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ANTIOXIDANT ACTIVITY OF 5-HYDROXYTRYPTOPHAN, 5-HYDROXYINDOLE, AND DOPA AGAINST MICROSOMAL LIPID PEROXIDATION AND ITS DEPENDENCE ON VITAMIN E

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The antioxidant capacity of 5-hydroxy-tryptophan, 5-hydroxy-indole, and DOPA (3,4-dihydroxy-phenyl-alanine) was tested in the Fe-induced lipid peroxidation of liver microsomes of normal- and vitamin E-deficient rats, using ascorbate as a reductant. Lipid peroxidation was monitored as low-level chemiluminescence, indicative of generation of electronically-excited states arising from the recombination of secondary lipid peroxyl radicals.

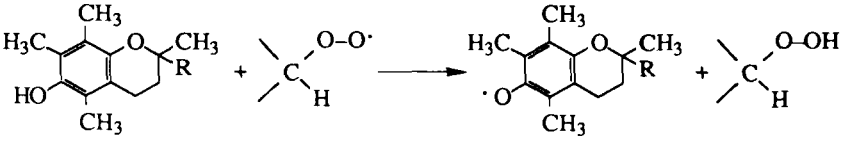
The lag phase (τ_0) preceding the rise in chemiluminescence intensity was used as indicator of antioxidant efficiency, in the absence (τ_0) and the presence (τ) of these polar compounds. The increase in duration of the lag phase exerted by these hydroxy compounds was expressed and quantified as the relationship: τ-τ_0. The τ-τ_0 values were considerably higher in the presence of vitamin E and almost negligible in the absence of tocopherol.

It is postulated that the observed increased protection against lipid peroxidation by the above 5-hydroxy derivatives is displayed in a fashion dependent on the presence of vitamin E and probably involving recovery of the chromanoxyl radical by means of an electron-transfer process

KEY WORDS: lipid peroxidation, vitamin E, peroxyl radicals, antioxidants, low-level chemiluminescence, 5-hydroxytryptophan, 5-hydroxyindole, DOPA or 3,4-dihydroxy-phenylalanine.

INTRODUCTION

Vitamin E (mainly as α-tocopherol) is the major antioxidant whose role is to protect the integrity of biological membranes by inhibiting peroxidation reactions of polyunsaturated fatty acids.[1] The inhibition in the membrane occurs *via* a reaction of vitamin E with peroxyl radicals of fatty acids,[2] generating the corresponding chromanoxyl radical.[3] The reaction can proceed either by an electron- or by a H-atom transfer mechanism (reaction *1*).



[1]

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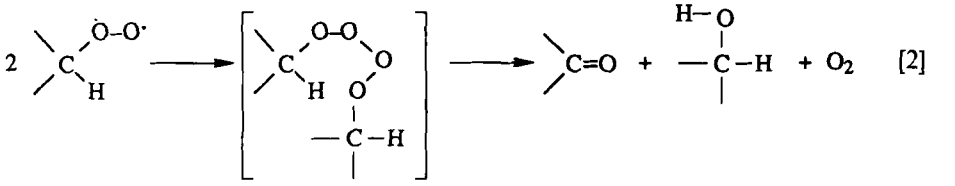
The interaction of the vitamin E radical in the membrane with more polar physiological constituents present in the saqueous phase has been subject of numerous studies. The recovery of the vitamin E radical by ascorbate (vitamin C) has been studied (see review ref.[4]) in homogeneous aqueous model systems[5,6a] and in liver microsomal fractions.[7,8]

It has become clear that physiological compounds, such as urate, bilirubin, carnosine, and anserine, might be of interest as biological antioxidants.[9-11]. The present study was carried out to examine a potential antioxidant activity of some water-soluble hydroxy-aromatic compounds. We investigated the antioxidant activity of 5-hydroxy-tryptophan, 5-hydroxy-indole, and DOPA in a system previously employed with other antioxidants, that is, measuring the low-level chemiluminescence originating from the recombination of lipid peroxyl radical formed during Fe^{2+}-induced lipid peroxidation of rat liver microsomal fractions.[7,12,13] The recombination of secondary lipid peroxyl radicals yields a tetroxide intermediate, which decomposes yielding electronically-excited states, singlet molecular oxygen ($^1O_2$) and/or triplet carbonyl compounds ($^3[>C{=}O]^*$) (as illustrated in reaction *2*). Low-level chemiluminescence originates from the decay of the above excited species to the ground state.



