

DISINFECTION BY FREE RADICALS AND UV-RADIATION

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ABSTRACT

In bacteria and viruses the target most sensitive to free-radical attack is their DNA. The present concept of the mechanism of $\cdot\text{OH}$ radical induced DNA strand breakage is discussed. Although under certain conditions DNA strand breakage is the main process of inactivation, it is suggested that upon exogenous $\cdot\text{OH}$ radical attack membrane (or coat) damage is the major contribution to inactivation and that damage of DNA associated with these structural elements plays only a minor role. In contrast to the rather advanced concepts of DNA strand breakage no detailed mechanism for $\cdot\text{OH}$ -induced membrane damage can as yet be forwarded. Near UV-radiation-induced inactivation of bacteria and viruses is mainly due to a dimerization of the pyrimidine residues of DNA. Whether at $\lambda < 220 \text{ nm}$ there is also a component to be associated with membrane (coat) damage remains to be investigated.

INTRODUCTION

If one considers a hierarchy of potential targets for killing a living cell or a virus, DNA would be placed at the top position. For example, it has been shown that one single non-repaired DNA double-strand break leads to reproductive cell death (e.g. Frankenberger et al. 1981). Although other functions of the cell might not be affected, it can no longer propagate, a requirement of disinfection.

Our own work over the last ten years or so has been centred on the question of the mechanism behind free-radical induced DNA strand breakage (for review see von Sonntag et al. 1981, von Sonntag, 1984). More recently we also studied free-radical induced changes of the nucleobases, which may be related to radical-induced mutation (for a review on the pyrimidine component see von Sonntag and Schuchmann, 1986; for a review on the purine component see Cadet and Berger, 1985). It is not easy to bridge the gap between these more fundamental studies which have been obtained with a model system, DNA in aqueous solutions, and the challenging title of my talk: disinfection by free-radicals. After a brief account on free-radical formation in some disinfecting systems examples of the free-radical chemistry of DNA will be given. The question will be raised whether these processes play a role in the real world of water disinfection. Subsequent to this some aspects of disinfection by UV-radiation will be dealt with.

FREE-RADICAL SOURCES

In disinfection by ozone one component is due to the formation of $\cdot\text{OH}$ radicals (Horrige and Bader, 1975). The slow reaction of the hydroxide ion with ozone leads to superoxide radicals, $\text{O}_2^{\cdot-}$ (reaction 1; $k(1) = 48 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ (Forni et al. 1982)).



An additional source of superoxide radicals comes from many peroxy radical reactions (see below).

The superoxide radicals are usually rather inert radicals but readily react with ozone (overall -1^{-1} reaction 2, the first step, the electron transfer, is diffusion-controlled $k = 1.6 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ (Böhler et al. 1984)).

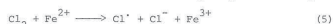


In our studies we generate $\cdot\text{OH}$ radicals using ionizing radiation such as $^{60}\text{Co}-\gamma$ rays or high-energy (e.g. 3 MeV) electrons. If the ionizing radiation is absorbed by water $\cdot\text{OH}$ radicals, H atoms and solvated electrons (e_{aq}^-) are formed (reaction 3). By saturating water with nitrous oxide the latter are converted into further $\cdot\text{OH}$ radicals (reaction 4).



The system thus consists of 90 % $\cdot\text{OH}$ radicals and 10 % H -atoms as free-radical species. This radiation-chemical way of generating $\cdot\text{OH}$ radicals is superior to many other procedures, e.g. the Fenton-reaction or photolysis of H_2O_2 , because the radiation technique avoids the addition and formation of reactive substances such as high concentrations of H_2O_2 , Fe^{2+} , EDTA etc. which can interfere with intermediates and products.

As in the case of ozone disinfection by chlorine will largely involve ionic rather than free-radical reactions, possibly also involving the hypochlorite ion which is in equilibrium with free chlorine. Some Cl radicals might, however, be formed in redox processes with adventitious transition metal ions present in the water or within the cell by enzymatic redox processes (e.g. reaction 5).



