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Note

Effects of Tetrahydropterines on the Generation of Quinones Catalyzed by Tyrosinase

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Tetrahydrobiopterine ($6BH_4$) can diminish the oxidative stress undergone by keratinocytes and melanocytes by reducing the *o*-quinones generated by the oxidation of the corresponding *o*-diphenols. We found that $6BH_4$ and their analogs reduced all the *o*-quinones studied. The formal potentials of different quinone/diphenol pairs indicate that the *o*-quinones with withdrawing groups are more potent oxidants than those with donating groups.

Key words: tetrahydropterines; quinones; *o*-diphenols; voltammograms; tyrosinase

The oxidation of diphenols to quinones in keratinocytes and melanocytes involves an increase in oxidative stress in these cells, since H_2O_2 is generated at the same time.[1,2] However, topical applications of formulations containing high concentrations (mM) of coenzyme Q_{10}-quinol, hydroquinone (HQ), or estrogens can contribute to increasing oxidative stress in the human epidermis.

A reduction in quinones can contribute to decreasing oxidative stress. A possible mechanism for *o*-quinone reduction is the thioredoxin reductase/thioredoxin (TR/T) system.[3] In human keratinocytes and melanocytes, the presence of the TR isoform has been demonstrated,[4] while $6BH_4$ can reduce *o*-quinone L-dopaquinone back to L-dopa.[5]

It has been found that keratinocytes and melanocytes have a capacity for *de novo* synthesis, recycling, and regulation of 6-tetrahydrobiopterin ($6BH_4$). TR reduces *p*-quinone 1,4 benzoquinone and coenzyme Q10-quinone back to HQ and coenzyme Q10-quinol.[6] On the other hand, $6BH_4$, can reduce coenzyme Q10-quinone back to coenzyme Q10-quinol, while 1,4 benzoquinone is not reduced to HQ. However, there is controversy concerning the reduction of *o*-quinones by BH_4, and it has been proposed that BH_4 does not reduce 1,4 benzoquinone or 1,2 benzoquinone. It has been suggested that the presence of electron-donating subtituents in the quinone structure is needed before reduction by $6BH_4$ can occur.[6]

The objective of this work was to demonstrate that quinones are reduced by $6BH_4$ and their analogs MBH_4 (6-methyltetrahydropterin) and $DMBH_4$ (6,7-dimethyltetrahydropterin). All the *o*-quinones were assayed, including 1,2-benzoquinone, oxidise BH_4, and its analogs MBH_4 and $DMBH_4$ (Scheme 1). In addition we studied the influence of withdrawing and donating groups in C-1 on the redox potential of the *o*-quinones.

First, the formal potentials of the different compounds under study were determined by square wave voltammograms. From these results, it can be seen that the oxidation/reduction potential was greater for compounds (*o*-quinones) that contained a withdrawing group in C-1 (see below). The potential of *p*-benzoquinone was near that of BH_4 and its derivatives, which means that they are not oxidised by the quinone.

Of the *o*-diphenols studied, catechol, L-dopa, and 4-*tert*-butylcatechol (TBC) were easily oxidised by tyrosinase, generating the corresponding *o*-quinones. In this study, we oxidized them with tyrosinase or with a deficiency of periodate. The results obtained with catechol are shown in Figs. 1 and 2.

1,2-Benzoquinone was generated enzymatically by the action of tyrosinase on catechol. In the presence of $6BH_4$, a lag period in the accumulation of 1,2-benzoquinone was observed (Fig. 1). Similar results were obtained in the case of MBH_4 (Fig. 1, inset A) and $DMBH_4$ (Fig. 1, inset B). Note that in all cases the *o*-quinone generated by the enzyme oxidized to tetrahydropterines and the system returned to the initial steady state (in the absence of a reductant). Compounds such as L-dopa and TBC, which are easily oxidized by tyrosinase, showed the same behavior (results not shown).

Another experimental focus involved the generation of *o*-quinone from catechol by oxidation in a deficiency of $NaIO_4$. Successive addition of tetrahydropterines led to a reduction in *o*-quinone (Fig. 2 and Fig. 2, insets A and B). However, when the quinone was 1,4 benzoquinone, the addition of tetrahydropterin caused no reduction (results not shown).

With respect to the influence of withdrawing or donating groups in the *para* position on the *o*-quinone redox potential, the oxidation capacity increased in the presence of withdrawing groups but not of donating groups, as described in ref. 6. All the above was demonstrated by generating *o*-quinones of TBC (which has a donating group), of 4-nitrocatechol (which has a

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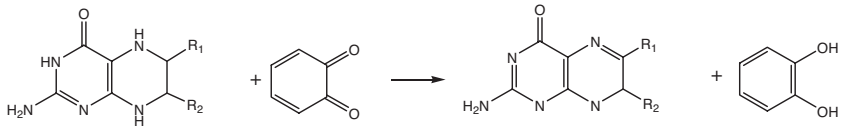

Scheme 1. Oxidation/Reduction Reaction of Tetrahydropterines by 1,2-Benzoquinone to Dihydropterines and Catechol.
The tetrahydropterines used were 5,6,7,8-tetrahydrobiopterine ($R_1 = CH_2OH–CH_2OH–CH_3$, $R_2 = H$), 6-methyl-5,6,7,8-tetrahydropterin ($R_1 = CH_3$, $R_2 = H$), and 6,7-dimethyl-5,6,7,8-tetrahydropterin ($R_1 = CH_3$, $R_2 = CH_3$).

