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OXYGEN ACTIVATION AND DEACTIVATION IN THE PRESENCE OF SIMPLE METAL-CHELATES

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Molecular oxygen is a paramagnetic molecule having a triplet ground state. In the presence of some metal-complexes, e.g. of cobalt, copper and iron this ground state is abolished, and oxygen is in an activated state.[1a-c] First of all the addition of molecular oxygen to a metal complex could involve a formal oxidation of the metal center and a concomitant reduction of the bound oxygen. The question is, whether this state of oxygen is the reactive species in oxygenation reactions.

With some chemical and physicochemical experiments we would like to show evidence for the singlet character of such a bound oxygen in some cases. The reaction is the oxygenation of 1.5-dihydroxynaphthalene (1) to give juglone (2) in the presence of Co-salen in high yield.[2] This reaction is affected by specific singlet oxygen quenchers, such as DABCO, sodium azide and Ni(II)-chelates. By measuring the ultraweak chemiluminescence, these findings could be supported.

KEY WORDS: Oxygen activation, Co-salen, singlet oxygen, quencher, juglone.

INTRODUCTION

In enzymological oxygenase reactions, the central problem is the mechanism of the oxygen activation. Figure 1 shows the activated species of oxygen. In biological systems three of them are very interesting:

1) The superoxide radical anion,
2) the first singlet state,
3) the metal bound oxygen.

For a simple reaction, I would like to show evidence for similarity and differences between singlet and metal-bound oxygen. In both cases the triplet ground state is abolished, and oxygen reacts very easily with substrates in their singlet states.

MATERIALS AND METHODS

Measurement of Low-Level Chemiluminescence and Spectophotometric Assays

Low level chemiluminescence was detected as described in ref.[3] with a red sensitive photomultiplier (EMI 9658AM, EMI Gencom, Plainview, NY, USA) cooled to −25°C and connected to an amplifier discriminator (model 1121; EG & G Applied Research, Princeton, NJ, USA) adapted for single photon counting.

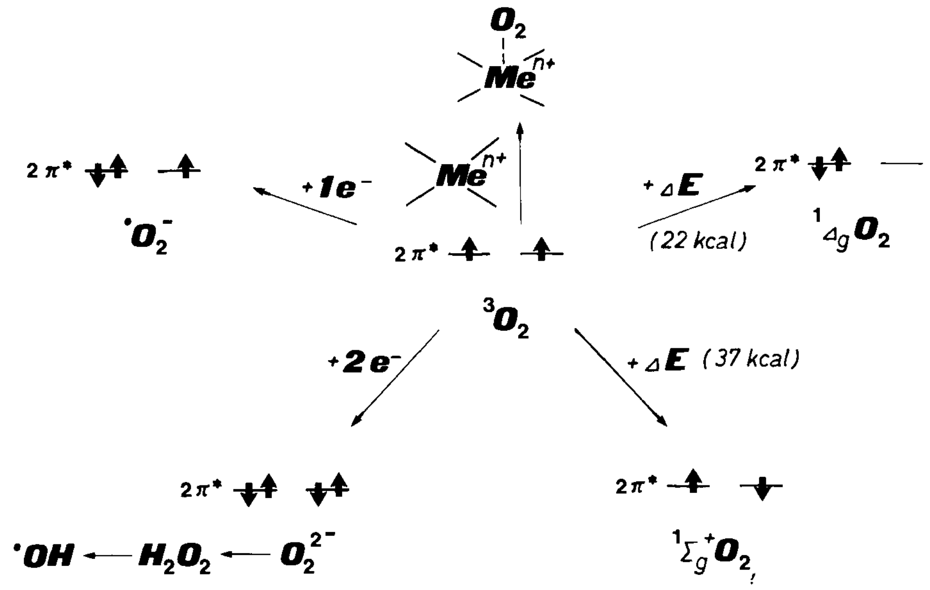


FIGURE 1 Ground state and activated species of oxygen.

Assay Conditions

Chemiluminescence reactions were carried out in a 35 × 5 × 56 mm cuvette provided with a lid for tubing connections for addition and gas exchange.[3] All additions were made from outside of a light-tight box while the photomultiplier was in operation in order to avoid intervals without recording. Under constant stirring and oxygen bubbling, substrate (1) or (4) was added to a Co-Salen-containing acetonitrile solution, 5 to 6 min after this solution was saturated with oxygen.

