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Kinetic evidence that cysteine reacts with dopaminoquinone *via* reversible adduct formation to yield 5-cysteinyl-dopamine: an important precursor of neuromelanin

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The reaction of cysteine (cys) with dopaminoquinone (DQ) to form (mainly) 5-cysteinyl-dopamine (5-cys-DA) is of interest because it is known to play a role in the production of melanin in the mammalian brain. To gain insight into this important reaction, an *in vitro* detailed kinetic study was undertaken. It has been established that cys reacts with DQ via the initial reversible formation of an intermediate adduct or complex and that this adduct then decomposes to form 5-cys-DA. A little 2-cys-DA, is almost certainly formed at the same time but its presence could not be kinetically investigated. Clarification of the kinetic data was aided by following the reaction of DO with a cys analogue, mercaptoacetic acid (maa). Maa was found to react in a similar fashion, but also forms, reversibly, a bis-complex. This bis-complex, 2,5-(maa)₂-dopaminoquinone, is in equilibrium with the di-protonated compound but neither of these species reacts further over the timescale employed in these kinetic studies. Equilibrium constants and first-order rate constants have been extracted from the data and the cys complex is found to be weaker than its maa analogue by an order of magnitude ($K_{cys} = (1.09 \pm 0.02) \times 10^{-3}$; $K_{1,maa} = (7.45 \pm 0.11) \times 10^{-3}$). (Note that the possibility that cys also forms a bis-complex at much higher cys concentrations cannot be excluded.) The rates of decomposition differ markedly—the cys complex has the value $k_{cys} = 1830 \pm 50 \text{ s}^{-1}$ whereas the rate constant for the decomposition of the maa complex is $k_{maa} = 69.3 \pm 0.02 \text{ s}^{-1}$ and we attribute this difference to the effect of the positive charge carried by the amino-group on cys. Finally, the constants obtained are used to compare the reactivity of thiol addition with ring cyclization (U. El-Ayaan, E. Herlinger, R. F. Jameson, and W. Linert, J. Chem. Soc., Dalton Trans., 1997, 2813–2818) and we show how this has important implications concerning the production of neuromelanin.

Introduction

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5-cysteinyl-dopamine (5-cys-DA) is found in certain dopaminergic regions of the mammalian brain,¹⁻³ including caudate nucleus, putamen, globus pallidus and substantia nigra. Indeed chemical degradation of neuromelanin in the substantia nigra has suggested that 5-cys-DA is a precursor of melanin,⁴⁻⁶ via its co-polymerisation during the oxidation of dopamine[†]. During the progression of Parkinson's disease, however, increased ratios⁷ of 5-cys-DA over dopamine are observed in the substantia nigra. It has therefore been postulated that, rather than being involved in melanin production, the formation of 5-cys-DA *diverts* production. In fact, with increasing molar excess of cysteine, the formation of melanin *in vitro* is decreased and ultimately stopped.⁴ Moreover, due to its lower redox potential, 5-cys-DA can further oxidize and cyclize intramolecularly to form toxic dihydrobenzothiazines.⁸

Although 5-cys-DA can be formed by nucleophilic attack by cysteine (cys) on dopaminoquinone (DQ), concentrations of cysteine in the brain are generally low. Alternatively, or in parallel, glutathione, present in much higher concentrations, could attack to form 5-glutathionyl-dopamine (5-glu-DA), which could then undergo enzymatic cleavage by γ -glutamyl transpeptidase and peptidase to form 5-cys-DA. Evidence for the presence of 5-glu-DA is not forthcoming but elevated activ-

ity of γ -glutamyl transpeptidase has been reported⁹ in the substantia nigra of those patients that suffered from Parkinson's disease.

Previous studies have focused on the interaction of cysteine with quinones because only cysteinyl catechols have been unequivocally detected in vivo⁸ and these studies have employed continuous electrolytic production of the quinones^{8,10,11} in the presence of excess L-cysteine. Because of oxidation of the initial substitution product (i.e. 5-cys-DA) and intramolecular cyclization by the amine group, a bewildering number of products were produced and the initial stages of the reaction were obscured. Xu et al.^{12,13} have, however, studied this initial step by first oxidizing dopamine in the absence of a nucleophile and then adding the nucleophile. They were able to determine the initial products through a mixture of spectroscopic techniques; mainly 5-cys-DA, some of the di-substituted product 2,5-dicys-DA and a small amount of 2-cys-DA were formed. A mechanism was proposed ^{10,12} but no kinetic data was supplied to back their claims. The interpretation of their results was also hampered by the fact that oxygen was not excluded.

