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Oxygen Toxicity Group Report

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INTRODUCTION

Since the appearance of O_2 on earth, microorganisms have been exposed to the ever increasing threat of this very strong oxidant. During evolution organisms have adapted to cope with oxygen to various degrees. For example, mere traces of oxygen may be toxic for methanogenic bacteria, while some cyanobacteria are able to grow in the presence of a partial pressure of 1 atmosphere oxygen. In between these two extremes detante exists between the potentially lethal effects of O_2 and its requirement for life.

It is only recently that most microbiologists have become aware of the fact that oxygen is not only toxic to obligate anaerobes or microaerophiles but also presents a direct threat for aerobic organisms. The toxicity of oxygen has been traced back to the products which are formed (directly or indirectly) when oxygen is partially reduced in or outside the living cell to give the superoxide anion radical $O_2^{\cdot-}$ or the peroxide anion (O_2^{2-}), rather than oxygen in its completely reduced form, H_2O. Superoxide and peroxide, if not already toxic by themselves,

may produce the hydroxyl radical (OH·), which is an extremely reactive and damaging free radical. Furthermore, it has now become clear that any organism containing a suitable photosensitizing pigment may, by exposure to light, produce electronically excited pigments which may generate, in the presence of oxygen, the very reactive species, singlet O_2 ($^1O_2$). During evolution the biological world has developed a variety of strategies to both deal with the dangers and exploit the usefulness of oxygen

The more one becomes aware of the dual role that oxygen plays on our planet, the more one comes to realize that although to the human eye it is the anaerobic environment which is alien and hence extreme, for the majority of the biological world it could be the aerobic situation which is the extreme environment.

The workshop has focused its attention primarily on the microbiological and biochemical aspects of oxygen toxicity. Studies on the genetic level are still in their infancy.

WHAT DO WE MEAN BY OXYGEN TOLERANCE AND HOW DO WE MEASURE IT?

From the paper by Morris (this volume), it has become clear that the toxic action of oxygen on growth and survival of organisms is "a many splendored thing." Toxic effects of oxygen are multifactorial, and at the same time protective mechanisms ("tolerance") are similarly numerous. Not only do physical and chemical factors play a crucial role in prompting O_2 toxicity or determining tolerance, but this is also true for biological factors such as the state of culture growth, the nature of growth, limitation, the previous growth history, the nature of the substrate, the presence of light, etc. In fact, one should envisage oxygen tolerance (oxygen sensitivity) as the resultant of "two contending sets of tendencies" (Table 1, Morris, this volume). It is clear that an analogous table could be made for aerobic (micro-)organisms. From the above it becomes clear that extrapolation from any one situation to another will not necessarily be valid, and precise quantification of oxygen toxicity or O_2 sensitivity becomes impracticable.

Some very peculiar effects of oxygen were mentioned:
a) Organisms which die as a result of sudden exposure to oxygen may survive when they are exposed to gradually increased concentrations of oxygen. Under the latter conditions one can assume that protective mechanisms are induced. This phenomenon lends itself to experimental investigation of the nature of O_2 tolerance.
b) Short-term effects of sudden exposure of anaerobically grown cells to oxygen may lead to metabolic changes different from those observed after long-term exposure. Thus in batch cultures or resting cells of propionibacteria, formation of acetate and propionate from lactate were inhibited by oxygen (9,21,24). In contrast, when a continuous culture of Propionibacterium shermanii had adapted to oxygen through a graded sequence of increasing pO_2 levels, acetate formation was strongly enhanced (18). When the partial pressure of oxygen in the gassing mixture reached 20-40 mm Hg, cell yield of the chemostat culture diminished. Further increasing the pO_2 in the gassing mixture did not only relieve the inhibition but even led to much higher yields than under anaerobic conditions. Apparently the "aerobic" enzymes and/or the aerobic complement of the "anaerobic" electron transport chain were only induced above a threshold concentration of 20-40 mm Hg of O_2. Above a pO_2 in the gassing mixture of approximately 500 mm Hg, when a dissolved oxygen concentration was just detectable, the oxygen became toxic and the culture was washed out.
c) Catalase may stimulate in unknown ways growth of Beggiatoa, some cyanobacteria, some species of Clostridium, and a Peptostreptococcus species ((5,12,14), and H.G. Schlegel, personal communication).

