(which initiate sedimentation) and their reversals (*r*). Formation of particulate metals (*p*) can proceed by (microbially mediated oxidation) oxidation (Fe(II) → $Fe(OH)_{3(s)}$, Mn(II) → $MnOOH_{(s)}$), by incorporation into the biomass and by adsorption at inorganic particulates ($Fe(OH)_{3(s)}$, $MnOOH_{(s)}$, clay minerals). The actual extent of these reactions is dependent on the solution parameters (pH, pE, presence of ligands) *i.e.* the *chemical speciation of the metal in the aquatic environment.* Resolubilisation is caused by low redox potential ($Fe(OH)_{3(s)}$ → Fe(II), $MnOOH_{(s)}$ → Mn(II)), by oxidation of the biological carrier and by desorption from inorganic particulate favored by low pH-values. In addition, metals such as Hg, Sn, Pb and Tl can be remobilized by biomethylation. The usual (biologically induced) distribution of pE and pH favors *p* over *r* near the surface and *r* over *p* near the bottom of the aquatic system. The resulting concentration gradients result in eddy-assisted back diffusion.

The marked increase in metal pollution (as documented by sedimentary record) gives rise to increasing concern for the *impact of metal ions upon the aquatic biosphere.* It is usually assumed that (some) metal ions are limiting at low concentrations and that most are toxic at higher concentrations. The sensitivity towards a given metal is largely dependent on the biological species. It is also generally agreed that availability of dissolved metals for phytoplankton is restricted to aquo ions. Hence, the biological impact of a given metal is again dependent on its chemical speciation. The situation is further complicated by the fact that some algae are able to release organic ligands (ferrichromes). Recent field studies seem to indicate that increase in metal concentration does not basically change the total amount of biota in a given aquatic system; the biological speciation is, however, greatly changed.

B34

The Electronic Structure of Iron Complexes of Bleomycin

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Bleomycin (BLM), a glycopeptide antibiotic, is activated in an Fe-dependent reaction and, when O_2 is present, catalyzes the cleavage of DNA. Drug activation takes place via two pathways. In the first, Fe(II)-BLM, an S = 2 species, and O_2 react to form a diamagnetic, ternary complex, 'oxygenated bleomycin'. The quadrupole splitting and isomer shift (ΔEq = 2.96 mm/sec, δ = 0.16 mm/sec) are suggestive of an electronic structure best described as low spin ferric bound to superoxide anion. Single electron reduction of oxygenated bleomycin yields 'activated bleomycin', a form of the drug that is kinetically competent to cleave DNA. As a second pathway for activation, Fe(III)-BLM reacts with peroxide; Fe(II)-BLM is not produced as an intermediate.

Activated bleomycin is an S = ½ species with a well resolved EPR spectrum (g = 2.26, 2.17, 1.94). When prepared with ^{57}Fe, the g = 1.94 EPR feature is split by 22 gauss, demonstrating that the electron spin resides primarily on the iron. When activated bleomycin is prepared from Fe(II)-BLM and $^{17}O_2$, the EPR spectrum is broadened, demonstrating the presence of at least a single oxygen atom derived from O_2.

Concomitant with DNA cleavage, activated bleomycin decomposes to form Fe(III)-BLM, an S = ½ species with EPR features (g = 2.45, 2.18, 1.89) and Mössbauer parameters (ΔEq = 2.85 mm/sec and δ = 0.20 mm/sec) closely resembling those of cytochrome P-450. Fe(III)-BLM, in the presence of phosphate or arsenate, or at pH below 4.5, becomes high spin with g_{eff} = 4.3 at 77 °K (E/D ~ 0.3). Yet, Fe(III) remains bound to the drug.

The bonding of iron to BLM is affected by the presence of DNA. Changes are observed in the g = 1.94 EPR feature for the activated complex, and in the hyperfine interaction with ^{14}N in the low spin ferric complex, as determined from differences in the electron spin echo spectrum. DNA also prevents the conversion of the iron in the low spin ferric complex to an S = 5/2 species by phosphate or arsenate.