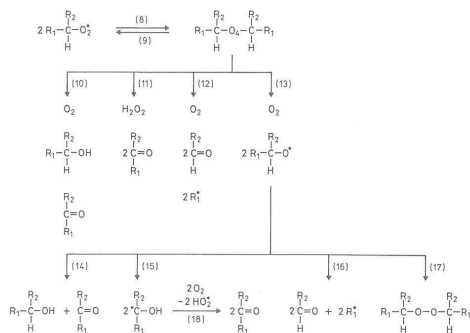
Both the OH radical and the Cl atom readily react with organic matter by adding to C=C double bonds and by abstracting carbon-bound H atoms thereby forming carbon-centered radicals (e.g. reaction 6; for rate constants of the OH radical see Farhatziz and Ross, 1977).



In the presence of oxygen these carbon-centered radicals are rapidly converted into the corresponding peroxy radicals (reaction 7, $k(7) \sim 2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$; Adams and Willson, 1969).



The product distribution is determined by the fate of these peroxy radicals. If in a radiation-chemical experiment a 4:1 mixture of N_2O and O_2 is used as a saturating gas instead of N_2O conditions are the most suitable for the investigation of peroxy radicals in aqueous solution. Reactions 8-18 depict the various routes that have been elucidated so far (Zegota et al. 1984 and references cited therein).



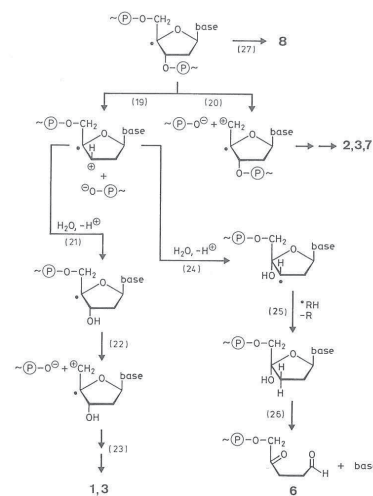
It will exceed the scope of this short review to discuss the various routes but it appears worth mentioning that in reaction 18 a superoxide radical is generated ($\text{HO}_2^\cdot \rightleftharpoons \text{H}^\cdot + \text{O}_2^\cdot$, $\text{pK}_a = 4.7$; Bielski, 1978) which in the presence of ozone can generate new OH radicals (cf. reaction 2). As one might expect sugars are one of the most efficient sources of $\text{O}_2^\cdot$ radicals if OH radicals are allowed to react with them in the presence of oxygen (for a review see von Sonntag, 1980).

FREE-RADICAL INDUCED DNA STRAND BREAKAGE

In the Introduction it has been mentioned that an unpaired DNA double-strand break is a lethal event. A double-strand break can occur by two single-breaks on opposite sites if these lesions are only a few base pairs apart. They can also occur when the cell tries to repair by excision damage on two opposite strands. A DNA double-strand break is per se not yet a lethal damage, because the cell might use the lacking information from another identical gen and repair this damage by recombinational repair. However, this attempt is not always successful.

Being chemists we asked ourselves how could such a DNA strand break occur if a radical, e.g. the OH radical attacks the DNA. Reactions 19-27 show a sequence of reactions (Dizdaroğlu et al. 1975b,

1977a, Beesk et al. 1979). The OH radical or a base OH-adduct radical (cf. Lemaire et al. 1984, Deebie and von Sonntag 1984, 1986, Deebie et al. 1986) produces the C(4') radical which breaks up by forming the radical cations.



The mechanism described only applies to the situation in the absence of oxygen, but similar experiments have been done in its presence (Dizdaroğlu et al. 1975a, 1977a,b, Isildar et al. 1981) and the reactions that lead to DNA strand breakage are on the way of being also understood (for a review see Schulte-Prohilde and von Sonntag, 1985). The altered sugars which have been recognized in the course of these studies are compiled in Figure 1.