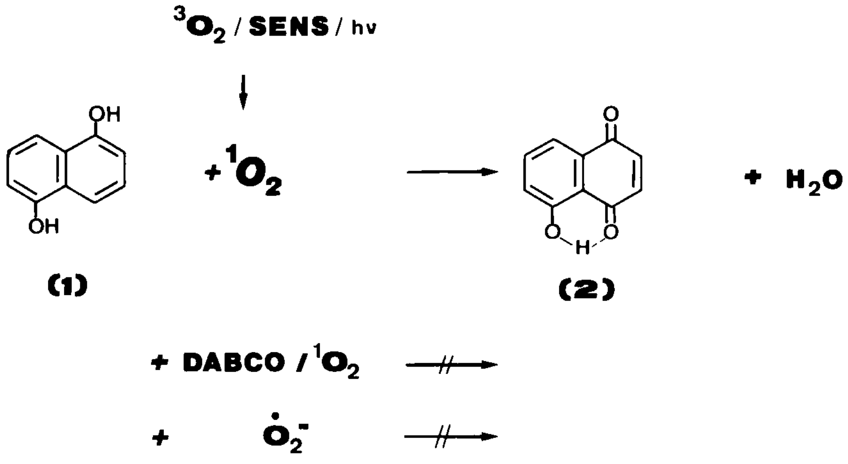


FIGURE 2 Synthesis of juglone (2) as a specfic $^1O_2$-reaction.

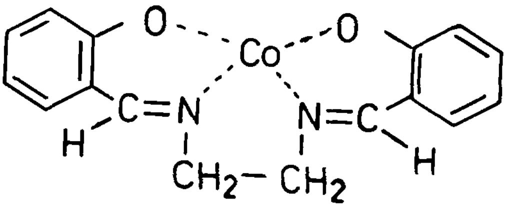
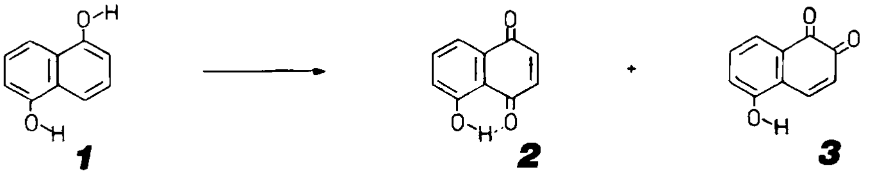


FIGURE 3 Co-salen.

Quenchers were added shortly after addition of the substrate and the chemiluminescence was detected again (see figure 8).

General Procedure for the Oxidation of the Substrates

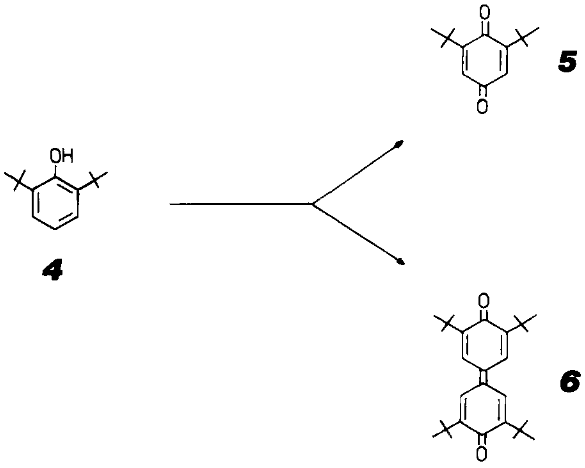
In a typical reaction, 500 mg of the substrate and 100 mg Co-salen were added to 300 ml acetonitrile. Pure oxygen was bubbled into the solution which is stirred for 2 to 4 hours in the dark at room temperature. The solvent was removed and the reaction mixture was purified by column chromatography using silaca gel and chloroform as solvent.



			2	3
1	Co-Salen/O_2		90 %	7 %
2	"	+ DABCO	10 %	7 %
3	"	+ DTBP	90 %	0 %
		Methylenblue / O_2 / hν		
4	$^1O_2$		85 %	12 %
5	"	+ DABCO	0 %	4 %
6	"	+ DTBP	80 %	0 %

DABCO = 1,4-Diazabicyclo[2.2.2]octane ; DTBP = 2,6-Di-tert.-butylphenol

FIGURE 4 Oxidation of 1,5-dihydroxy-naphthalene (1).



