

## Oxidative calcium release from catechol



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### ABSTRACT

Oxidation of 4-methylcatechol previously exposed to aqueous calcium chloride was shown by ion chromatography to be associated with release of calcium ions. The catechol was oxidised to the corresponding orthoquinone by the use of tyrosinase from *Agaricus bisporus*. The oxidative release of calcium from the catechol is ascribed to the diminution of the available hydroxyl functions able to act as chelating groups. Our results suggest that the redox status of melanin may regulate calcium binding and influence calcium levels in pigmented cells.

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In addition to the remarkable light absorption of the pigment, an important property of melanin is its ability to act as a powerful cation chelator.<sup>1–5</sup> Although the detailed structure of melanins is not known, eumelanin is regarded as a heteropolymer incorporating many indole-2-carboxylic acid residues.<sup>6</sup> Under conditions in which the carboxylic acid groups are ionised the pigment is able to act as a cation exchanger. In addition, the catecholic residues present in melanin are able to bind cations. Divalent cations, such as calcium, are known to form chelation complexes with catechols<sup>7</sup> and melanin has been shown to have a role in calcium homeostasis.<sup>8,9</sup> Since the proportion of catechols in melanin is subject to variation depending on the redox status of the pigment, we investigated whether oxidation of a catechol would result in calcium release.

Experiments were made with 4-methylcatechol exposed to calcium chloride and oxidized with mushroom tyrosinase as detailed below.<sup>10</sup> Separate determination of the oxidation efficiency was made using combined oximetry and spectrophotometry as previously described.<sup>11</sup> The extent of oxidation of 4-methylcatechol in the presence or absence of additions was measured by the total oxygen utilization or, in the case of the calcium incubations, by absorbance at 400 nm to take account of the tyrosinase inactivation reaction in the presence of catechols.<sup>12</sup> The rate of catechol oxidation was estimated from the rate of formation of the corresponding orthoquinone which was stable over the period of the investigations.

In initial experiments we showed that oxidation of the catechol by tyrosinase was not inhibited by calcium or by 20% methanol (MeOH) as shown in Table 1. The oxygen stoichiometry was consistent with the formation of the 4-methylquinone according to the formula given in the graphical abstract as judged by the quinone absorbance at 400 nm using the molar absorption coefficient of 1600.<sup>13</sup> The results of the calcium release experiment using 50 units of tyrosinase are shown in Table 2 and demonstrate that exposure to tyrosinase increases the amount of calcium released.

**Table 1**

Total oxygen utilization, estimated orthoquinone production (nmoles) and rate of generation of quinone (based on absorbance at 400 nm per min) from 4-methylcatechol (100  $\mu$ M) by tyrosinase in the presence of additions

Addition	O <sub>2</sub> utilisation (nmoles)	Quinone formation (nmoles)	Oxidation rate (400 nm) $\pm$ SD
Control	175	386	1.51 $\pm$ 0.18
MeOH 20%	189	392	1.48 $\pm$ 0.12
Calcium	217	375	1.92 $\pm$ 0.08
Ca + MeOH	168	392	1.64 $\pm$ 0.03

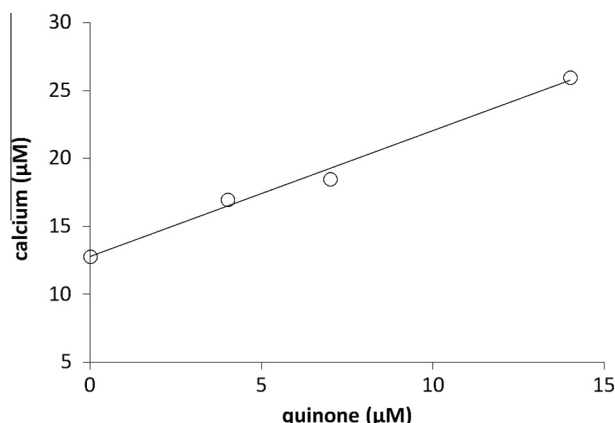
**Table 2**

Calcium release data

Sample	Addition	Calcium ( $\mu$ M) $\pm$ SEM
Control 1	H <sub>2</sub> O	0.0
Control 2	Tyrosinase	2.0
Catechol/Ca	H <sub>2</sub> O	5.9 $\pm$ 0.5
Catechol/Ca	Tyrosinase	29.6 $\pm$ 2.6

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**Figure 1.** Calcium release as function of the amount of quinone formed by oxidation by varying amounts of tyrosinase.