In the present study the oxidation of dopamine to the quinone was achieved electrolytically and the reactions with cysteine and mercaptoacetic acid‡ (maa) followed spectrophotometrically in a stopped-flow apparatus. Mercaptoacetic acid was included in this investigation in order to clarify the data

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[†] Dopamine: 2-(3,4-dihydroxyphenyl)ethylamine.

and to understand the role played, if any, by the $-NH_3^+$ group present in cysteine. The absorption of dopaminoquinone¹⁴ at 385 nm ($\varepsilon = 1650 \text{ M}^{-1} \text{ cm}^{-1}$) was used to follow its consumption and, in order to make use of pseudo first-order kinetics, the respective thiols were always used in large excess. Furthermore, the exclusion of all oxygen has enabled us to study, for the first time, the initial stages of the basic reactions and to establish that they involve the initial reversible formation of adducts (or 'complexes') between the quinones and the thiols, which then react to form the final product, 5-cys-DA or 5-maa-DA.

Materials and methods

Chemicals

Dopamine was obtained from Merck; L-cysteine and citric acid from Sigma; mecaptoacetic acid and acetic acid from Aldrich. All were used without further treatment.

Generation of dopaminoquinone

Solutions of 1 mM dopamine were made in 0.4 M KCl and 0.1 M HCl. Low pH's were used to hinder the ring closure reaction ¹⁴ of the resultant quinone by protonating the amine group; high ionic strength was used to facilitate fast oxidation, thus also reducing ring closure and minimizing the chance of further oxidation. An IsoTech IPS 1810H Laboratory DC power supply unit produced a constant voltage across two platinum electrodes in a custom two-compartment glass cell separated by a glass frit. A current of 40 mA at an emf of 5 V was maintained for 3¹/₂ min to ensure fast conversion of dopamine to quinone (10–40% conversion depending on the concentrations required; the UV-Vis spectrum was measured and checked before each run of experiments). Anaerobic conditions were maintained by bubbling argon through the solutions before, during and after electrolysis.

Stopped-flow

Solutions of cysteine (2–16 mM) and mercaptoacetic acid (1–12 mM) were made in various buffers (acetate, citric acid, or HCl) depending on the pH. The ionic strength was kept constant at 0.5 M with KCl. All solutions (both thiol and quinone) were transferred to the SX-17MV stopped-flow spectrometer (fitted with a photo-multiplier detector) from Applied Photophysics *via* gas tight Hamilton syringes. Single wavelength measurements were carried out at 385 nm and time-dependent spectra by combining many single wavelength measurements. Final pH's were measured using a Radiometer PHM64 instrument.

Results

Cysteine

Experiments were performed over a large range of pH values (pH = 1–5), concentrations of cysteine (2–16 mM) and of quinone (0.1–0.4 mM) and over a range of ionic strengths, *I*. Time-dependent spectra (see Fig. 1) showed the disappearance of the quinone and increases in absorbances at 255 and 300 nm corresponding to the formation of both 5 and 2-cys-DA¹² (they absorb in the same regions of the electronic spectrum).

The rate of consumption of dopaminoquinone (DQ) obeyed the rate law (1) and was found to be essentially independent of ionic strength (Table 1).

$$-\frac{d[DQ]}{dt} = k_{cys}^{obs} [DQ]_{T}$$
(1)

 рН	<i>I</i> /M	$k_{\rm cys}^{\rm obs}/{ m s}^{-1}$
1.08	0.200	0.918
1.09	0.300	0.895
1.15	0.400	0.814
1.09	0.500	1.16
4.65	0.300	345
4.65	0.370	350
4.65	0.460	353
4.65	0.500	333



Fig. 1 Time-dependent electronic spectra of the reaction of dopaminoquinone with L-cysteine, showing the disappearance of quinone at 385 nm and appearance of products absorbing at 255 and 300 nm. Spectra were taken on a logarithmic timescale from 1 ms–1 s. Initial concentrations: [DQ] = 0.32 mM, [cys] = 4 mM; pH = 3.15.