[2]

Thus, the present experimental model consists of [a] a peroxyl radical-generating system ultimately reflected as formation of electronically-excited states detected by low-level chemiluminescence (reaction *2*), [b] a chain-breaking antioxidant, α-tocopherol, present in microsomal membranes, and [c] potential antioxidants, hydroxy-aromatic compounds, such as 5-hydroxy-tryptophan, 5-hydroxy-indole, and DOPA which may be involved in the recovery of chromanoxyl radicals formed as in reaction *1*.

MATERIALS AND METHODS

Chemicals and biochemicals

Chemicals were from Merck (Darmstadt, FRG) and biochemicals from Boehringer (Mannheim, FRG) and Sigma Chemical Co. (Munich, FRG).

Biological material

Microsomes were obtained from livers of control- and vitamin E-deficient rats as previously described.[12,14] Vitamin E deficiency was achieved by feeding the rats a vitamin E-deficient diet (Altromin GmbH, Lage, FRG) for 14–16weeks. The vitamin E content from control- and vitamin E-deficient microsomes was 0.38 ± 0.08- and 0.07 ± 0.03 nmol × mg protein^{-1}, respectively, measured by a fluorimetric method.[15]

Assay conditions

The standard reaction mixture consisted of 1 mg microsomal protein × ml^{-1} (from either control- or vitamin E-deficient microsomes) in 0.1 M potassium phosphate buffer, pH 7.4, containing 2-mM ADP and 16-μM $FeSO_4$. The reaction was started upon addition of 0.5 mM ascorbate. The reaction mixture was exposed to 100% pure O_2 during the measuring period.

Chemiluminescence measurements

Low-level chemiluminescence was measured by photon counting as described previously[13] with a red-sensitive EMI 9658AM photomultiplier (EMI-Gencom, Plainview, NY, USA). Chemiluminescence assays were carried out in a glass cuvette (35- × 56- × 5 mm) provided with a lid with ports for gassing and additions. The reaction mixture was maintained at 37°C and constant stirring was applied during the measuring period. Additions were made from outside of a light-tight box through thin polyethylene tubing.

RESULTS AND DISCUSSION

Lipid peroxidation-supported low-level chemiluminescence

The experimental model utilizes the Fe-catalyzed autooxidation of microsomal lipids using ascorbate as electron donor. In the course of chain-propagation reactions within lipid peroxidation, secondary lipid peroxyl radicals are formed, whose recombination yields electronically-excited states, singlet molecular oxygen ($^1O_2$) and/or excited triplet carbonyl compounds, as illustrated in reaction *2*.

The typical time course of photoemission associated with lipid peroxidation shows three phases (Figure 1): [a] a lag phase (τ_0), [b] a rise in chemiluminescence intensity, in general of a sigmoidal shape, and [c] a plateau, indicating a steady-state formation of electronically-excited states. The duration of the lag phase (τ_0) is directly proportional to the content of vitamin E[12] and it ranges from 8 to 12 min in microsomes from untreated rats. The duration of the lag phase in microsomes from vitamin E-deficient rats is considerably shorter than that of microsomes from control rats.[8,12]

Effect of physiological compounds as antioxidants

With the above experimental model, several antioxidants have been shown to exert a delay of the lag phase preceding the rise in chemiluminescence intensity, probably by inhibition of free radical reactions.[7,8,12,16]

