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Notes

Generation of superoxide anion and hydrogen peroxide by (+)-cyanidanol-3

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Summary

The generation of superoxide anion from cyanidanol has been studied. At low concentration, it can act as scavenger but at higher concentrations it undergoes autooxidation during which superoxide is generated. The studies also show the generation of hydrogen peroxide during autooxidation.

(+)-Cyanidanol-3, isolated from *Acacia catechu*, has been reported to be useful in protecting against hepatotoxicity (Perrissoud and Weibel, 1980) and reperfusion injury (Vander et al., 1988). It is also known to be an effective scavenger of hydroxyl radical (Bader et al., 1986). Many of the biological activities of cyanidanol have been attributed to its hydroxyl radical scavenging properties. Since in many biological systems, hydroxyl radical is generated from the superoxide anion radical (Halliwell and Gutteridge, 1984), we were interested in studying cyanidanol for its capacity to scavenge superoxide anions. The present study shows that cyanidanol acts as scavenger of superoxide anion at low concentrations, but at higher concentrations it can undergo autooxidation to generate superoxide anion and hydrogen peroxide. Previously, we observed that curcumin was a potent scavenger of oxygen radicals but at low concentrations it activated the Fenton reaction to generate hydroxyl radicals (Elizabeth and Rao, 1989, 1990).

(+)-Cyanidanol-3, nitroblue tetrazolium (NBT), cytochrome *c*, catalase, horseradish peroxidase and superoxide dismutase (Cu,Zn-SOD, type 1) were from Sigma. All other chemicals were of analytical grade. Cyanidanol was dissolved in phosphate buffer pH 7.4 (20 mM) and prepared fresh before use.

The effect of cyanidanol on the generation of superoxide anion by alkaline DMSO was determined by the NBT reduction method (Elizabeth and Rao, 1990). Reduction of cytochrome *c* was studied in phosphate buffer pH 7.4 (20 mM) in the presence and absence of superoxide dismutase by measuring the absorbance at 550 nm (Markert et al., 1984). Hydrogen peroxide generation was assayed by the aminoantipyrine method (Monica and Hill, 1984).

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TABLE 1

Effect of cyanidanol on the generation of superoxide anion by alkaline DMSO measured by NBT reduction

[Cyanidanol] (μM)	Scavenging (%)
0.0043	23.9
0.0086	11.9
0.0172	4.5
0.0345	−4.5 [a]
0.0690	−9.0 [a]
0.3450	−24.8 [a]
0.6900	−109.5 [a]

[a] In these cases, instead of scavenging, increase in superoxide anion production was observed.

Table 1 lists the data on the effect of cyanidanol on the generation of superoxide anion by alkaline DMSO. At low concentrations, cyanidanol acts as scavenger but at higher concentrations it induces activation resulting in the increased generation of superoxide as measured by NBT reduction. The generation of superoxide anion by cyanidanol was further confirmed by using a cytochrome *c* system. Superoxide reduces cytochrome *c* to ferrocytochrome *c* (Markert et al., 1984). Table 2 gives the results on the effect of cyanidanol on cytochrome *c*. A concentration-dependent reduction of cytochrome *c* by cyanidanol was observed. This reduction is prevented in the presence of superoxide dismutase. Further, when a heat-denatured enzyme was used such prevention was abolished. Thus, the reduction of cytochrome *c* can be attributed to superoxide generated by cyanidanol.

TABLE 2

Cyanidanol-induced reduction of cytochrome c in the presence of superoxide dismutase (SOD)

[Cyanidanol] (μM)	Cytochrome *c* reduction (%)		
	Without SOD	With SOD [a]	Denatured SOD [b]
3.45	77.6	42.3	75.1
0.69	69.4	38.5	63.5
0.35	43.2	29.3	41.8

[a] In the presence of 1 μg/ml of SOD.
[b] Preparation was denatured by immersion in boiling water for 15 min.

TABLE 3

Generation of hydrogen peroxide by cyanidanol

[Cyanidanol] (μM)	Hydrogen peroxide liberated (mmol/l)	
	Without catalase	With catalase [a]
3.450	0.0614	0.0248
0.690	0.0273	0.0219
0.345	0.0211	0.0201
0.069	0.0197	0.0186
0.035	0.0181	0.0170

[a] Catalase (0.1 mg/ml) was added to the test solution.

In aqueous solution the superoxide anion undergoes dismutation to yield hydrogen peroxide (Halliwell and Gutteridge, 1984). Also, many compounds containing a catechol nucleus, such as epinephrine, 6-hydroxydopamine and 6-aminodopamine, are known to undergo autooxidation during which superoxide and hydrogen peroxide are formed (Cohen, 1982). Since cyanidanol is also a catechol derivative, superoxide generation can be attributed to the autooxidation process. Since hydrogen peroxide is also generated during the autooxidation process, studies were carried out in order to determine whether cyanidanol generates hydrogen peroxide during its autooxidation. Table 3 shows that cyanidanol produces hydrogen peroxide in a dose-dependent manner. The production of hydrogen peroxide is inhibited in the presence of catalase enzyme which is specific for hydrogen peroxide.

Based on studies conducted for other catechol derivatives (Cohen, 1982), the following reaction sequences can be proposed for the autooxidation of cyanidanol:

$$\text{Cyanidanol} + O_2 \rightarrow \text{semiquinone } (SQ^{\cdot}) + O_2^- + H^+$$

$$SQ^{\cdot} + O_2 \rightarrow \text{quinone} + O_2^- + H^+$$

$$\text{Cyanidanol} + O_2^- + H^+ \rightarrow (SQ^{\cdot}) + H_2O_2$$

$$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Thus, the present study shows that cyanidanol can generate superoxide anion through autooxida-

tion which finally yields hydrogen peroxide. However, at lower concentrations cyanidanol can act as a scavenger of superoxide radical.

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