We concluded that one should <u>not</u> try to construct a "league" table, i.e., a list of species in invariant order of increasing O_2-tolerance (or toxicity) in precise quantitative terms (see Morris, this volume). Oxygen tolerance may be assessed usefully in qualitative terms (for example, for identification purposes). It was suggested that for qualitative measurements of O_2 tolerance simple methods should be used: particularly

cultivation in agar deeps exposed to air that will establish their own oxygen gradients (Pfennig, personal communication and (20)) but also growth of colonies on plates at different pO_2 values (4,17). Quantitative studies of O_2 tolerance, toxicity, or response of a certain organism should be undertaken with its particular physiology and ecology in mind: though it does not make much sense to try to grow a methane producer in the presence of (traces of) oxygen, it may be relevant to measure its survival in the presence of O_2. Similarly, it seems relevant to study the effect of 100% oxygen of 1 atmosphere on growth of cyanobacteria known to thrive in such high concentrations of oxygen.

RESPONSE OF MICROORGANISMS TO THE PHASED APPEARANCE OF DIFFERENT pO_2 IN THE ATMOSPHERE

Nature now provides habitats with a continuum of oxygen levels ranging from traces of oxygen to environments containing one atmosphere partial pressure of oxygen (pO_2 = 100%). One can roughly group organisms in clusters living at, or below, rather discrete levels of oxygen:

1) Many anaerobes are so intolerant of oxygen that no growth is possible in the presence of even traces of dissolved oxygen.
2) Microaerophiles, among which are facultative anaerobes and obligate aerobes, are limited to life at pO_2 not greater than 1 - 2%.
3) Normal aerobes can withstand levels up to about pO_2 of 40%.
4) Some aerobic organisms are able to live in aquatic environments which are occasionally supersaturated with oxygen up to pO_2 of 100%.

An interesting question arose as to whether one might relate the existence of these groups to the phased appearance of oxygen during evolution. The initial anaerobic environment would have harbored organisms of type 1 only. When the first traces of oxygen started to appear (perhaps initially from photolysis of water (23) and later from oxygenic photosynthesis), organisms of type 2 may have begun to emerge. Only

when most of the available reduced iron compounds and sulfide in the sea had been oxidized by the bulk oxygen production of oxygenic photosynthetic processes did the oxygen concentration rise to the much higher levels known today (see Pfennig, this volume and (8)). This may have then resulted in the development of type 3 organisms followed by the type 4 organisms.

It should be realized that the slow appearance of chemically produced O_2 and the later occurrence of diurnal production of O_2 by photosynthesis have allowed the development and selection of defense mechanisms against O_2 and other oxidants such as NO_3^- and ferric ions without killing the organisms. The first line of defense may have been diminishing oxygen toxicity by scavenging of the oxygen with reducing agents which thereby might be destroyed, followed by the development of reducing (regenerating) cycles and specific enzymes such as superoxide dismutase (SOD), catalases (CAT), and peroxidases (PER). Once microaerophilic and other aerobic microorganisms had emerged, other mechanisms became feasible, such as high respiration rates (e.g., Azotobacter) to protect oxygen sensitive targets or compartmentation (e.g., heterocysts). It should be tested whether the different mechanisms of protection are related to these clusters in order to find out to what extent defense mechanisms are congruent with an organism's habitat and ecological niche.

Particular attention was paid to the ecological advantage of microaerophiles and to the ecological niche of such organisms. They seem to be successful at low pO_2 for various reasons, some of which should be mentioned:

a) They possess a high affinity (low K_2) for oxygen, so that they can compete successfully with normal aerobes at low pO_2.

b) They may be able to utilize substrates labile in the presence of elevated concentrations of oxygen (for example, sulfide).

c) N_2-fixing species can fix molecular nitrogen without special need for protection of their nitrogenase.

d) Fixation of carbon dioxide may proceed more efficiently than at higher pO_2 (oxygenase activity of ribulose 1,5-bis-phosphate

carboxylase, leading to the production of glycollate, will be greatly diminished).