Exposure to water alone gave a value of 5.9 μM calcium whereas in the presence of tyrosinase the catechol yielded 29.6 μM ( $p < 0.03$ ). Allowing for the calcium associated with the enzyme solution (about 2 μM) the difference in the amount of calcium released by tyrosinase is over fourfold that released in its absence. We ascribe this result to the tyrosinase-catalysed oxidation of the catechol initially present. The effect of the conversion of the catechol to the corresponding orthoquinone is to diminish the available cation-binding sites and thus to facilitate the release of calcium (see Graphical abstract). The data from the second series of experiments using differing amounts of tyrosinase showed a linear increase in the calcium detected as a function of the amount of orthoquinone formed in the samples analysed (Fig. 1,  $R^2 = 0.98$ ).

The demonstration of calcium release resulting from catechol oxidation may indicate an additional mechanism by which melanin photoprotection may be exerted. One of the consequences of photon absorption by melanin is photo-oxidation of the pigment with an increase in the proportion of orthoquinone residues and a concomitant increase in conjugation in the polymer with delocalisation of  $\pi$ -electrons accounting for bathochromicity.<sup>14</sup> Our data suggest that an additional effect of photo-oxidation is to release bound calcium ions. Of interest in connection with this proposal are the results reported by Stauffer and Weber<sup>15</sup> showing the dissociation of a stable zinc-catechol complex upon illumination with visible light.

Since calcium is an important co-factor regulating several crucial cellular processes this influence on intracellular calcium homeostasis could be of importance. For example, intracellular calcium plays a key role in apoptosis by regulating the exodus of cytochrome c from mitochondria,<sup>16</sup> hence calcium sequestration by melanosomes may play a crucial role in cytoprotection. As argued elsewhere,<sup>6</sup> there is indirect evidence of such an action in the case of the effect of inner ear melanin on acoustic injury. The pathogenesis of acoustic damage involves L-type calcium channels<sup>17</sup> and it has been suggested that melanin may regulate calcium levels in the endolymph.<sup>18,19</sup> The susceptibility of albino mice to acoustic injury is abrogated by generation of dihydroxyphenylalanine in ear melanocytes.<sup>20</sup> A similar example of the putative protective action of catechol is ectopic expression of tyrosine hydroxylase in the pigment epithelium which rescues retinal abnormalities and visual function in albino mice.<sup>21</sup> A corollary of this argument is that if calcium binding by catecholic functions

in melanin is cytoprotective, the absorption of sufficient radiant energy to cause significant photo-oxidation of melanin could result in large-scale release of calcium from melanosomes. The consequent substantial rise in intracellular calcium levels could initiate apoptosis in the irradiated cells. The biological significance of such a sequence of events could be to eliminate those cells that had been exposed to radiation doses sufficient to cause deleterious mutations; a process of genoprotection.<sup>22</sup> Increased light-induced cellular damage due to melanin content has been reported.<sup>23,24</sup> Studies are in progress to examine the proposal that this is brought about by calcium release.

