Early Steps in the Free Radical Polymerisation of 3,4-Dihydroxyphenylalanine (Dopa) into Melanin

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Pulse radiolysis and flash photolysis-initiated one-electron oxidation of 3,4-dihydroxyphenylalanine (dopa) and other catecholamines have allowed the direct measurement of the kinetics of semiquinone disproportionation leading successively to the corresponding melanin precursors dopaquinones and dopachromes.

Malignant melanoma, a cancer of pigment cells, is characterised by the accumulation of large amounts of melanin, and improved knowledge of the mechanism of melanin formation may ultimately provide a chemotherapeutic approach. The formation of melanin *in vivo* is a complicated process involving the conversion of 3,4-dihydroxyphenylalanine (dopa) into melanin *via* several oxidation steps involving intermediates such as dopaquinone and dopachrome. The reactions of dopasemiquinone, dopaquinone, and dopachrome are pivotal for the eventual formation of eumelanin and pheomelanin pigments, and may play an important role in the cellular metabolism of melanocytes, thus involving themselves in the general process of malignant melanoma induction. Previous studies[1,2] of dopa oxidation have often involved the use of enzymes. However, such studies do not lead to a detailed kinetic understanding of the early processes involved due to slow rate-determining steps lasting seconds, the enzymatic reaction probably including a 2-electron oxidation process. Fast time resolution pulse radiolysis and flash photolysis generate the semiquinone (one-electron oxidation) virtually instantaneously, and uniquely, within a few microseconds. This novel approach to dopa oxidation clearly can lead to a detailed kinetic understanding of the process of melanin formation and new insight into the chemistry involved.

In vivo, only the initial oxidation of dopa is believed to be under enzymatic control, so that the subsequent steps initiated by ionizing radiation or light are biologically relevant.

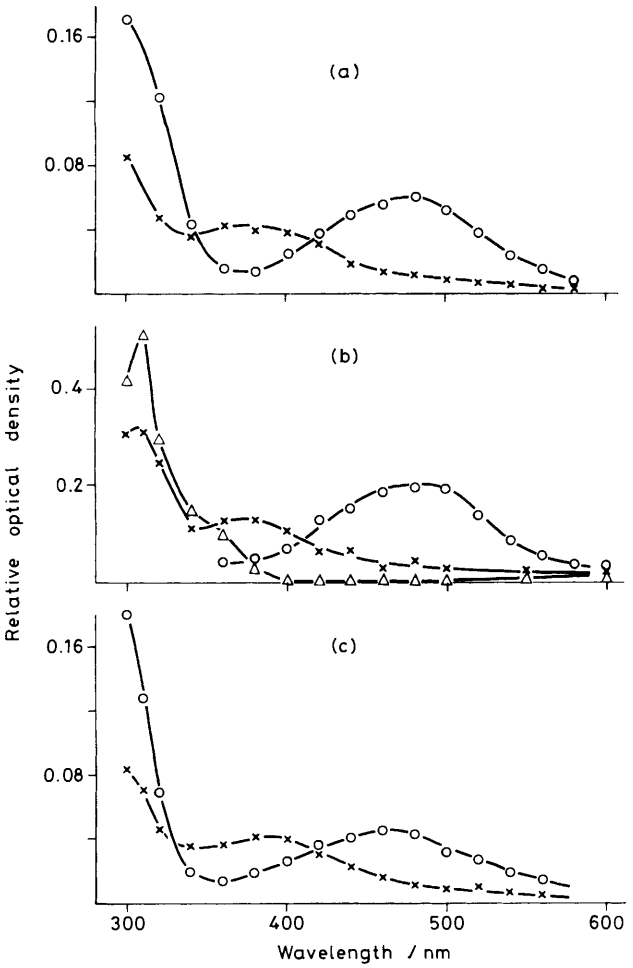


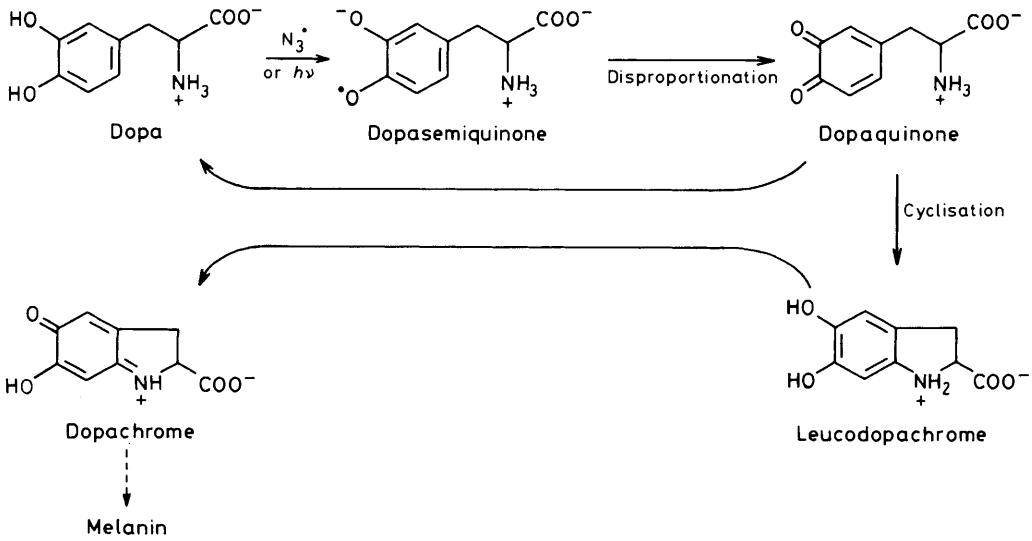
Figure 1. Changes in absorption at various times after pulse radiolysis of nitrous oxide-saturated solutions of 10^{-3} mol dm^{-3} catecholamines in water containing 5 × 10^{-2} mol dm^{-3} NaN_3, pH 7.7, dose *ca.* 30 Grays, optical path length 2.5 cm. (a) L-Dopa: ×, 10 ms; ○, 410 ms. (b) L-α-Methyldopa: △, 20 μs; ×, 9 ms; ○, 180 ms. (c) Dopamine: ×, 0.1 s; ○, 4 s.