Intuitively one would assume that the possession of characteristics such as (a) will make the organisms vulnerable to inhibition by high pO_2, too, and that is in fact what is found (22).

Quite interesting is the occurrence of partial oxygen pressures up to 1 atmosphere in many habitats (11). The cyanobacteria thriving in these environments apparently have developed powerful defense mechanisms against reactive oxygen compounds, which include singlet oxygen in the case of photodynamic damage. But also heterotrophic organisms that grow in these habitats must have a high capacity to detoxify the reactive oxygen species. A thorough investigation of the defense mechanisms of these groups of bacteria may provide valuable information on defense mechanisms against oxygen toxicity.

MULTIPLE MECHANISMS OF TOLERANCE TO O_2

As has been mentioned before there is no unitary explanation for oxygen toxicity (or tolerance) in (an)aerobes (see Table 1, Morris, this volume). Oxygen toxicity is due to multiple mechanisms depending on a number of conditions. From an ecological standpoint, it is useful to look at defense mechanisms both from the point of view of survival and from the point of view of growth of an organism.

Survival

The effect of oxygen on a "resting" cell will not be uniform. On the one hand, a spore has a high resistance to oxygen compared to that displayed by vegetative cells. On the other hand, a resting vegetative cell may be more vulnerable to oxygen than a growing cell, since it does not respire at such a high rate and hence may not be able to protect possible O_2-sensitive targets. Survival may not only depend on the defense mechanisms (see also Maintenance of Low O_2 in Mixed Populations, below), but will also be highly dependent on

the conditions created for recovery in the field or laboratory. For example, low E_h and the presence of hydrogen gas will be essential for recovery of methanogens. Another example is that lyophilized cultures often recover more easily when resuspended in O_2-free medium even if they are aerobes. Quantitative data on these phenomena are lacking. In conclusion we can say that in the study of the survival of microorganisms upon exposure to various concentrations of oxygen, we should always consider that there are two important factors: survival during fluctuations and survival during dispersal.

Growth

Tolerance of organisms to O_2 during growth is multifactorial and extremely dependent on the growth conditions. There are various lines of defense of increasing complexity or sophistication, ranging from the simple diversion of reducing power, to protection by respiration with cytochrome oxidase (which does not produce free reactive oxygen species), and the highly effective protective action of carotenoids against photodynamic damage by oxygen. An interesting example is the sensitivity of cyanobacteria to photodynamic damage (by singlet $^1O_2$) in the absence of CO_2 and at low temperature. The CO_2 effect is unexplained. As the induction of repair mechanisms can be inhibited by chloramphenicol, it has been suggested that the low temperature also inhibits induction of repair mechanisms (11).

It must be stressed that oxygen is toxic for both anoxybiontic and oxybiontic organisms and that the utilization of oxygen as a terminal electron acceptor increases the dangers. This is even more the case when O_2 is produced during photosynthesis. Some instances of tolerance to oxygen seem to implicate a functional indifference towards oxygen. Can an organism be entirely oxygen indifferent? The conclusion is that one should look for defense mechanisms of organisms against the background of their ecological niche. A methanogen that is entirely dependent on products of anaerobic metabolism of other anaerobes is so much restricted to an anaerobic mode of life that the possession of elaborate (and

therefore "expensive") defense mechanisms against O_2 would seem an ecological disadvantage rather than an advantage!

Methodological Assessment of the Role of Different Mechanisms

It appears that the use of mutants of E. coli lacking superoxide dismutase (SOD), catalase (CAT), and peroxidase (PER) or lacking CAT and PER (see Hassan and Fridovich, this volume) is most promising. These studies should be extended to other mutants and to other organisms. Investigations on mutants with various degrees of oxygen tolerance should also be carried out at different partial pressures of oxygen. Studies on the induction and repression of defense mechanisms in E. coli (see Hassan and Fridovich, this volume) should be extended to other organisms and should include studies of other (high and low molecular) defense mechanisms. Study of cyanobacteria and heterotrophs resistant to exceptionally high oxygen concentrations (up to 100% O_2) might be rewarding.