The dependence of k_{cys}^{obs} on the concentration of cysteine followed the expression (2).

$$k_{\rm cys}^{\rm obs} = \frac{A[\rm cys]_{\rm T}}{(1 + B[\rm cys]_{\rm T})}$$
(2)

An example of the fit obtained using (2) for a particular pH is given in Fig. 2 and the results are summarised in Table 2. It was also established that both A and B are inversely proportional to the proton concentration (Fig. 3).



Fig. 2 Variation of the observed first-order rate constant k_{cys}^{obs} with cysteine concentration for a representative pH of 5.04. The solid line through the data points follows eqn. (2) using the constants A = 1500 and B = 3.



Fig. 3 Plots of $A(\bigcirc)$ and $B(\bigcirc)$ vs. $1/[H^+]$ for the reaction of cys with DQ. The solid lines represent the best fit lines through the origin. The slopes are $A: 2.00 \pm 0.03$ and $B: (1.09 \pm 0.02) \times 10^{-3}$.

Table 2 Kinetic data obtained for a range of pHs and of cysteineconcentrations for the reaction of cysteine with dopaminoquinone

pН	$[cys]_T/M$	$k_{\rm cys}^{\rm obs}/{ m s}^{-1}$	A	В
2.72	0.002	1.72	1500	3
	0.004	5.83		
	0.006	8.58		
	0.008	11.1		
	0.010	15.4		
2.76	0.004	5.44	1400	5
	0.006	8.22		
	0.008	10.8		
	0.010	12.7		
4.11	0.004	77.3	21000	15
	0.006	113		
	0.008	126		
	0.010	162		
4.22	0.004	90.5	33000	18
	0.006	148		
	0.008	211		
	0.010	293		
	0.016	438		
4.45	0.004	159	55000	30
	0.006	264		
	0.008	355		
	0.010	422		
5.04	0.002	361	220000	120
	0.004	602		
	0.006	835		
	0.008	1060		

Table 3 The variation of ionic strength, *I*, with the observed first-order rate constant, $k_{\text{maa}}^{\text{obs}}$, of the reaction of maa with DQ ([maa] = 8 mM)

рН	I/M	$k_{ m maa}^{ m obs}/{ m s}^{-1}$
4.66	0.300	24.7
4.63	0.460	26.3
4.65	0.500	25.6

Mercaptoacetic acid

The rate of consumption of dopaminoquinone once again followed the pseudo first-order expression (1) and $k_{\text{maa}}^{\text{obs}}$ was once again independent of ionic strength (Table 3). The dependence of $k_{\text{maa}}^{\text{obs}}$ on mercaptoacetic acid concentration was shown to follow eqn. (3). A representative example of the fits obtained is presented in Fig. 4 and the results are summarized in Table 4. Although *A* and *B* are inversely proportional to the proton concentration, the plot of *C vs.* $1/[\text{H}^+]^2$ is linear with an intercept on the ordinate axis (Figs. 5 and 6).



Fig. 4 Variation of observed first-order rate constant, $k_{\text{maa}}^{\text{bbs}}$, with maa concentration at pH = 2.74. The solid line is a theoretical fit using equation (3) with A = 950, B = 3, and C = 48000.

Table 4 Kinetic data obtained at a range of pHs and concentrationsof the reaction of DQ and maa

pН	[maa] _T /M	$k_{\rm maa}^{\rm obs}/{ m s}^{-1}$	A	В	С
2.74	0.002	1.60	950	3	48000
	0.004	2.09			
	0.006	2.15			
	0.008	1.92			
	0.010	1.53			
	0.012	1.35			
3.16	0.002	2.73	2650	40	50000
	0.004	5.40			
	0.006	4.92			
	0.008	4.68			
	0.010	4.20			
	0.012	3.78			
4.07	0.001	8.06	5800	75	55000
	0.002	9.83			
	0.004	10.6			
	0.006	10.8			
	0.008	8.99			
	0.010	7.53			
	0.012	6.43			
4.22	0.002	14.2	8800	90	51000
	0.004	16.6			
	0.006	15.6			
	0.008	13.9			
	0.010	11.3			
	0.012	11.2			
4.45	0.001	18.0	16000	210	53000
	0.004	23.7			
	0.006	23.2			
	0.008	22.8			
	0.010	18.1			
4.84	0.006	30.1	36000	520	80000
	0.008	28.8			
	0.010	25.4			
	0.012	23.8			
5.04	0.002	35.0	56000	820	120000
	0.004	35.4			
	0.006	32.5			
	0.008	29.1			
	0.010	26.5			
	0.012	24.5			



Fig. 5 Plots of $A(\bigcirc)$ and $B(\bigcirc)$ vs. $1/[\mathrm{H}^+]$ for the reaction of maa with dopaminoquinone. The solid lines represent the best fit straight lines through the origin. The slopes are $A = 0.516 \pm 0.003$ and $B = (7.45 \pm 0.11) \times 10^{-3}$.

