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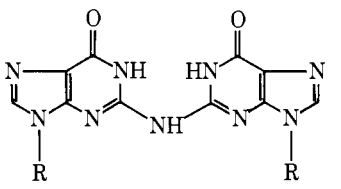
Isolation and Identification of Cross-Linked Nucleosides from Nitrous Acid Treated Deoxyribonucleic Acid

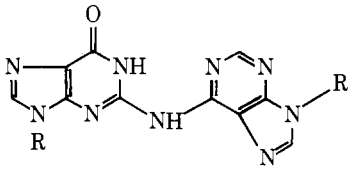
Sir:

Treatment of DNA with nitrous acid covalently cross-links the two strands of the double helix.[1,2] This reaction leads to inactivation[3] and perhaps to deletion mutations[4] in bacteriophage. The chemical structure of the cross-links has not been determined, although this knowledge is needed in the study of DNA repair processes.[5] A recent suggestion that the cross-links arise from the reaction of aldehyde groups, liberated by depurination, with amino groups on the opposite chain[6] has now been withdrawn.[3] We have approached this problem directly, by isolating cross-linked nucleosides from nitrous acid treated DNA. We wish to propose I and tentatively, II, as the structures of two such products.

Calf thymus DNA (500 mg) was treated with 1 M $NaNO_2$ at pH 4.2 and 25 °C for 24 h. At the end of this time, the product (T_m 75 °C) had at least one cross-link per molecule,



I



II

a, R = 2-deoxy-β-D-ribofuranosyl 5'-phosphate
b, R = 2-deoxy-β-D-ribofuranosyl

even after sonication, as measured by the ultraviolet assay for reversible denaturation.[1,2] The product was freed of salt by dialysis, and hydrolyzed with deoxyribonuclease I and snake venom phosphodiesterase. The mixture was fractionated by DEAE-Sephadex chromatography using a LiCl gradient in the presence of 7 M urea.[7] Mononucleotides were eluted, followed by dXMP, and then a series of small peaks containing the cross-linked dinucleotide Ia, and oligonucleotides resulting from inhibition of enzymatic hydrolysis of the modified DNA. Each of these latter peaks was desalted,[8] treated with alkaline phosphatase, and subjected to Sephadex G-25 chromatography, in water. This procedure converted Ia (Ve/Vo 1.0) to Ib (Ve/Vo 1.9). The mobility of the oligonucleotide peaks (Ve/Vo 1.0) was not altered substantially by this treatment, as they retained internal phosphates.

Compound Ib (yield 12.5 A_{290} units) was homogeneous in an anion exchange high pressure liquid chromatography system, and had the following properties: λ_{max} (nm) 260, 300 (pH 2.5), 292 (pH 7.0), 250, 260, 290 (pH 13); pK_a values, 5.6 and 10.8, the NMR (in D_2O, Fourier transform) showed an aromatic proton (δ 8.1) and a set of peaks due to deoxyribose[9] in a 1:1 ratio. The compound resisted reduction by sodium dithionite, which excluded the presence of nitro, nitroso, azo, azoxy, or diazoamino functions. The pK_a of Ib was shifted from 5.6 to 7.8 in the presence of glyoxal,[10] which suggested the presence of the adjacent N-1 and amino functions of guanine.

After trimethylsilylation[11] the mass spectrum[12] of Ib showed a molecular weight of 1021, which from comparison with the trimethylsilyl-d_9 derivative[13] (M = 1075) gave a molecular weight of free Ib of 571. Measurement of exact mass (1021.4426, found) supported $C_{20}H_{16}N_9O_8(SiMe_3)_7$ (1021.4436, calcd), corresponding to two deoxyguanosine molecules minus NH_3. The principal fragmentation pathway showed sequential loss of each sugar moiety with hydrogen rearrangement to give *m/e* 761 and 501, which are analogous to common nucleoside reactions[14] and militate against a sugar-sugar linkage. N,O-Permethylation (CD_3)[15] produced a similar mass spectrum (M = 618) in which the base-base linkage was maintained in the principal fragment ions, as required for the proposed structure. Treatment of Ib with D_2O for 1 h at 80 °C resulted in 2 amu shifts for all base-containing ions, in accord with two unsubstituted C-8 moieties.