A different line of research has been focused on the role of carotenoids in the protection against photodynamic damage (see Krinsky, this volume). Further work in this area should also consider the protective role which pigments in many (airborne) microorganisms may play in the defense against photodynamic damage, and whether other compounds can carry out any of the functions of the carotenoid pigments in this process.

A helpful tool for a variety of structures will be the use of chemical, photochemical, and enzymic methods that allow generation of reactive oxygen species (see Krinsky, Hassan and Fridovich, and Halliwell, this volume).

Maintenance of Low O_2 in Mixed Populations

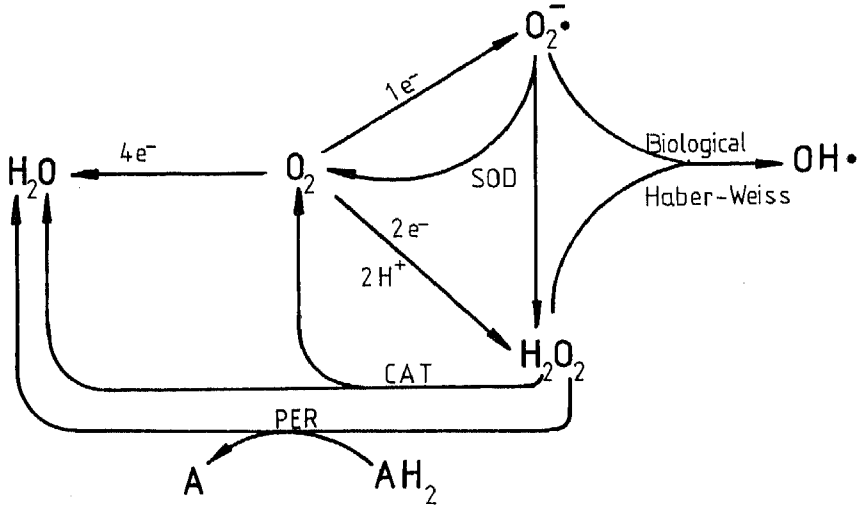
Organisms in nature usually grow in communities rather than as single cells. Hence the presence of oxygen tolerant or respiring organisms may confer oxygen tolerance upon an otherwise oxygen sensitive organism.

A very important protection against oxidative damage resides in the ability of many organisms to display negative and positive aerotaxis (for example, the "Beijerinck figures" created by microaerophiles under a microscopic cover slip). Alternatively, the survival of a single cell of an oxygen sensitive species may be ensured by death of its companions.

On the other hand, the presence of mixed populations need not necessarily be beneficial to another organism. Many organisms are known to excrete toxic O_2-species. For example, polymorphonuclear leucocytes even use reactive O_2 intermediates to kill engulfed bacteria (See Halliwell, this volume).

WHAT ARE THE SOURCES OF REACTIVE OXYGEN SPECIES?

In order to give the basic information needed for further discussion the following scheme may be helpful.



It has been suggested that singlet oxygen ($^1O_2$) may be produced during the nonenzymic dismutation of superoxide or by a Haber-Weiss reaction, but the evidence for this is very indirect. However, it is likely that in the so-called "biological Haber-Weiss reaction" (O_2^- + H_2O_2) the highly reactive and often lethal hydroxyl radical (OH·) is produced. The

hydroxyl radical has not yet been detected directly, but indirect evidence is available.

The enzyme superoxide dismutase (SOD) produces H_2O_2 and O_2 from the dismutation of 2 molecules of the superoxide radical (O_2^-). The oxygen produced is in the triplet (ground) state, and not in the singlet form. The hydrogen peroxide can be removed by catalase (CAT), leading to the formation of triplet oxygen and water, or by peroxidase (PER), yielding water and an oxidized substrate.