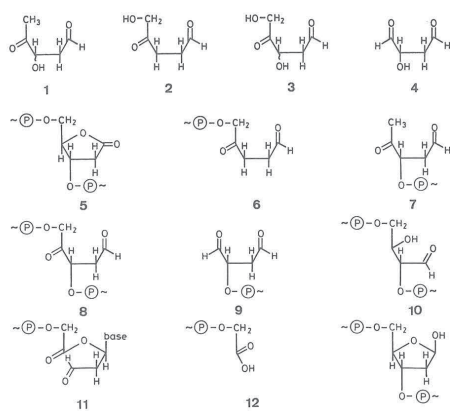


Fig. 1. Alterations at the sugar moiety of DNA observed after OH radical attack in the absence (1-3, 5-8, 13) and presence (4-6, 8-13) of oxygen.

In the case of ionizing radiation there are, albeit with a low probability, clusters of radicals which cause the potentially lethal double-strand breaks. If one induces DNA strand breaks with hydrogen peroxide via a Fenton-type reaction within the cell only single-strand breaks are induced which are sufficiently distant from one another to be effectively repaired without causing double-strand breaks (Ward et al. 1985). This is an important observation with respect to water disinfection by free-radicals. Hydroxyl radicals generated in the ozone system, for example, would attack the DNA also at random as do those from the Fenton's reaction, i.e. they would not induce DNA double-strand breaks unless very high doses are given.

The next important question is whether or not the OH radicals generated in the bulk solution can reach the DNA of a bacterium or a virus at all. Both bacteria and viruses are protected by either a membrane or a protein coat against external agents such as free-radicals. The OH radical is a very reactive radical and the rate constant for the addition to C=C double bonds and for H-abstraction are usually near to diffusion-controlled and rarely drop below $10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$. For this reason they are readily scavenged by any organic impurity in the water, and if they attack a virus or a bacterium they predominantly react with the outer wall, membrane or coat. The inactivation of viruses has been studied under such conditions, and it has been shown that it is mainly (> 70 %) due to a destruction of the protein coat (Freifelder, 1966; Clarkson and Dewey, 1973). This destruction can result in a release of the encapsulated DNA into the water where it will be further destroyed. Quite in contrast to our rather advanced knowledge of mechanistic details of DNA strand breakage very little is known about the breakdown processes that follow free-radical attack on proteins. As it stands, we have to accept that it occurs, without being able to say much more.

The membranes of bacteria are much more complex than the protein coat of viruses. The target most often discussed are the lipids, among them the polyunsaturated ones, because they tend to undergo autooxidation. This autooxidation is a chain reaction (cf. reaction 26) and hence the effect of the primary process 6 (followed by 7) is multiplied.



Although at the first sight such a mechanism is very convincing, there are a few points which put a question mark behind this type of reaction as the sole or most important reaction. In membranes the fatty acid component is not necessarily dominating and in erythrocytes, for example, the lipid

content is only about 5 %, i.e., proteins are dominating (De Robertis and De Robertis, 1980). Furthermore, only the polyunsaturated fatty acids undergo this autooxidation with some efficiency and these polyunsaturated fatty acids are usually not the major component. In addition, membranes usually contain sufficiently high levels of vitamin E to stop the chain. In the membranes the unsaturated part of the lipids is buried inside and the OH radicals which attack the membrane from the outside stand a reduced chance to react with an allylic hydrogen (Patterson and Hasegawa, 1978). Although all this does not exclude the possibility of lipid peroxidation as an important component in the destruction of the cell membrane one certainly will have to consider other sites as well, certainly more than has been done so far.

In bacteria the DNA is attached to the cell membrane. Thus damage to the membrane can have a severe effect on these attachment sites and hence on DNA functioning. Although this line of thought is being actively followed at present, our knowledge is still limited.

