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THE EFFECT OF OXYGEN AND THIOLS ON THE RADIATION DAMAGE OF DNA

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In 1960 Howard-Flanders[1] suggested that the protecting effect of thiols (RSH) against high-energy irradiations of phages besides OH scavenging could be due to H donation from RSH to DNA radicals. The protective action of sulhydryls on mice by H donation was already suggested by Prevot-Bernas,[2] 1953. Alexander and Charlesby,[3] 1955, introduced the competition theory but interestingly rejected H donation by thiols as protective mechanism in biological systems with several arguments. Studying radiation effects on biologically active DNA in aqueous solution, Hutchinson[4] clearly showed in 1961 that an oxygen effect is only observed when thiols are present in the solution. In the absence of thiols the biological consequences of a DNA damage are virtually the same in the presence and absence of oxygen. From these and further results it was concluded that oxygen and thiols compete for the reaction with DNA radicals. Reaction with thiols "repair" and reaction with oxygen lead to biologically significant damage. This is the chemical basis for the oxygen fixation hypothesis.[5] Adam and Jameson[6] and Michaels and coworkers[7] using pulse methods presented kinetic evidence that in bacterial and in mammalian cells oxygen and thiols compete for DNA radicals on a ms time scale and that this competition determines survival. Revesz[8] demonstrated that this hypothesis is able to explain the effect of added thiols obtained by steady state irradiation of mammalian including human cell lines deficient in glutathione (GSH). The author discussed the possibility that GSH may act as a cofactor in enzymatic repair processes.[8] Unexpectedly, Bresler and coworkers[9] found with *E. coli* strains that the protection effect due to added thiols decreased when bacterial strains were used with various deficiencies in enzymatic repair capacities. The validity of these experiments was confirmed.[10] Breslers results create a problem. Why is a strong influence of slow enzymatic repair observable on the one hand when on the other hand the effect of thiols and oxygen can basically be explained by fast free radical reactions in the cell? This question was further studied in our laboratory by measuring the survival of γ-irradiated *E. coli* strains carrying various deficiencies in their enzymatic repair capacities.[10,11] The survival was determined as a function of the concentration of various thiols in the presence of air. The results show that at concentrations of cysteamine up to 1 mM a small protection (dose modifying factor, DMF = 1.2–1.4) was found in wild type cells but not in enzymatic-repair-deficient strains. The DMF values obtained are in the range of values determined also by other methods.[12] Above a concentration of $\sim$ 5 mM of cysteamine a strong increase of the protection was found with wild type cells but a smaller increase with enzymatic repair-deficient strains. These results may be explained as follows. At concentrations of cysteamine up to 1 mM (dithiothreitol which is not oxidized in aerated solution gives similar results) the influence is restricted to that on enzymatic processes since interaction with free radicals is not expected at such low concentration, since the concentration of endogenous glutathione is already high (7 mM).[13] At concentrations

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above 5 mM a competition sets in between the reaction of oxygen and the added thiol for DNA radicals. At a concentration of ~ 50 mM all DNA radicals which can react with thiols have reacted. If this reaction would lead to restoration of the original DNA the DMF values should be the same for the various *E. coli* strains. However, this is not the case. In enzymatic-repair-deficient strains smaller DMF values were observed throughout. With decreasing D_0 (D_0 characterzies the linear part of the slope of a survival curve) the various repair-deficient strains show a decrease of the DMF value for protection by cysteamine.[11] One possible explanation is the following hypothesis which we call the damage modification (dm) hypothesis. It is known that the "repair" by H donation from the thiols to DNA radicals does not lead to the original structure but to a modified one. The dm hypothesis postulates now that the modified structures in DNA are easily repaired enzymatically in wild type strains but in enzymatic repair-deficient strains the modified strutures are not repaired as well. Depending on the kind of repair deficiency and on the kind of modified damage the DMF varies with the strain studied, with pH and with the kind of thiol. Examples which demonstrate the change of the structure due to "repair" by H donation from a thiol are known.[14-18] The dm hypothesis leads to the consequence that the oxygen enhancement factor (OER) should decrease with the DMF values for protection by thiols by a comparable extent because in the extreme case of DMF = 1 (absence of protection by added thiols) the radical sites at the DNA should result in the same yield of biological damage as if they had reacted with oxygen. A decrease of the OER has been observed with decreasing D_0 values for *E. coli* strains[19,20] and also with glutathione-deficient fibroblast cells.[21] Alper explains the decrease in OER by assuming two different primary targets, the DNA and the membrane, and two different kinds of damage.[20] Davies assumed that in the case of Chlamydomonas with an OER = 1 the cells lack a repair system for lesions incurred in the absence of oxygen.[22] In the dm hypothesis it is assumed that the non-repaired lesions in strains with low OER are those which have been modified by reaction with a thiol.

The dm hypothesis demands that differently damaged sites at the DNA should be repaired differently well by different enzymatic repair systems. This has been shown to be generally the case by several authors.[23,24]

In addition to the modification of the structure of the DNA by "repair", the thiols have an effect on various enzymatic reaction of cells. To what extent the differences observed in the effects of thiols as a function of their structure are due to an influence on enzymatic repair processes or due to differences in their chemical reactivity with DNA radicals has to be clarified by further experiments.

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