		5	6
1	*Co-Salen / O_2*	89%	5%
2	*" + DABCO*	90%	7%
	Methylenblue/O_2/hν		
3	$^1O_2$	33%	56%
4	*" + DABCO*	40%	50%

FIGURE 5 Oxidation of 2.6-di-t-butyl-phenol (4).

	Yield of juglone (2)	
Co-salen / O_2	0%	30%
Methylenblue / O_2/hv	10%	20%

FIGURE 6 Ni-chelates as singlet oxygen quenchers in the synthesis of juglone (2) from 1.5-dihydroxy-naphthalene (1).

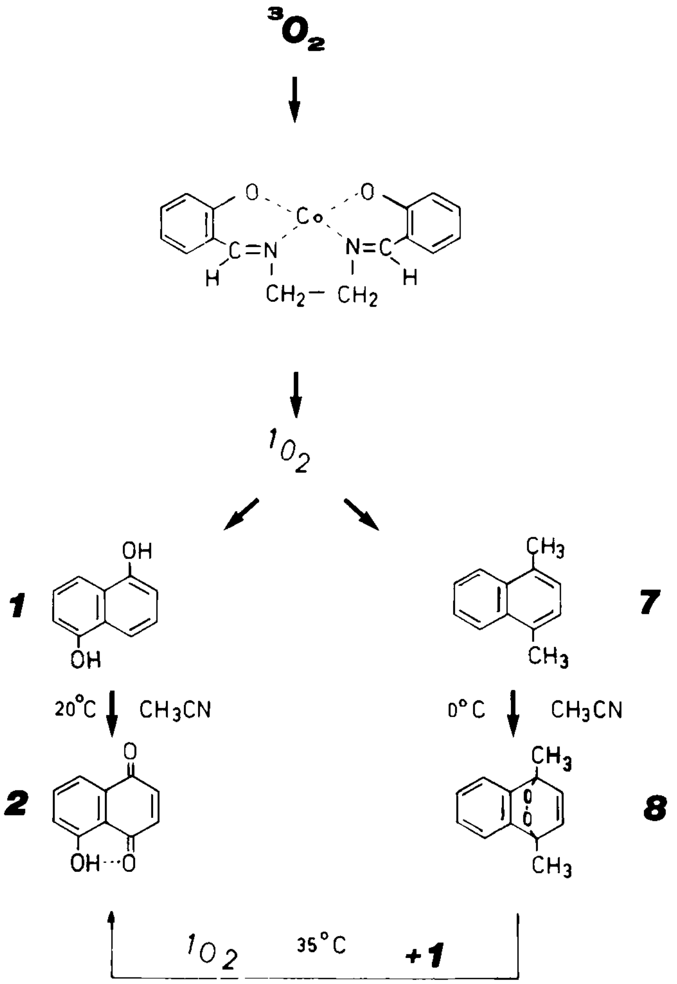


FIGURE 7 Oxidation of 1.4-dimethyl-naphthalene (7).

Quenching experiments were carried out in the same procedure adding the quencher in various amounts and determining the yields of the reaction-products.

RESULTS AND DISCUSSION

To show you these facts, I will focus your interest on figure 2 with a typical singlet oxygen reaction.[2] The reaction is the oxygenation of 1.5-dihydroxy-naphthalene (1) in the presence of methylenblue, light and oxygen. In acetonitrile, the yield of juglone (2) is about 85%, and the reaction is suppressed by singlet oxygen quenchers, but not by radical scavengers (see Fig. 4). The $^{\cdot}O_2^-$-radical does not react with the substrate to give juglone (2).