It has been shown that only the combination of O_2^- and H_2O_2 (also produced from O_2^-!) wreaks maximum havoc to the cell. If either one of the two species is removed by SOD or CAT, the toxicity is largely suppressed. This indicates that a product of the $H_2O_2 + O_2^-$ reaction is the real damaging species and lends support to the view that the OH· radical acts as the active, damaging oxygen species.

For details concerning the production of superoxides and peroxides by enzymatic and (photo)chemical means, the reader is referred to Hassan and Fridovich, and Halliwell (this volume).

MECHANISMS OF PROTECTION AGAINST SINGLET O_2 BY CAROTENOIDS

Singlet oxygen ($^1O_2$) can be produced photodynamically and chemically (for a review, see (15)). Thus far there is little or no evidence that singlet oxygen is produced in biological systems in any way other than photodynamically. Since singlet oxygen is highly reactive and therefore highly toxic, biological systems must be protected from this damage. This is particularly true for photosynthetic organisms that use photosensitizers ((bacterio)chlorophyll) for their photochemical reaction. After reaction with a photon (light), chlorophyll (Chl) is converted to the singlet state (^{1}Chl) and a fraction of this is transformed to the triplet state (^{3}Chl), which can react with O_2 to yield singlet O_2. Carotenoids can provide the necessary protection against this singlet oxygen and may even prevent the formation of this oxygen species. Carotenoids which possess more than 7 conjugated double bonds are able to quench singlet

oxygen very effectively. This is due to the fact that the energy needed to convert ground state carotenoid into triplet is less than 94 kJ/mol. Hence the energy difference between $^1O_2$ and $^3O_2$ (94 kJ/mol) can be transferred directly from $^1O_2$ to a ground state carotenoid in a spin-conserved reaction.

The carotenoids also are very effective in quenching triplet state chlorophyll directly. In both cases the quenching process inflicts very little damage on the carotenoids. However, carotenoids also protect against the damaging effects of radicals produced in low quantities during the photosynthetic reaction. Carotenoids are damaged in the radical reaction. To date no specific repair mechanisms for the damaged carotenoids have been found.

In photosynthetic organisms the carotenoids should be considered as a first line of defense against the very lethal effects of singlet oxygen. The possession of carotenoids appears to be essential for any O_2 producing phototroph. Therefore, it has been suggested that during evolution the development of carotenoids - initially used as accessory pigments for energy transfer in anaerobic photosynthetic organisms - must have preceded the development of the process of oxygenic photosynthesis.

An important field of future research can be seen in investigating the possible production of singlet oxygen in reactions other than photosynthetic reactions. Singlet oxygen has been reported to occur in polluted water (30). As mentioned above the possible role of carotenoids and other pigments in protecting nonphotosynthetic organisms against photodynamic damage should be investigated.

MECHANISMS OF PROTECTION AGAINST SUPEROXIDE O_2^-

In general it is found that eukaryotes have superoxide dismutases that carry either a copper-zinc complex in the reactive center of the molecule (CuZn-SOD), or manganese (MnSOD). This MnSOD is found in the mitochondria. Prokaryotes usually have

an iron SOD (FeSOD) or a manganese SOD (MnSOD) or both. The bacterial MnSOD has a great deal of homology with the mitochondrial MnSOD.

Mechanisms for Induction of Superoxide Dismutase (SOD)

Hassan and Fridovich (this volume) show that in E. coli MnSOD is an inducible enzyme while FeSOD is constitutive.

There is convincing evidence that O_2 is essential for the induction of MnSOD. Methylviologen (MV, also called "paraquat") can generate O_2^- in the presence of reducing power and oxygen. MV added to cultures leads to production of the MV radical that amplifies the inductive effect of O_2. This strongly indicates that O_2^- or a product of this radical is involved in the induction of MnSOD. It is not yet known whether the appearance of MnSOD is due to induction or derepression. It is important that the MV radical does not induce MnSOD under anaerobic conditions; this rules out the involvement of radicals other than O_2^- in the induction process.

When E. coli is exposed to air, not only SOD but also CAT and PER are induced. Very interestingly the appearance of cyanide resistant respiration coincides with increasing MnSOD concentrations.