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## References and notes

- White, L. P. *Nature* **1958**, *158*, 1427.
- Potts, A. M.; Au, P. C. *Exp. Eye Res.* **1976**, *22*, 487.
- Horcicko, J.; Borovansky, J.; Duchon, J.; Prochtzkova, B. *Hoppe-Seyler's Z. Physiol. Chem.* **1973**, *354*, 203.
- Larsson, B.; Tjalve, H. *Acta Physiol. Scand.* **1978**, *104*, 479.
- Hong, I.; Liu, Y.; Simon, J. D. *Photochem. Photobiol.* **2007**, *80*, 477.
- Borovansky, J.; Riley, P. A. In *Melanins and Melanosomes*; Borovansky, J., Riley, P. A., Eds.; Wiley-VCH GmbH: Weinheim, 2011; p 343.
- Pierpoint, C. G. *Coord. Chem. Rev.* **1983**, *38*, 45.
- Hoogduijn, M. J.; Smit, N. P.; Van Der Laarse, A.; Van Nieupoort, A. F.; Wood, J. M.; Thody, A. J. *Pig. Cell Res.* **2003**, *16*, 127.
- Bush, W. D.; Simon, J. D. *Pig. Cell Res.* **2007**, *20*, 134.
- 4-Methylcatechol and tyrosinase (ex *Agaricus bisporus*) were obtained from Sigma-Aldrich. Tyrosinase was made up as a stock solution of 500 units/ml in distilled water. The calcium release experiments used 4-methylcatechol at a concentration of  $5 \times 10^{-2}$  M dissolved in a solution of calcium chloride (1 mM). Separations were made using Supelco DSC-18 solid phase extraction columns after wetting with 1.0 ml methanol, followed by a 1.0 ml H<sub>2</sub>O wash. Samples (1.0 ml) of the test solution were applied to a DSC-18 column, and the retained material washed with 1.0 ml H<sub>2</sub>O to remove unbound calcium. Samples of the catechol-calcium complex were then eluted with 1.0 ml 20% methanol. The eluate was divided into aliquots to which were added either distilled water (control) or tyrosinase solution. A control of tyrosinase alone was also examined. Aliquots were incubated at room temperature for ten minutes. The methanol was then removed by rotary evaporation and the samples applied to new (wetted and washed) DSC-18 filters and the aqueous eluates examined for released calcium; the expectation being that calcium bound to residual catechol would be retained whilst unbound calcium was eluted. The determination of the calcium levels present in the samples was made by ion chromatography using a Dionex IonPac CS16 5 μm column (250 × 5 mm). Elution was at a rate of 1.0 ml/min with methane sulphonic acid (40 mM) using Dionex ED-40 detection with autosuppression.
- Cooksey, C. J.; Garratt, P. J.; Land, E. J.; Pavel, S.; Ramsden, C. A.; Riley, P. A.; Smit, N. P. *M. J. Biol. Chem.* **1997**, *272*, 26226.
- Land, E. J.; Ramsden, C. A.; Riley, P. A. *Tohoku J. Exp. Med.* **2007**, *212*, 341.
- Cooksey, C. J.; Land, E. J.; Ramsden, C. A.; Riley, P. A. *Anti-cancer Drug Design* **1995**, *10*, 119.
- Chedekel, M. R. In *Melanin: Its Role in Human Photoprotection*; Zeise, L., Chedekel, M. R., Fitzpatrick, T. B., Eds.; Valdenmar: Overland Park, 1995; p 11.
- Stauffer, M. T.; Weber, S. G. *Anal. Chem.* **1999**, *71*, 1146.
- Mattson, M. P.; Chan, S. L. *Nat. Cell Biol.* **2003**, *5*, 1041.
- Uemaetomari, I.; Tabuchi, K.; Nakamagoe, M.; Tanaka, S.; Murashita, H.; Hara, A. *Tohoku J. Exp. Med.* **2009**, *218*, 41.
- Meyer zur Göttesberge, A. *Pig. Cell Res.* **1988**, *1*, 238.
- Gill, S. S.; Sait, A. N. *Hearing Res.* **1997**, *113*, 191.
- Lavado, A.; Jeffery, G.; Tovar, V.; De la Villa, P.; Montoliu, L. *J. Neurochem.* **2006**, *96*, 1201.
- Murillo-Cuesta, S.; Contreras, J.; Zurita, E.; Cediell, R.; Cantero, M.; Varela-Nieto, I.; Montoliu, L. *Pig. Cell Melanoma Res.* **2009**, *23*, 72.
- Riley, P. A. In *The Physiology and Pathophysiology of the Skin*; Jarrett, A., Ed.; Academic Press: London and New York, 1974; p 1101.
- Johnson, B. E.; Mandel, G.; Daniels, F., Jr. *Nature* **1972**, *235*, 147.
- MacDonald, C. J.; Snell, R. S.; Lerner, A. B. *J. Invest. Derm.* **1965**, *45*, 110.