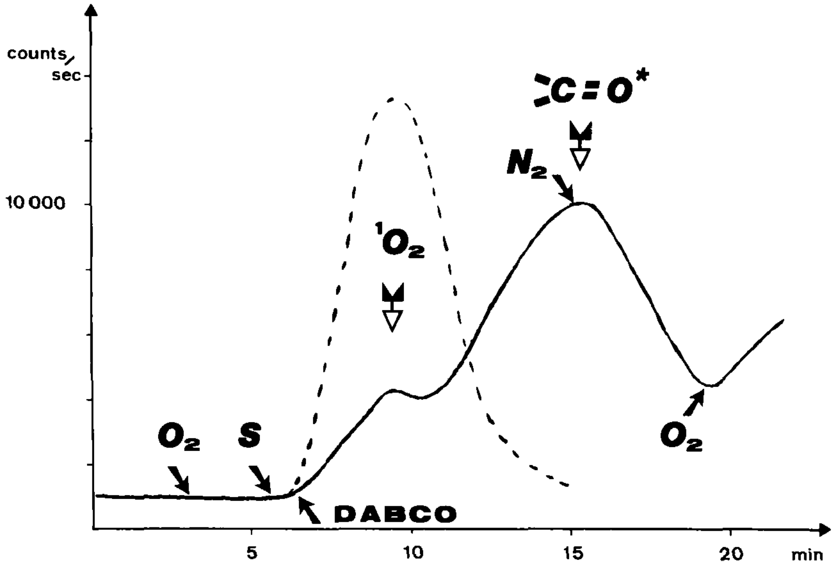


FIGURE 8 Oxygen-dependent low-level chemiluminescence in the presence of Co-salen.

The same reaction you can also find in an oxygenation reaction with a model compound for enzymological oxygenations, the cobalt(II) Schiff's base complex Co-salen [salen = bis(salilcyliden)-ethylendiamine[4a–b]] (see figure 3). The oxygenation was carried out by bubbling of oxygen at room temperature through a acetonitrile solution of 1.5-dihydroxy-naphthalene (1) and catalytic amounts of Co-salen (figure 4). The two products are juglone (2) and the 1.2-naphthoquinone (3),[5] the first should be a singlet oxygen product, because of an assumed (4 + 2)-cycloaddition at the naphthalene. The second product probably is a radical by-product.

Quenching reactions support these considerations. With singlet oxygen quenchers such as DABCO, sodium azide, β-carotene and furan derivatives, the main product (2) is suppressed, whereas the 1.2-naphthoquinone (3) is not. With radical scavengers, opposite results are found. Nishinaga *et al.*[6a] have shown that radical scavengers like 2.6-di-t-butyl-phenol (DTBP) (4) react with the activated oxygen. Since this reaction is not influenced by DABCO (Figure 5) and since similar results are obtained for the methyleneblue-sensitized photooxidation of (4), singlet oxygen should not be involved. The only difference between these two reactions of (4) is the ratio of (5) and (6). It is very difficult, only from this relationship to assume the mechanism of these reactions. Matsuura *et al.*[6b] have found that mainly dimer (6) is generated with chemically produced singlet oxygen, but in the Co-salen-dependent oxygenation the benzoquinone (5) predominates, so that a radical mechanism shhould be possible. More quenching reactions and the dependence of the reaction rate in the presence of DABCO are under investigation.

Other quenchers of singlet oxygen reactions are Ni-chelate complexes (figure 6) such as Ni-salen and the bisdithiodiketone complex Ni-BDTK.[7a–b] Both complexes are quenchers of the photoxidation as well as of the Co-salen-dependent oxygenation of (1) with the yields of juglone (2) shown in figure 6.

Another important experiment is shown in figure 7. During the reaction of Co-salen/ oxygen with 1.4-dimethyl-naphthalene (7) at 0°C the relatively stable 1.4-endoperoxide (8) is generated, probably by a (4 + 2)-cycloaddition of singlet oxygen to (7).[8] Endoperoxide (8) is able to release an activated oxygen molecule at 35°C, that is able to form juglone (2) in the presence of 1.5-dihydroxy-naphthalene (1). This endoperoxide formation shows also the evidence, that the activated oxygen in Co-salen mediated reaction should be in the singlet state.

The chemical experiments are supported by measuring of the ultraweak chemiluminescence of the dimol-emission of singlet oxygen.[3,9] We were able to observe this luminescence in Co-salen-dependent oxygenations. In figure 8, the full line shows the oxygen and substrate [1.5-dihydroxy-naphthalene (1)]-dependent chemiluminescence of a Co-salen satured acetonitrile solution. The luminescence increased by the addition of DABCO (dotted line), and decreased by the addition of β-carotene (not shown in figure 8). With (4) as a substrate, chemiluminescence was not observed, either in the absence or in the presence of DABCO.

CONCLUSION

The Co-Salen catalyzed oxygenation reactions of 1.5-dihydroxy-naphthalene (1) and 2.6-di-t-butyl-phenol (4) and the inhibition of these reactions by several quenchers were examined. The activation of oxygen in these two reactions is different. Based on these reactions and the measurement of ultraweak chemiluminescence it is shown that Co-salen may, depending on the nature of the substrate, activate oxygen in either the singlet state or in the radical state. The substrate-dependent activation of oxygen appears to be important with regard to cobalt complexes as model-catalysts of enzymological oxygenation reactions.

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