The Role of MnSOD vs FeSOD and Other "SOD" Enzymes

The role of FeSOD that is present in E. coli under anaerobic conditions is unclear. On the one hand the possession of FeSOD alone is not enough to protect E. coli against oxygen toxicity. If E. coli is transferred from anaerobic to aerobic conditions in the presence of puromycin the induction of MnSOD is inhibited and the cells are killed by exposure to air (Hassan and Fridovich, this volume). On the other hand (leaky) mutants that possess low amounts of FeSOD do not die under anaerobic conditions. A further study of the role of FeSOD appears necessary.

To date there is no indication that the genetic information for SOD is located on a plasmid. As mentioned above the further use of mutants of SOD and/or PER and CAT both in E. coli and

particularly also in cyanobacteria may provide valuable information on the role of these and other possible mechanisms for the protection against superoxide.

Studies on homologies between the MnSOD enzymes of different bacteria and mitochondria may give information about a possible common ancestor SOD.

The circle of defense mechanisms is not complete with SOD. Organisms preferably should also have CAT and PER to deal with the product of SOD catalysis. Other defense mechanisms may exist, for example, one-electron acceptors (such as cyt c) that can react with O_2^- and may be a possible defense mechanism in the lipid bilayer membrane, where the O_2^- radical has a longer half-life than in an aqueous environment. The SOD enzymes known to date may not be effective against the action of O_2^- in the membrane since they all are cytoplasmatically located. Other metal containing proteins may also react with O_2^- (Cu(I) chromophores of many copper proteins). More work is certainly needed to further our knowledge in this field.

The question arises whether SOD and also CAT and PER are more resistant than other proteins to the attack by reactive oxygen species.

The conclusion is that our knowledge of reactive species of oxygen is just emerging. Halliwell (this volume) has indicated several biological systems and processes (Leucocytes, ageing, cancer) which may provide novel information on the role of O_2^-.

WHY HAS AN SOD-ENZYME EVOLVED IF SMALL CU^{++} COMPLEXES CAN ACT AS SOD?

Superoxide dismutase produces H_2O_2 and O_2 from the dismutation of two molecules of O_2^-. The oxygen produced is in the triplet (ground) state. Recent work (2,3,16,28) has shown that Cu^{++}-complexes can exert an SOD-function at an even higher rate than the enzyme. It may be that these complexes generate

singlet oxygen ($^1O_2$), which would explain why these complexes usually are not found in living cells. Other reasons that would explain the development of a protein-SOD rather than a Cu^{++} complex are: compartmentation, genetic control, protection of the active site, etc. The state of our knowledge at present does not allow us to decide whether SOD may react with compounds other than O_2^- as has recently been suggested. A reaction of SOD with transient radicals before O_2^- is generated would provide some raison d'être for the presence of this enzyme in anaerobes (19,25,26,27,29).

In view of the important role that copper ions play in proteins catalyzing oxygen reductions, it may be interesting to mention that the toxicity of Cu^{++} ions (for example, for algae) may be due to the extreme reactivity of the divalent ion with sulfhydryl groups of proteins. This yields monovalent copper that can react with hydrogen peroxide to give the hydroxyl radical:

$$2Cu^{++} + 2RSH \longrightarrow 2Cu^{+} + RSSR + 2H^{+}$$
$$Cu^{+} + H_2O_2 \longrightarrow Cu^{++} + OH\cdot + OH^{-}$$

THE ROLE OF PARTIALLY REDUCED SPECIES OF O_2 AS ESSENTIAL INTERMEDIATES OF CERTAIN REACTIONS

The stepwise electron transfer during reduction of molecular oxygen proceeds as follows:

$$\begin{array}{ccccccccccc} {}^3O_2 & \overset{e^-}{\underset{(1)}{\rightleftharpoons}} & O_2^- & \overset{e^-}{\underset{(2)}{\rightleftharpoons}} & O_2^{2-} & \overset{e^-}{\underset{(3)}{\rightleftharpoons}} & O^- & \ldots & O^{2-} & \overset{e^-}{\underset{(3)}{\rightleftharpoons}} & 2O^{2-} \\ & & & & \Updownarrow 2H^+ & & \Updownarrow H^+ & & \Updownarrow 2H^+ & & \Updownarrow 4H^+ \\ & & & & H_2O_2 & & OH\cdot & & H_2O & & 2H_2O \end{array}$$