Discussion

Cysteine

The departure from first-order dependence of the rate on one of the reactants that is illustrated in Fig. 2, has been known for some time. It has been shown to be explicable in terms of the prior, reversible, formation of a reactive intermediate.^{15,16} Indeed similar curves have been observed while investigating the addition of sulfite to *p*-benzoquinone.¹⁷ Note that independence of the observed rate constants with ionic strength (Table 1) when using cysteine as nucleophile neither supports nor disproves the existence of this intermediate because one of



Scheme 1 The proposed reaction mechanism of cysteine or mercaptoacetic acid with dopaminoquinone. Note that only the formation of 5-cys-DA and of 5-maa-DA is depicted. Formation of the 2-substituted products could occur in a similar manner but is disfavoured, possibly for steric reasons.



Fig. 6 Plot of *C vs.* $1/[H^+]^2$ for the reaction of maa with dopaminoquinone. The solid line represents the best fit straight line through the data points (the ordinate axis is depicted logarithmically to show clearly the goodness of fit). The slope is $(5.85 \pm 0.11) \times 10^{-6}$ and the intercept is $(5.02 \pm 0.08) \times 10^4$.

the feasible reactants, namely the cysteine, bears an overall charge of zero at most of the pH's under investigation. This is not the case with mercaptoacetic acid.§

To take into account the form of the rate eqn. (2) the following reaction sequence is proposed, eqns. (4) and (5). (See also Scheme 1.) Please note that proton ambiguity means that it

$$cys + DQ \xrightarrow{K_{cys}} cys - DQ + H^+$$
 (4)

$$cys-DQ \xrightarrow{k_{cys}} 5-cys-DA$$
(5)

§ The protonation constants²⁴ for cysteine are: $\log K_1 = 10.29$, $\log K_2 = 8.15$, $\log K_3 = 1.88$; and maa $\log K_1 = 10.11$, $\log K_2 = 3.48$.

would be equally valid to describe reversible formation of the intermediate cys-DQ as an attack by the deprotonated cysteine thiolate on the quinone. In other words the proton could be lost before reaction rather than during (as described by eqn. (4)). This ambiguity would of course be resolvable if the reaction produced directly the product, without the reversible formation of an intermediate. In the absence of a unique expression, the following formulation was adopted because the resulting equations are simpler and only differ in a numerical factor, namely the protonation constant of the S⁻ group of the cysteine anion.§

The total amount of quinone is given by eqn. (6).

$$[DQ]_{T} = [DQ] + [cys-DQ] = [DQ] \{1 + (K_{cys}[cys]_{T}/[H^{+}])\}$$
(6)

Therefore the rate of consumption of dopaminoquinone is given, from eqns. (4) and (5), by eqn. (7):

$$-\frac{\mathrm{d}[\mathrm{DQ}]}{\mathrm{d}t} = k_{\mathrm{cys}}[\mathrm{cys}\text{-}\mathrm{DQ}]$$
(7)

Making use of eqns. (6) and (7), and putting $[cys] = [cys]_T$ because cysteine is in large excess yields eqn. (8).

$$-\frac{d[DQ]}{dt} = \frac{k_{cys}K_{cys}[cys]_{T}[DQ]_{T}/[H^{+}]}{(1 + K_{cys}[cys]_{T}/[H^{+}])}$$
(8)

Thus, comparing eqn. (8) with eqns. (1) and (2), the slope of A vs, $1/[H^+] = k_{cys}K_{cys} = 2.00 \pm 0.03$, and that of B vs. $1/[H^+] =$

 $K_{\rm cys} = (1.09 \pm 0.02) \times 10^{-3}$. The ratio of the two slopes gives $k_{\rm cys}$. The values accepted are $K_{\rm cys} = (1.09 \pm 0.02) \times 10^{-3}$ and $k_{\rm cys} = 1830 \,{\rm s}^{-1}$.

