concentration and time dependence of the release of iron from ferritin in vitro by reduction with 6-OHDA. Iron was monitored spectrophotometrically as iron(II) with the colour indicator ferrozin.

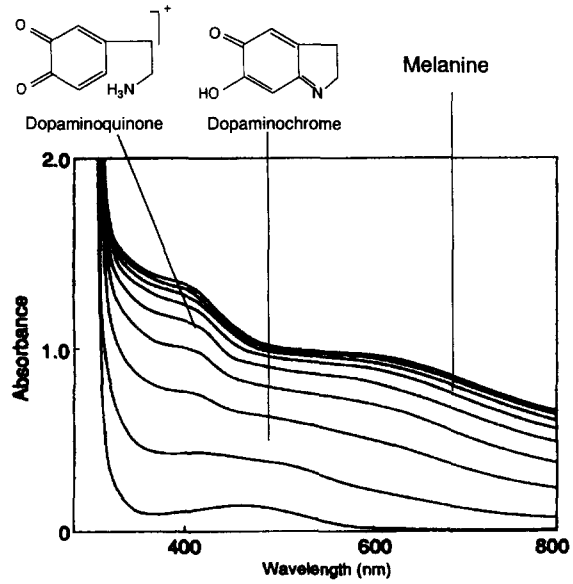


Fig. 6. Time-dependent UV-Vis-Spectra during the autoxidation of dopamine. Spectra were recorded every 300 s, the first (bottom) one 200 s after the start of the reaction. $[DAM]_T = 10.0$ mM, $[O_2]_0 = 1.32$ mM, pH 7.65, $I = 0.100$ M, $T = 298$ K.

7, it is interesting to note that (a) general absorption increases and (b) the peak at 405 nm is suppressed by the presence of iron(III)-ions, whereas the peak at 620 nm is unchanged. Thus, even taking into account that suppression of the 405- nm peak might be due to the increase of general absorption, it can be said that iron(III) affects the course of melanine-formation, even as it does not catalyse the autoxidation reaction. Also it can be seen that the peak at 495 nm, due to $Fe(LH)_2^+$ and masking the less intense dopaminochrome peak, disappears during the reaction. Since DA is in large excess and its complex a sensitive indicator for iron(III), it can be concluded that iron is removed from solution, certainly by inclusion into the melanin (adsorption of iron at the surface of melanin can only account for 5% of the total iron present under our conditions [14]). It should be noted that under these conditions, iron(II) is rapidly oxidised to iron(III).

3.5. General discussion

The in vitro experiments described above would appear to be important and relevant to an understanding of the progress of Parkinson's disease:

1. By use of the confocal fluorescence microscope, it can be demonstrated histochemically that excessive hydrogen peroxide accumulation is found in the post mortem frontal cortex of a Parkinsonian patient, while in control tissue no such associated fluorescent spots could be detected [39]. This strongly suggests that H_2O_2 is in fact present in the brain (strictly speaking, its presence has been shown a few hours after extraction of a brain). It is important to note here that the ability of cells to destroy peroxides is reduced due to ageing processes.

2. The fact that 6-OHDA and MPTP (and other reducing agents) experimentally induce lesions of the nigrostriatal dopaminergic system (i.e., that they induce effects analogous to those evident in Parkinson's disease) is well described in the literature [40,41]. That the neurotoxic action of MPTP leading to damage of the dopaminergic neurons in primates can be blocked by NMDA antagonists [42] is also of interest here.

3. The production of 6-OHDA by a Fenton-type reaction followed by the release of iron — as iron(II) — from ferritin enables one to suggest the following Scheme 2 by which the production of cytotoxic chemicals is sustained. As soon as Fe(II) is present (it might occur from ferritine by reduction due to 6-OH-DA, MPTP, or other outer sphere-reducing agents), OH-radicals or rather Ferryl-