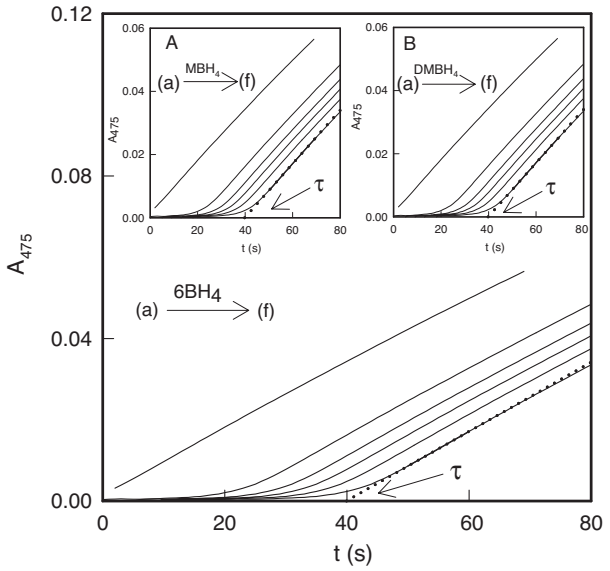

Fig. 1. Catechol Oxidation by Tyrosinase in the Presence of Tetrahydropterines.
Spectrophotometric recording at $\lambda = 475$ nm of the oxidation of 0.5 mM catechol by 1 nM tyrosinase in the presence of $6BH_4$ (μM): (a) 0, (b) 100, (c) 125, (d) 150, (e) 175, and (f) 200. The buffer used was 30 mM sodium phosphate (pH 7.0), 25 °C. Inset A, the same conditions as in Fig. 1, but in the presence of MBH_4 (μM); (a) 0, (b) 100, (c) 125, (d) 150, (e) 175, and (f) 200. Inset B, the same conditions as in Fig. 1, but in the presence of $DMBH_4$ (μM); (a) 0, (b) 100, (c) 125, (d) 150, (e) 175, and (f) 200.

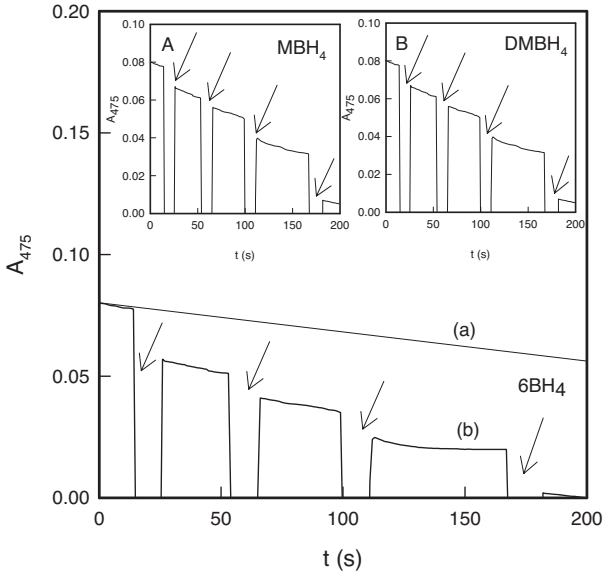

Fig. 2. Reduction of 1,2-Benzoquinone by Tetrahydropterines at $\lambda = 475$ nm.
Catechol (1 mM) was oxidized in a deficiency of sodium periodate (0.4 mM), and the evolution of *o*-quinone was followed spectrophotometrically, recording (a). At the times indicated by arrows, $6BH_4$ (100 μM) was added, recording (b). The buffer used was 30 mM sodium phosphate (pH 7.0), 25 °C. Inset A, exactly the same as in Fig. 2, but with the addition of MBH_4 (100 μM). Inset B, exactly the same as in Fig. 2, but with the addition of $DMBH_4$ (100 μM).

withdrawing group), and of 3,4-dihydroxybenzaldehyde (which has a withdrawing group) by oxidation in a deficiency of $NaIO_4$, and in these cases, the tetrahydropterines were also oxidized by these *o*-quinones (results not shown). Taking into account the redox potentials of the tetrahydropterine oxidized/reduced pairs (−0.099, −0.105, and −0.110 for BH_4, $DMBH_4$ and MBH_4 repectively) and those of the *o*-quinone/*o*-diphenol pairs (0.192, 0.163, 0.156, 0.152, 0.114, and 0.036 for 4-nitrocatechol, 3,4-dihydroxybenzaldehyde, catechol, L-dopa, 4-*tert*-butylcatechol, and *p*-benzoquinone respectively), it was deduced that tetrahydropterines are in general oxidized by *o*-quinones. Moreover, compounds with electron-withdrawing groups facilitated oxidation.

Acknowledgments

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