Mercaptoacetic acid

The method employed above can be used, *mutatis mutandis*, to explain the rate eqn. (3) obtained for the reaction of maa with DQ but with the reversible attachment of a second maa allowed for. This second maa is presumably bound *para* to the first, *i.e.* in the 2-position. (The structure of the bis-complex is formulated in Scheme 1.) The following equilibria are involved (eqns. (9) and (10)).

$$maa + DQ \xrightarrow{K_{1,maa}} maa-DQ + H^+$$
 (9)

$$\max + \max -DQ \xleftarrow{K_{2\max}} \max_{2} -DQ + H^{+}$$
 (10)

The total amount of quinone is thus given by eqn. (11):

$$\begin{bmatrix} DQ \end{bmatrix}_{T} = \begin{bmatrix} DQ \end{bmatrix} + \begin{bmatrix} maa-DQ \end{bmatrix} + \begin{bmatrix} maa_{2}-DQ \end{bmatrix} \\ = \begin{bmatrix} DQ \end{bmatrix} \left\{ 1 + \frac{K_{1,maa}[maa]_{T}}{[H^{+}]} + \frac{K_{1,maa}K_{2,maa}[maa]_{T}^{2}}{[H^{+}]^{2}} \right\}$$
(11)

Because both reactants are charged species, the independence of the observed rate constants with ionic strength confirms the existence of a reactive intermediate. Furthermore, the form of the experimental rate equation, eqn. (3), taken in conjunction with the speciation, shows that only the mono-adduct reacts, *i.e.*:

$$-\frac{d[DQ]}{dt} = k_{maa}[maa-DQ]$$
(12)

Making use of eqns. (11) and (12) and putting $[maa] = [maa]_T$ yields:

$$-\frac{d[DQ]}{dt} = \frac{k_{maa}K_{1,maa}[maa]_{T}[DQ]_{T}/[H^{+}]}{(1 + K_{1,maa}[maa]_{T}/[H^{+}] + K_{1,maa}K_{2,maa}[maa]_{T}^{2}/[H^{+}]^{2})}$$
(13)

Comparison of eqns. (1), (3), and (13) enables k_{maa} and $K_{1,\text{maa}}$ to be calculated from the plots of A and B vs. 1/[H⁺]. The values accepted are $K_{1,\text{maa}} = (7.45 \pm 0.11) \times 10^{-3}$ and $k_{\text{maa}} = 69.3 \pm 0.1$ s⁻¹. Although in large excess, maa did not remove all quinone. This implies that the formation of product (5-maa-DA) is in fact reversible and therefore the rate constant obtained is a combination of the forward and backward reactions (k_{maa}^+ and k_{maa}^-). The complicated nature of the overall reaction means that it is not possible to measure an equilibrium constant for this reaction and therefore not possible to separate k_{maa}^+ and k_{maa}^- .

The plot of *C vs.* $1/[H^+]^2$ is linear with a slope of $(5.85 \pm 0.11) \times 10^{-6}$, and with an intercept of $(5.02 \pm 0.08) \times 10^4$. This implies that the bis-complex exists in two forms described by eqn. (14):

$$\operatorname{maa}_{2}$$
-DQ + 2H⁺ $\rightleftharpoons \frac{\beta_{2}^{H}}{\swarrow}$ maa_{2} -H₂DQ (14)

We suggest that the protonation is of the two $-O^-$ functions on the ring (see Scheme 1). This means that the last term in the denominator of the theoretical rate equation, eqn. (13), becomes eqn. (15):

$$K_{1,\text{maa}}K_{2,\text{maa}}[\text{maa}]_{\text{T}}^{2} (1 + \beta_{2}^{\text{H}}[\text{H}^{+}]^{2})/[\text{H}^{+}]^{2}$$
(15)

Thus the plot of *C* vs. $1/[\text{H}^+]^2$ yields the following: the slope = $K_{1,\text{maa}}K_{2,\text{maa}} = 5.85 \times 10^{-6}$ which makes $K_{2,\text{maa}} = (7.85 \pm 0.11) \times 10^{-4}$. The intercept = $K_{1,\text{maa}}K_{2,\text{maa}}\beta_2^{\text{H}} = (5.02 \pm 0.08) \times 10^4$ which gives $\beta_2^{\text{H}} = 8.58 \times 10^9$ (or $\log \beta_2^{\text{H}} = 9.94$).