B35

Chemical Aspects of Structure, Function and Evolution of Superoxide Dismutases

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The superoxide dismutases (SOD) constitute a class of metalloproteins having either Cu/Zn, Mn or Fe as their prosthetic group and their function is to dismute O_2^- to H_2O_2 and O_2. The distribution of the SODs has to be considered in the light of the acquisition of a permanent defence by organisms against any form of toxicity arising from the increase, by photosynthetic organisms, at atmospheric oxygen. Anaerobic sulphate reducing bacteria and fermenta-

tive anaerobic bacteria were found to contain the enzyme, probably the iron form. This is considered to be the most primitive form of SOD. In general terms the distribution of SOD can be stated to be that the Cu/Zn enzyme is essentially a eukaryotic enzyme and the iron enzyme is essentially a prokaryotic enzyme. The manganese enzyme can also be considered to be a prokaryotic enzyme. It is, however, also found in mitochondria. Whilst certain species of bacteria have been found to have one form of SOD *i.e.*, either the Fe or the Mn form, there are species with both forms as well. It appears that which form of the enzyme is present depends on the growth conditions. The phototrophic purple sulfur bacterium *Thiocupsa roseopersicina* grown in the presence of acetate or glucose contains Fe-SOD. Growth on media with glucose but not acetate leads to the formation of Mn-SOD [1]. The synthesis of either Fe- or Mn-SOD with apparently identical protein moiety by *Photobacterium shermanii* was found to be conditional on the metal supply [2]. All eukaryotic species have the Cu/Zn and Mn form of the enzyme. Some plant species have the Fe form as well whilst two bacterial species have the Cu/Zn enzyme [3].

The Cu/Zn SOD is a homodimer of about 32,000 daltons. The molecular weight determined for the enzyme from a variety of sources are all substantially in agreement. By contrast, the Mn- and Fe-forms have a slightly higher molecular weight of about 40,000 daltons. Whilst all the Cu/Zn and the Fe-SODs have been shown to be dimeric, the manganese enzyme has been found to form tetramers. The extent of polymerisation does not apparently depend on the source of the enzyme.

The Cu/Zn SOD contain up to 2 g atoms of both Cu and Zn calculated on the basis of a molecular weight of 32,000 daltons. The values reported in the literature is 1.8 g atoms. The metal content of the Fe and Mn enzymes varies between 1 and 2 g atoms per dimer. Whether this variation is due to loss of metal content can only be determined once the number of metal binding sites is determined from the X-ray structure. Only preliminary data has so far been presented on the X-ray structure for this protein. The complete sequence has only been determined for the Mn enzyme from *E. coli* and *B. stearothermophilus* and yeast mitochondria [4–6]. The sequence homology is low compared to the homologies observed between the Cu/Zn enzyme. The nature of the Mn and Fe ligands is as yet unknown. Preliminary spectroscopy evidence, however, indicates that a tyr could not be a ligand.

The Cu/Zn SODs have been more extensively investigated than the Fe- and Mn-SODs. The primary structure has been determined for the enzyme from bovine [7] and human erythrocytes [8, 9], horse liver [10], yeast [11, 12], swordfish liver [13] and the free living symbiotic bacterium *Photobacterium leiognathi* [14]. Extensive homology has been found to occur between the eukaryotic enzymes.The presence of a Cu/Zn SOD in *P. leiognathi* has led to speculation about a possible gene transfer from a eukaryotic to a prokaryotic species. However, the amino acid sequence only shows a 20–25% structural homology between this enzyme and the other eukaryotic SODs indicating an independent evolutionary line. All the Cu/Zn SODs have, however, a conserved metal binding site determined from the X-ray structure of the bovine enzyme. These are the histidines 47, 49, 76, 81, 134 for the Cu site and the histidines 76, 85, 94 and aspartate 97 for the zinc site. Arginine 157 claimed to be essential for activity is also conserved. However cysteine 125 is only present in the human enzyme.

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B36

Iron and Manganese Superoxide Dismutases

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Fe and Mn SDs are enigmatic metalloproteins for several reasons: They are found in strict anaerobes as well as aerobes. In some cases the Fe and Mn ions