5-Hydroxy-tryptophan exerted a prolongation of the lag phase of chemiluminescence associated with lipid peroxidation (Fig. 1A). This effect can be quantified with the expression τ-τ_0, in which τ and τ_0 represent the duration of the lag phase in the presence and absence of 5-hydroxy-tryptophan, respectively. The τ-τ_0 value observed with microsomes from control rats was 47 min and half-maximal effect was obtained with 410 μM 5-hydroxy-tryptophan. Conversely, the τ-τ_0 value observed upon supplementation of 5-hydroxy-tryptophan to microsomes from vitamin E-deficient rats was dramatically shorter: 3 min. This indicates that the antioxidant activity displayed by 5-hydroxy-tryptophan — as increasing the τ-τ_0 value — is dependent on the occur-

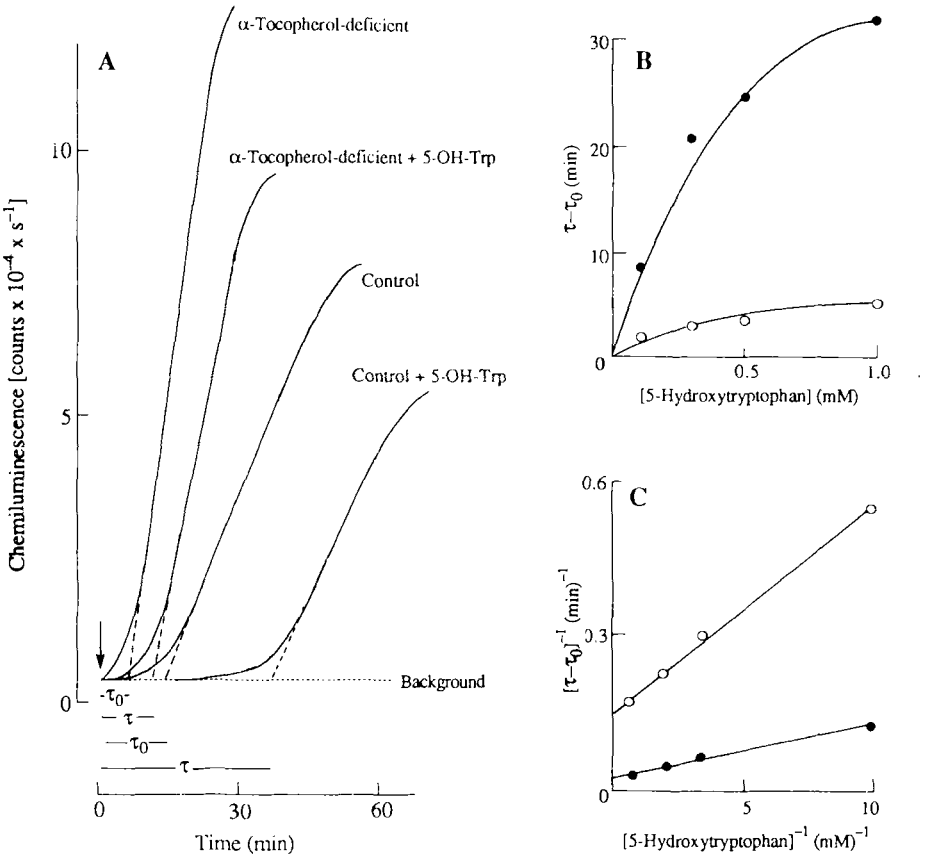


FIGURE 1 Effect of 5-hydroxytryptophan on the lag phase of lipid peroxidation as detected by low-level chemiluminescence