Conclusions

There are relatively few mechanistic studies of the addition of sulfur nucleophiles to quinones and until now none has provided clear evidence for the existence of a tetrahedral intermediate (Scheme 1), similar to those found in other addition– elimination reactions. The existence of such an intermediate is kinetically important and has mechanistic ramifications.

Firstly, production of the product is a first-order process and not, as is often assumed, a second-order step. This fundamental difference may sometimes be over-looked when the range of concentrations used is too small. This has been the case in previous studies of additions to quinones^{13,18,19} and is because at lower cys concentrations the curvature of plots of $[cys]_T vs. k_{cys}^{obs}$ (*e.g.* Fig. 2) is less pronounced and can be assumed to be linear and thus obeying simple second-order kinetics. Indeed, we only became aware of this problem when investigating the reaction of maa with DQ when the deviation from second-order kinetics is very pronounced (see Fig. 4).

Secondly, intermediate formation allows us to explain the difference in behaviour between cysteine and mercaptoacetic acid. The stability constant for the formation of maa-DQ is almost an order of magnitude greater than that for cys-DQ $(7.45 \times 10^{-3} \text{ in contrast to } 1.09 \times 10^{-3})$. This could be ascribed to steric effects, but the great difference in the rate constant for the decomposition step (*ca*. 70 s^{-1} for the maa intermediate and 1830 s⁻¹ for the corresponding cys complex) strongly supports the suggestion that this is due to the positive charge on the amino group in cysteine, possibly by stabilizing build-up of negative charge on the carbonyl oxygen of C4 (see Scheme 1). Furthermore, the relative weakness of the first association equilibrium for cysteine with dopaminoquinone coupled with its high rate of reaction is sufficient to explain why a second association step is not observed-at least with the cysteine concentrations employed in this study.

The detailed mechanistic information provided in this paper is of great value when discussing the relative importance of possible pathways in the formation of pheomelanin and neuromelanin, and especially when abnormal conditions prevail as is the case in neurodegenerative diseases such as Parkinson's disease. Indeed, this provided the impulse to undertake this study. Our previous work on catecholamine oxidations 14,20-22 has shown that the initial product, a quinone, undergoes intramolecular Michael addition of the amine side-chain at C6 to form leucodopaminochrome. Further oxidations and condensations lead finally to melanin. Neuromelanin is found to contain 5-cysteinyl conjugates and levels of 5-cys-DA are more pronounced during the development of Parkinson's disease.⁷ Addition of cysteine is therefore in competition with the ring cyclized product. With this study we are now in a position to compare the relative importance of these two reactions. The dependence of the observed first-order rate of decay of dopaminoquinone to leuchodopaminochrome on pH is given in El-Ayaan et al.¹⁴ At physiological pH (\approx 7) k_{cyc}^{obs} is calculated to be 0.37 s⁻¹. Should cysteine be present we can use eqn. (8) to calculate k_{cys}^{obs} for the same pH and any required cysteine concentration. It is believed that cysteine concentrations in the brain are in the order of 31–95 nmol g^{-1} ²³ or approximately 50 μ M. This produces a value for k_{cys}^{obs} of 644 s⁻¹.

This is an interesting result in that it suggests that the presence of cysteine would completely inhibit ring cyclization. The fact that neuromelanin contains lower amounts of 5-cysteinyl conjugates suggests two possibilities. (i) The quinone concentration is higher than that of cysteine but this in turn implies that oxidation of dopamine is relatively high. (ii) Oxidation of dopamine occurs in a region where cysteine is largely excluded as suggested by Carstam et al.4

It is possible, however, that 5-cys-DA is formed by the addition of glutathione followed by cleavage of the initial product (5-glutathionyl-dopamine) by γ -glutamyl transpeptidase and peptidase. Credence is given to this mechanism by the report of elevated activity⁹ of this enzyme during Parkinson's disease. Preliminary experiments in our laboratory suggest that, although complicated, glutathione reacts with dopaminoquinone in an analogous fashion to cysteine but slower. Glutathione is present in much higher concentrations than cysteine and therefore k_{glu}^{obs} will again be several orders of magnitude higher than the corresponding rate constant for ring cyclization. These results suggest that (ii) above is the more likely explanation for the 5-cys-DA concentrations observed in vivo but much more work, outwith the scope of the present purely chemical study, needs to be done.

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