It has been mentioned above that cells are capable of repairing DNA damage. This is of course also true for membrane damage. In fact if radiation-biological studies are a good guide typical effects of membrane damage such as potassium loss and response to trypan blue staining only show up at doses exceeding those required for reproductive cell death (Wolters and Konigs, 1985). It is therefore not unreasonable to believe that there must be considerable (multiple) membrane damage to cause cell death and to bring about disinfection.

In reaction 5 we envisaged that Cl atoms could be formed during chlorine treatment of water. For reactions 5 to be of any importance in disinfection of water there must be a high pool of low-valent metal ions. If this pool is consumed and the metal ions are converted to higher oxidation states reaction 5 ceases and hence the contribution of the Cl atom. The Cl atoms are much less reactive than the OH radicals, hence they are more selective. Our knowledge about the reaction of Cl atoms with subcellular structures is too limited to allow a detailed discussion, but it might be of interest to note that Cl atoms (as other halogen and pseudohalogen radicals; e.g. Adams et al. 1965; Davis and Gilbert, 1984) readily complex with halogenide ions (reaction 29)



and that these complexed halogen atoms are strongly oxidizing agents which extract, for example, an electron from DNA nucleobases leaving radical cations behind (reaction 30).



The fate of such radical cations which can also be produced by other reactive species are presently under investigation (Schuchmann et al. 1986).

In conclusion, it appears that disinfection by exogenously produced OH radicals may be largely due to membrane damage in bacteria and coat damage in viruses, and DNA damage is possibly only a minor component. Mechanistic details of the latter are in part fairly well understood while the mechanism of the more complex membrane damage still awaits elucidation.

UV-RADIATION

We have discussed above that OH radicals (and to some extent also Cl atoms) react more or less unselectively with all organic material which they encounter. This is no longer so with another disinfecting agent, UV-radiation. UV-radiation can only bring about changes in material which is capable of absorbing the UV-quanta. For water disinfection two sources of UV-radiation are under discussion, the mercury-low-pressure arc and the mercury-high-pressure arc. They considerably differ in their emission spectra. Whereas the former only emits the wavelengths 254 and 185 nm (which can be retained by vycor quartz, cf. Fig. 2), the latter exhibits a broad continuum up to 200 nm (and below) which is overlain by a number of more or less intense lines (Fig. 3).

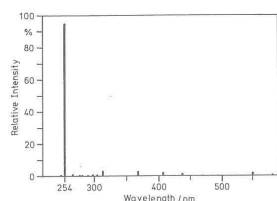


Fig. 2. Emission spectrum of the Hg-low-pressure arc.

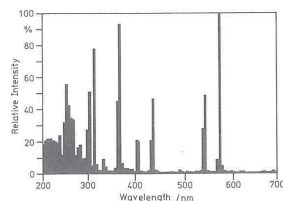


Fig. 3. Emission spectrum of the Hg-high-pressure arc.

The 254 nm line is not absorbed by lipids, carbohydrates, cholesterol and the saturated amino acids which make up the major part of the proteins. Only the aromatic amino acids show some absorption at this wavelength. The first absorption maximum of DNA (Fig. 4), however, is very near to this wavelength and most of the 254 nm light which is absorbed by bacteria and viruses is absorbed by their DNA. This also pertains to other wavelengths. Hence it is not too surprising that inactivation spectrum of bacteria and viruses is very close to the absorption spectrum of DNA (Fig. 4), at least at the longer wavelengths.

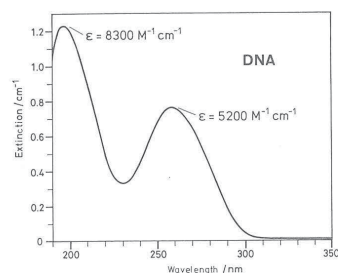


Fig. 4. UV-absorption spectrum of DNA.

