Control of DNA photocleavage by oligothiazole derivatives

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ABSTRACT
Mechanism of DNA damage by the p-cyanobenzamide (pCyBz)-oligothiazole derivatives were investigated by HPLC analysis. The oxidation product of dG by the photocleaver was imidazolone (dlz).

INTRODUCTION
Oxidative DNA damage by small ligands is currently attracting a great deal of attention. We have been studying a series of compounds consisting of an oligothiazole moiety linked to the p-cyanobenzamide (pCyBz) group (Fig. 1). The bis- and ter-thiazole derivatives 2b and 3b bind to duplex DNA in the minor groove and selectively cleave DNA at the 5'-G of 5'-GG-3' sequences on UV irradiation. These photocleavers are thought to abstract one electron from a guanine base of DNA. To examine this hypothesis, the oxidation products of guanine were analyzed by HPLC.

RESULTS AND DISCUSSION
The pCyBz-oligothiazole derivatives oxidize guanines especially 5'G of 5'-GG-3'. The analysis of the guanine oxidation products shall reveal sites of attacks on DNA and at the same time, the oxidation pathways by the photocleavers. Thus, the pCyBz-terthiazole derivatives, 3b (Fig. 1) was irradiated in the presence of mononucleoside 2'-deoxyguanine (dG) and the reaction products were analyzed by HPLC. A new peak (peak 1) appeared concomitant with the disappearance of the peak corresponding to dG (Fig. 2b). To identify the photooxidation products, the retention time was compared with that of an authentic sample of one electron oxidation product of dG by riboflavin. Various conditions such as column materials, elution buffers and gradients were tested beforehand and the two oxidized samples prepared by 3b and riboflavin were coinjected. Peak 1 showed the same chromatographic behavior as the peak of the authentic sample and thus identified as (2S)-2,5'-anhydro-l-(2'-deoxy-D-erythro-pentofuranosyl)-5-guanidinylidene-2-hydroxy-4-oxoimidazolidine, (imidazolone: dlz). It was also shown that upon incubation of the photolysate prepared by 3b or by riboflavin at 37 °C for 6 h, a new peak (peak 2) appeared with concomitant disappearance of peak 1 (Fig. 2c). Raoul et al. have shown that the initially generated oxidized nucleoside dlz decomposes spontaneously to 2,2-diamino-4-[2-deoxy-β-D-erythro-pentofuranosyl]amino-2,5-dihydroxazol-5-one (oxazolone: dZ) in aqueous solution. To identify the oxidation products by the photocleaver, hydrolysis of the photoprodut corresponding to peak 1 was performed and monitored by UV spectroscopy as a function of time (Fig. 3).
presence of an isosbestic point ($\lambda_{\text{max}} = 242\, \text{nm}$) and absorption maximum at 255 nm are similar to the reference data$^3$ (isosbestic point: $\lambda_{\text{max}} = 234\, \text{nm}$ and absorption maximum: $\lambda_{\text{max}} = 254\, \text{nm}$) indicating that peak 2 corresponds to dZ. Together with this spectroscopic evidence, it is no doubt that the oxidation products is d1Z. Compound d1Z and its hydrolysis product dZ were first described by Cadet et al.$^4$ as the major oxidation products of dG in the presence of hydroxy radicals or direct electron transfer by type I photosensitizers. The presence of 8-oxoG, the well known oxidation product of guanine, in the reaction prod

Fig. 2
HPLC profiles of the photoreaction mixture produced by irradiation of dG and dA (a), irradiation of dG and dA with 3b in a sodium cacodylate buffer (50 mM, pH 7.0) at 310 nm for 12 h at 4°C (b), and incubation of (b) for 6 h at 37°C (c). The concentration of dG and dA were 250 µM and that of 3b was 300 µM.

Fig. 3
UV absorbance spectrum, as a function of time, of an aqueous solution d1Z prepared by 3b. Incubation was performed at 37°C.

REFFERENCES