Reaction 1 is a xanthine oxidase-like reaction. Reactions 1 and 2 are an SOD-like reation, while reaction 3 is a CAT or PER-like reaction. This chain of reactions may have been a primitive terminal oxidase. The challenge to evolution has been to design a protein which allowed the stepwise (or direct) addition of 4 electrons to O_2 without releasing the toxic

intermediates. Today's cytochrome oxidase is such a protein. It produces H_2O directly via intermediates that may involve bound O_2^- and O_2^{2-} (6,7) without any release of intermediates. There is clear evidence that cytochrome oxidase is reduced by stepwise additions of one electron at a time. Relevant to this finding is the observation that the Mn-protein involved in splitting water during photosynthetic oxygen evolution is thought to operate in an analogous manner (10).

In nature a variety of other proteins can be found which bind oxygen in a reduced form. For example, hemerythrin (in marine worms) and cytochrome P450 (in liver) bind oxygen in the state of O_2^- and hemocyanin binds oxygen in the state of O_2^{2-}.

STRATEGY OF LIFE IN RESPONSE TO O_2

For an overview of the strategy of life under anaerobic conditions see Pfennig, and Morris (this volume).

Recent papers have reinforced the view that oxygen may have appeared on this planet long before oxygenic photosynthesis evolved. It has been proposed that the early oxygen was produced by photolysis of water. Theoretical analyses have shown that the rate of photolysis was not self-limiting through shielding of the light by the produced oxygen, as was previously assumed (13,23). Accepting this view, as opposed to the hypothesis that oxygen production was due to oxygenic photosynthesis only, considerably changes our thoughts and speculations about the strategy of life in the presence of oxygen.

Initially the atmosphere of the earth was so reducing that the evolved O_2 must have reacted immediately with the reduced gases of the atmosphere. However, when the hydrogen concentration in the atmosphere was slowly decreasing (by escape into interstellar space and perhaps by biological activity), early life on this planet may have become exposed to the first traces of oxygen. Thus the proteins and enzymes necessary for dealing with this reactive compound and products of its reduction may

have started to evolve. Meanwhile, anaerobic photosynthetic organisms may have evolved.

Pfennig's proposition (this volume) is that the sulfur cycle preceded the O_2 cycle as the predominant cycle to provide reducing power for photosynthesis. One may speculate that this started as a simple $S^{2-} \rightleftharpoons S^{o}$ cycle that developed to a $S^{2-} \rightleftharpoons SO_4^{2-}$ cycle. This seems a very attractive hypothesis, since photosynthetic bacteria can oxidize S^{2-} to SO_4^{2-} under anaerobic conditions, thereby providing a reactive electron acceptor for anaerobic respiration. Furthermore, they could have developed carotenoids which were, later in evolution, needed for protection against singlet oxygen in oxygenic photosynthetic organisms. At the same time the anaerobic photosynthetic organisms might have acquired some mechanism to deal with traces of oxygen in the atmosphere. This supposition makes it easier to envisage those circumstances under which oxygenic photosynthesis could have evolved. It is important to realize that many proteins that contain identical chromophores can have completely different reactivity and redox potential depending on the character of the protein chain. Thus by a change in the protein, a chromophoric protein originally designed for detoxifying oxygen may have evolved into an oxygenic protein.

Once oxygenic photosynthesis started, the rate of oxygen production is thought to have become several orders of magnitude larger than the rate of oxygen production by photolysis. By that time, or even earlier, organisms may have evolved that could take advantage of ever increasing thermodynamic potential (between oxidized and reduced compounds). The use of higher potentials for energy transduction increased the danger of oxidative damage. This was the price to be paid for the higher gain of ATP. And so more and more sophisticated means of protection against the toxicity of oxygen were developed.

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