(A) Time course of low-level chemiluminescence associated with lipid peroxidation showing the lag phase in the absence (τ_0) and presence (τ) of 5-hydroxy-tryptophan (0.5 mM) for control- and vitamin E-deficient microsomes. (B) Dependence of duration of the lag phase (τ-τ_0) on 5-hydroxytryptophan concentration: (●), control microsomes; (○), vitamin E-deficient microsomes. (C) Double-reciprocal plot from data from (B). Assay conditions as detailed in the Materials and methods section. 5-OH-Trp, 5-hydroxy-tryptophan.

rence of vitamin E in the microsomal membrane. The dependence of the antioxidant effect, in terms of τ-τ_0 values, on 5-hydroxy-tryptophan concentration in control- and vitamin E-deficient microsomes is shown in Fig. 1B and the corresponding double reciprocal plot in Fig. 1C. The τ-τ_0 values were obtained by extrapolation of the double reciprocal plots: $[\tau\text{-}\tau_0]^{-1}$ versus $[5\text{-hydroxy-tryptophan}]^{-1}$.

5-Hydroxy-indole, also a good electron donor,[17] is effective as an antioxidant: the maximal $\tau - \tau_0$ value observed with control microsomes was 112 min, whereas it was shortened to 2 min when the hydroxy-aromatic compound was supplemented to vitamin E-deficient microsomes. The half-maximal effect with 5-hydroxy-indole was achieved in the range 10–30 μM (not shown).

Likewise, DOPA (3,4-dihydroxyphenylalanine) was observed to produce an effect

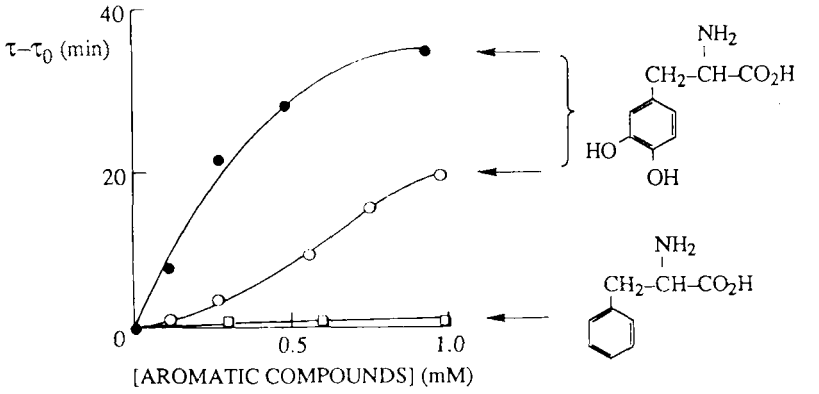


FIGURE 2 Effect of DOPA (3,4-dihydroxy-phenylalanine) and phenylalanine on the lag phase preceding the rise of low-level chemiluminescence associated with lipid peroxidation

Assay conditions as in Fig. 1, with varying amounts of DOPA or phenylalanine. Assays with DOPA were carried out with control (●) or vitamin E-deficient (○) microsomes; phenylalanine (□) did not produce any changes in the τ-τ_0 values when supplemented to either control- or Vitamin E-deficient microsomes.

similar to 5-hydroxy-tryptophan with a half-maximal concentration of 390 μM: maximal $\tau - \tau_0$ values were 56- and 6.9 min, for control- and vitamin E-deficient microsomes, respectively, obtained from the double-reciprocal plot of data in Figure 2. Phenylalanine, lacking a $-$OH substituent, exerted no effect on the $\tau - \tau_0$ values of either control- or vitamin E-deficient microsomes.

The antioxidant effect exerted by 5-hydroxy-tryptophan, 5-hydroxy-indole, and DOPA seems to depend on the presence of the $-$OH group in the benzene ring, for the parent compounds, that is, tryptophan and phenylalanine, lacking the $-$OH group, were devoid of a protective effect (Table 1).