The UV-induced damage of DNA is quite well known (Wang 1976). It is mainly due to a dimerization of two neighbouring thymine residues which form a cyclobutane-type dimer such as shown by Fig. 5.

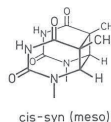


Fig. 5. Structure of the thymine dimer

The photochemistry and biochemistry of these DNA photodimers is rather complex. There is a photochemical equilibrium between DNA and DNA-thymine dimers. The equilibrium at a given wavelength is determined by the extinction coefficients of these two species, and the quantum yield for the forward and backward reactions (reactions 31 and 32).



At long wavelengths the dimer does not absorb very much, hence the equilibrium lies quite on the right hand side. At short wavelengths the dimer also absorbs, and considerable resplitting of the dimers can occur.

Because UV-radiation has always been a hazard to living organisms, evolution has developed a defense system which copes with this type of damage, i.e. this damage is continually repaired by enzymes. In fact the production of these enzymes can be stimulated by light. However, intense UV-radiation can produce so many of such lesions that the enzymes can no longer cope with all the damage induced. This leads to inactivation, i.e. disinfection.

It has been mentioned above that around 254 nm only DNA and to a minor extent aromatic amino acids have to be considered as absorbers of the UV-quanta. This is no longer true for quanta at around 200 nm. In this wavelength region nearly all organic matter starts to absorb, not only the aliphatic amino acids but also carbohydrates and lipids. Furthermore the energy of these quanta is substantially higher than those of longer wavelengths and quantum yields might go up and new processes might start to play a role (e.g. von Sonntag and Schuchmann, 1977). Light of this shorter wavelength is emitted to a considerable extent by the mercury-high-pressure arc (Fig. 3). Hence disinfection using such lamps as light sources might not be only due to DNA impairment but membrane damage might come in as well, as a second component. Too little is known about the photochemistry and the photobiology at this wavelength region to make a educated guess. It is evident that more research should be devoted to elucidate this point.

CONCLUSION

Disinfection of bacteria and viruses by free radicals such as the OH radical is likely to be largely due to destruction of the cell membrane or coat and to a minor extent of the DNA associated with these structural elements. In contrast disinfection by near-UV-radiation is mainly caused by DNA impairment via pyrimidine dimer formation. Whether or not membrane (coat) damage significantly contributes at $\lambda \leq 220$ nm remains to be investigated.

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INACTIVATION OF VIRUSES BY DISINFECTION METHODS

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ABSTRACT

Inactivation of viruses is depending upon several parameters such as their environment and physico-chemical factors such as pH and temperature. Moreover viral disinfection is depending upon virological factors such as virus strain. Results presented in this general review propose also an approach of the molecular mechanism of inactivation by chlorine which is demonstrated as a single hit phenomenon able to inactivate 99.9 % of the virus with no visible action on its RNA or proteins nor on its attachment process to cells. In these conditions alteration of the physiological function of the virus can be strongly suspected.

KEYWORDS

Disinfection; viruses; oxidizing agents; inactivation kinetics; mechanism of action; physico-chemical factors; virological factors.

INTRODUCTION

If we try to find our way in such a broad subject we have to consider the things in a logical order even if they were random distributed since the beginning of this 80 years old story.

So far as I know oxidizing agents were firstly used for industrial purposes such as drinking or raw water treatment plants. There was then a first bloom of papers dealing with the eradication of viruses by oxidizing agents as used in water plants. People became then interested to know the interference of what we could call the "coarse factors". If we forget about floating things these factors can be as different as fecal homogenates, sewage effluents, turbidity, and clay.

The next logical step was laboratory assays with two main subjects: the study of physico-chemical influencing factors and the study of virological influencing factors. The results of both ways led to an important amount of inactivation kinetics which are still under investigation and lead to the final discovery of the mechanisms of action.

Going as usual from simple experimentations to the more sophisticated ones we have first to consider briefly the role of what we could call the "coarse factors".