Oxidation of L-dopa with ionizing radiation-induced $N_3^{\bullet}$ radicals [$k(N_3^{\bullet}$ + dopa) = 3.4 × 10^9 dm^3 mol^{-1} s^{-1} at pH 8.4] by means of pulse radiolysis results in the formation of dopasemiquinone, $\lambda_{max.}$ 305 nm.[3,4] The decay of this semiquinone has now been shown to follow second order kinetics [$2k$ = 9.8 × 10^7 dm^3 mol^{-1} s^{-1}, at pH 7.7 based on ε(305 nm) = 12 000 dm^3 mol^{-1} cm^{-1}[4]] and lead initially to the formation of a species with an absorption maximum around 380 nm [Figure 1(a), spectrum 10 ms after pulse]. Since dopaquinone is known to have a maximum at this wavelength,[5] it is suggested that the decay found corresponds to disproportionation of dopasemiquinone into dopaquinone and dopa. The species absorbing at 380 nm subsequently decays *via* a process following overall first order kinetics into a species with a maximum around 480 nm, indicative of dopachrome [Figure 1(a), spectrum 0.41 s after pulse]. This first order process, which can be followed either *via* the decay at 380 nm or *via* the growth at 480 nm, is strongly pH dependent (see Table 1). The pH dependence we report cannot be directly related to a pK_a typical of an amino acid, and the complex behaviour we observe may involve a base-catalysed reaction. This will be the subject of further study.

The first order kinetics observed may be understood in terms of Scheme 1 if the conversion of dopaquinone ultimately into dopachrome is rate determined in the pH range studied by a cyclisation of dopaquinone into leucodopachrome, the latter as soon as it is formed reacting rapidly (k > 10^9 dm^3 mol^{-1} s^{-1}) with unchanged dopaquinone to give dopa and dopachrome. According to this interpretation, at the

Table 1. First order rate constants for the decay of dopaquinone and formation of dopachrome following pulse radiolysis of aqueous N_2O-saturated 10^{-4} mol dm^{-3} dopa and 5 × 10^{-2} mol dm^{-3} NaN_3 in the presence of 10^{-2} mol dm^{-3} phosphate or borate buffer.

	First order rate constant (s^{-1})	
pH	380 nm (dopaquinone) decay	480 nm (dopachrome) growth
8.6	23 ± 1.3	23 ± 1.7
7.6	7.6 ± 0.36	6.9 ± 0.26
6.6	0.91 ± 0.075	0.99 ± 0.033
5.6	0.20 ± 0.014	0.20 ± 0.013



Scheme 1

doses employed (*ca.* 30 Grays), the maximum concentration of dopaquinone formed is around 2×10^{-5} mol dm^{-3} and so the leucodopachrome initially formed would decay into dopa and dopachrome with a half life of *ca.* $0.693/2 \times 10^{-5} \times 10^{9}$ s, *i.e.* 35 μs, short compared with the observed half lives of formation of dopachrome.

These techniques will also be useful in studies of the oxidation of other catecholamines. Indeed we have obtained similar spectral data for the decay of azide-radical induced L-α-methyldopa and dopamine[6] semiquinones into the corresponding dopaquinones and, subsequently, dopachromes [Figure 1(b), (c)], although the time scales over which these transformations occurred were different. The semiquinones can also be formed by addition of OH· radicals to the catecholamines followed by water elimination.[6,7] Evidence for the corresponding formation of adrenaline quinone and adrenochrome from adrenaline after OH· radical oxidation has been previously obtained.[8]

The above series of reactions of dopa were also initiated *via* 265 nm laser flash photolysis, in this case the dopasemiquinone radicals being formed *via* photoionization. A similar strong pH dependence in the rate of the transformation dopaquinone → dopachrome was noted after photoinitiation.

Since high urinary levels of the toxic metabolite 5-*S*-cysteinyl dopa are associated with metastasing melanomas, reactions of the various melanin precursors with cysteine were investigated. Cysteine was found to react with pulse radiolytically produced dopaquinone with a rate constant of 2.4×10^{7} dm^{3} mol^{-1} s^{-1}, preventing the formation of dopachrome. The corresponding rate constant for dopaminequinone was 7.3×10^{7} dm^{3} mol^{-1} s^{-1}. The relevance of this quenching process to the production of 5-*S*-cysteinyl dopa will be further investigated. No reaction of the dopasemiquinones with cysteine could be detected.

We consider that our studies have led to a new understanding of the kinetics and mechanism of the early processes following dopa oxidation and we believe that the further applications of these techniques will lead to new insight into melanogenesis and related processes.

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