CONCLUSIONS

By applying the low-level chemiluminescence technique to monitor lipid peroxidation, the presence and duration of a lag phase (τ) preceding the rise of photoemission can be accurately measured. The dependence of this lag phase on vitamin E content of microsomal membranes[12] and the use of control- and vitamin E-deficient microsomes

TABLE I
Compounds effective in prolonging the lag phase (τ-τ_0 values) preceding microsomal lipid peroxidation as detected by low-level chemiluminescence

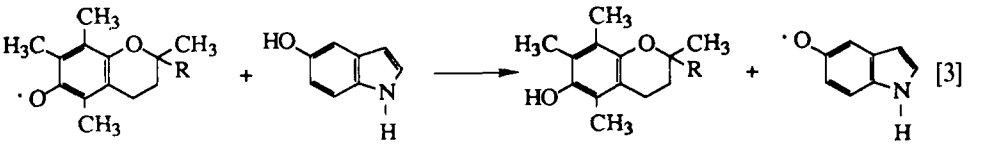
COMPOUNDS			
Active antioxidants	$\tau - \tau_0$ (min)		*Non active analogues*
	Control	Vit. E-deficient	
5-Hydroxy-tryptophan	47	3.0	Tryptophan
DOPA	56	6.9	Phenylalanine
5-Hydroxy-indole	112	2.0	

has permitted to distinguish the antioxidant effect exerted by several substances in terms of either a direct free radical scavenging activity or a vitamin E-mediated activity.

5-Hydroxy-tryptophan, 5-hydroxy-indole, and DOPA were shown to exert their antioxidant effect in a vitamin E-dependent fashion. Their full effectiveness in the protection against membrane peroxidation appears to be associated, at least in part, with vitamin E, in spite of the similarities in antioxidant properties of 5-hydroxy-tryptophan and DOPA with α-tocopherol.

5-Hydroxy-tryptophan, 5-hydroxy-indole, and DOPA exert substantial antioxidant activity in both normal (control)- and vitamin E-deficient microsomes (Figures 1 and 2). From the lag phases in vitamin E-deficient microsomes it may be inferred that the antioxidant activity of these physiological constituents cannot fully match that of vitamin E. Hence, the effectiveness of the protection of a membrane depends primarily on the presence of vitamin E, similar to what was concluded for GSH or ascorbate.[8] In microsomes containing full amount of vitamin E, the tested antioxidants showed protection capacity beyond that of the tocopherol.

The higher antioxidant capacity of the examined antioxidants in the presence of vitamin E indicates a possible role of these neurotransmitters in the repair of the chromanoxyl radical of vitamin E, as hypothesized in reaction *3*. The likelihood of this reaction is supported by kinetic- and reduction potential measurements.[18] The decay of the hydroxy derivative radical does not seem to include electron transfer to O_2, but rather electron-transfer reaction at expense of a reducing agent, such as ascorbic acid.



[3]

Because lipid peroxidation in this experimental model is monitored as low-level chemiluminescence, the possibility might be considered that α-tocopherol could react with the formed electronically-excited states arising from the recombination of secondary lipid peroxyl radicals, i.e. $^1O_2$ and triplet carbonyl compounds (reaction *2*). The reaction of $^1O_2$ with α-tocopherol proceeds at considerably high rates ($k = 6.7 \times 10^8 M^{-1}s^{-1}$) and involves oxidation of the phenol and quenching of $^1O_2$ by the phenol without reaction;[19] hence, it is unlikely that the antioxidant activity displayed by the hydroxy-derivatives tested here could imply recovery of the α-tocopherol products originating from its reaction with $^1O_2$. Triplet carbonyl compounds are known to react with α-tocopherol ($k = 6.8 \times 10^9 M^{-1}s^{-1}$) in a fashion that involves H-atom abstraction from the $-OH$ group in the phenol, thereby yielding the corresponding chromanoxyl radical.[20] However, the low concentration of triplet species within the limits of the lag phase τ as well as the preliminary requirement of peroxyl radical collisions to produce these species, makes the above reaction unlikely.

Acknowledgements

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