PHOTODYNAMIC DEGRADATION OF GUANINE.

J.S. Sussenbach and W. Berends.

Biochemical and Biophysical Laboratory of the
Technological University, Julianalaan 67, Delft,
The Netherlands.

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The photodynamic inactivation of deoxyribonucleic acid (DNA) by visible light in the presence of certain dyes was reported by Bellin and Oster in 1960. Recently it was observed (Simon and Van Vunakis, 1962) that visible light caused destruction of the guanine moieties in DNA if methylene blue and oxygen were present. We investigated the photodynamic action of the dye lumichrome on DNA (Sussenbach and Berends, 1963). The B.subtilis transforming principle was found to be very rapidly inactivated on irradiation. In spectrophotometric studies of deoxyribonucleotide solutions containing lumichrome only deoxyriboguanylic acid (dGMP) appeared to be changed on irradiation with a high-pressure mercury lamp (Philips HP 125). No reaction occurred in a nitrogen atmosphere so an oxidation of guanine was likely. The oxygen consumption was found to be 1.2 molecule per molecule nucleotide, while 0.8 molecule CO_2 was formed.

Experiments with DNA containing $8\text{-}^{14}C$ guanine revealed that also in this polymer the oxidation of guanine took place. At least 80% of the guanine could be oxidized in this way. The report deals with further studies on the photodynamic action of lumichrome on guanine.

Autoradiography of thinlayer chromatograms of irradiated solutions of $2\text{-}^{14}C$ and $8\text{-}^{14}C$ guanine showed many labelled compounds. Even when small doses of visible light were used at least four products could be detected. By using $6\text{-}^{14}C$ guanine it could be demonstrated that about 50% of the total radioactivity was eliminated as CO_2. This amount of CO_2 accounted for a great deal of the 0.8 molecule CO_2 per molecule dGMP observed on irradiation of this compound by visible light in the presence of lumichrome.

The carbon dioxide formation as well as the guanine degradation showed a short but significant lag period (fig. 1).

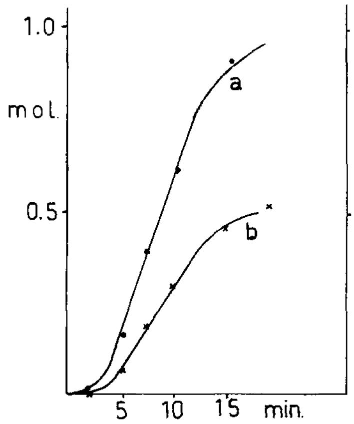

Figure 1. Guanine destruction(a) and carbon dioxide formation(b) upon irradiation of 2 µC 6-^{14}C guanine (specific activity 1,5 mC/mM) and 14 µg lumichrome in 3 ml water with two Philips HP125 light sources at a distance of 20 cm.

Though the main part of the radioactivity of 6-^{14}C guanine was found as $^{14}CO_2$ several ^{14}C-containing spots could also be detected on a thin-layer chromatogram. The most active spot, however, contained no more than 15% of the original radioactivity of the 6-^{14}C guanine.

If 2-^{14}C guanine was used as test compound a short lag period could also be demonstrated. On a two-dimensional chromatogram several spots could be demonstrated. One of these contained about 50% of the radioactivity. This product could be identified chromatographically as guanidine. So the C-2 atom from guanine is split off in guanidine.

The rate of the CO_2-evolution from 6-^{14}C guanine and the

rate of the guanidine formation from 2-^{14}C guanine were practically identical. The amount of CO_2 and guanidine were also the same so we assume that they arise from the same intermediate.
When 8-^{14}C guanine was irradiated, about 20% of the radioactivity was eliminated as CO_2. As mentioned before 0.8 molecule CO_2 is produced by irradiation of dGMP. From this 0.5 molecule CO_2 originates from the C-6 atom while the remainder is from C-8. At the moment we are engaged in the identification of the main labelled product of 8-^{14}C guanine which does not contain the C-2 or C-6 atom.
So the oxidation of guanine is rather complicated; one of the reactions is responsible for 50% of the degradation.
The reaction leading to guanidine and CO_2 among other products also takes place in DNA. The presence of guanidine could be demonstrated on chromatograms of DNA, irradiated in the presence of lumichrome and air. When DNA was irradiated in a nitrogen atmosphere no guanidine could be detected.

The lag period observed during the guanine degradation was very striking and we supposed peroxides might be involved in the oxidation of guanine. A great amount of peroxides was found indeed if irradiated solutions of guanine or DNA were tested with starch-KJ or ferrosulphate-rhodanide. No peroxides were formed in solutions of lumichrome alone. Almost immediately after the beginning of the irradiation peroxides could be detected in guanine solutions. The peroxide formation was measured according to Aebi et al. (1959), see fig. 2. If the irradiation was interrupted half-way in the reaction, no further degradation occurred while the amount of peroxides stayed unchanged. On renewed irradiation the reaction resumes its original velocity, however, without a lag period.

As described in earlier papers the reaction mechanism of this photodynamic destruction of guanine and guanine-derivatives must be based upon an energy transfer between excited lumichrome and guanine-residues. The activated guanine is supposed to react with oxygen, so the peroxides may arise. Apparently these peroxides are rather stable

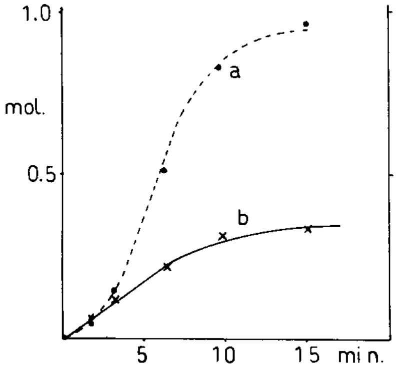

Figure 2. Guanine destruction(a) and peroxide formation (b) upon irradiation of 4 μC 6-^{14}C guanine (specific activity 1,5 mC/mM) and 36 μg lumichrome in 6 ml water with two Philips HP125 light sources at a distance of 20 cm.

for they only disintegrate if irradiated. One of the decomposition products also shows a positive peroxide-reaction (a maximum value of 0.3 mole of peroxide is reached).

References.

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