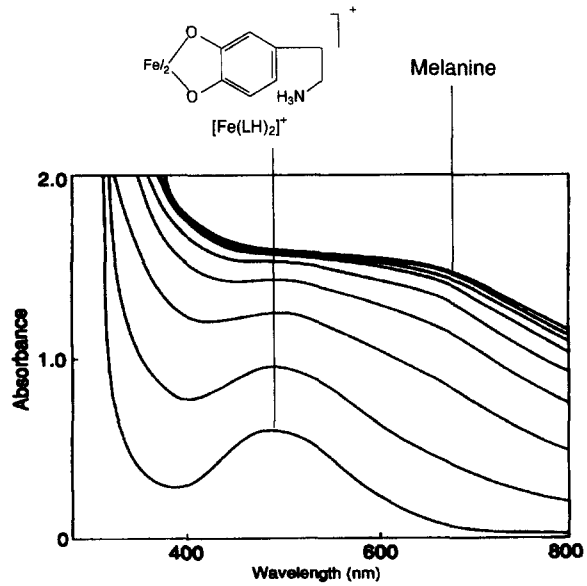
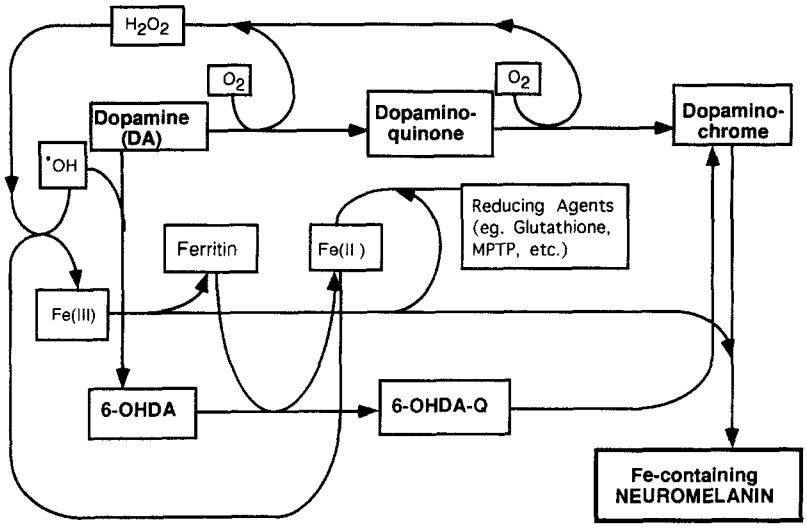


Fig. 7. Time-dependent UV-Vis-Spectra during the autoxidation of dopamine in the presence of 100 μM iron(III). Other experimental details are given in Fig. 6.



Scheme 2.

species are formed which hydroxylate DA to form 5-, and 6-OHDA. The latter in turn frees again Fe(II) from ferritine. In other words, the left hand, lower part forms an autocatalytic cycle continuously producing the cytotoxic species like especially OH-radicals or strongly oxidising ferryl species. In fact 5-OHDA and 6-OHDA are found in human urine — especially that of patients undergoing dopa therapy [43]. The removal of iron from its storage protein by reducing agents such as MPTP while at the same time inducing symptoms very similar to PD lends added weight to the suggestion that its role in the progression of Parkinson's disease is as presented in Scheme 2 above.

4. The trapping of iron(III) by melanin as demonstrated by the in vitro reaction of DA with O_2 in the presence of iron(III) and its subsequent complete removal from the system strongly suggests that one of the functions of melanin formation in the SN is the entrapment of any free iron(III) to prevent its toxic effects. As mentioned in the Introduction, iron-containing melanin has been shown to occur significantly in the SN of Parkinson brains; indeed, the presence of this melanin can be seen as a result of free iron ions in this area rather than as a pathogenic factor in its own right.

5. Investigations concerning the influence of nicotine strongly suggest that the development of Parkinsonism can be strongly inhibited by nicotine [44–46]. Nicotine is a strong complexing agent for Fe(II) (we obtained a formation constant for the $Fe(Nic)_2^{2+}$ complex of about 6.5×10^8 M^{-2} (more-or-less pH-independent) by spectrophotometric titration at 25°C). This observation again strongly reinforces the validity of the above proposed mechanism (Scheme 2). In fact this might be a hint that it should be possible to devise possible treatments for PD based on the use of Fe(II)-complexing agents.

6. Finally, the true catalytic effect of manganese(II) is obviously directly related to its toxic effects since it can effect the oxidation of DA without subsequently being effectively removed from the system (as is the case with iron).

These studies of in vitro chemical reactions considered to be important for the development of PD are being continued and extended in the belief that this will provide a sound chemical basis for the further in vivo studies that are required in order to clarify the mechanisms involved in the biology of ageing.

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