Structure Ib is consistent with the properties and origin of the compound, as well as the known thermal and base stability of the cross-links induced by nitrous acid in DNA.[16] To confirm its origin, we allowed 500 mg of dGMP to react with nitrous acid under the conditions used for DNA, and isolated 8.2 A_{290} units of Ia, after a DEAE-Sephadex workup. A possible mechanism for the formation of I involves diazotization of a guanine amino group, and attack at the position by a second

guanine amino group, with loss of nitrogen.

Each of the oligonucleotide peaks from the DNA-nitrous acid reaction was further hydrolyzed ith spleen phosphodiesterase and alkaline phosphatase. The hydrolysates were fractionated by anion exchange high pressure liquid chromatography. By this procedure, a second cross-linked nucleoside product, IIb, was obtained (yield 5 A_{310} units). This compound did not reduce with sodium dithionite and had the following ultraviolet spectrum: λ_{max} 306 nm (pH 6); λ_{max} 258, 301, 344 nm (pH 10.5), pK_a = 7.9. Mass spectra of the trimethylsilylation[11] and N,O-permethylation[15] (CD_3) products of II showed molecular weights (933 and 603, respectively) consistent with combined molecules of deoxyguanosine and deoxyadenosine minus NH_3. Exact mass measurement of the base-base + 2H ion (*m/e* 303.1454 found, 303.1445 calcd for $C_{12}H_5N_9OD_6$) confirmed the presence of the latter structural unit. Similar to the case of Ib, principal fragment ions showed retention of base-base units with typical sugar fragmentation.[14,15] The structure shown for II is based upon the above data, and analogy to I.

We believe that our results demonstrate the possibility of direct isolation and determination of the structures of DNA cross-links. (It should be noted that the conditions used above, and by others[1,2] for the study of cross-linking by nitrous acid involve a greater extent of reaction than those employed for mutagenesis. It is possible that other chemical transformations may predominate under the latter set of conditions.) Our isolation method, which will be published in detail elsewhere, should be applicable to other cross-links of interest.[17]

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Heterogeneous Photocatalytic Oxidation of Cyanide Ion in Aqueous Solutions at TiO_2 Powder

Sir:

There has been much recent interest in photoelectrochemical processes at single-crystal and polycrystalline TiO_2 electrodes and those of other semiconductors.[1-3] While the photoinduced oxidation of water to oxygen has been the subject of most investigations, oxidations of other species, such as I^-, Br^-, hydroquinone, and *p*-aminophenol at TiO_2 have also been demonstrated.[4,5] We were intrigued by the possibility that TiO_2 power could be employed as a heterogeneous photocatalyst for useful chemical processes and that the principles and measurements obtained with photoelectrochemical studies at semiconductor electrodes could be applied to these systems as well. Most studies of semiconductor photocatalysis have been concerned with gas phase reactions;[6] solution studies are much less common. An excellent review of this field has been given by Freund and Gomes.[7]

We report here the photocatalyzed oxidation of cyanide ion, a frequent industrial pollutant, with oxygen in the presence of TiO_2 in both the anatase and rutile forms. The reaction was studied at several cyanide concentrations and with illumination from either a 450-W xenon lamp, a 2.5-kW mercury-xenon lamp, or unfocused sunlight; under all conditions the reaction proceeded at a measurable rate. The general procedure involved irradiation of 10-ml solution samples of 0.1 M KOH containing 1 mM to 0.1 M KCN and 0.05 to 0.2 g of TiO_2 in quartz tubes with continuous bubbling of oxygen. The amount of cyanide which reacted was determined by potentiometric titration with a standard silver nitrate solution.[8] Typical results are given in Figure 1 and Table I. All TiO_2 was prepared from Matheson, Coleman and Bell reagent powder, with a particle size below 1 μm.

Four forms of TiO_2 were investigated: undoped anatase (the white form of the untreated commercial material), anatase reduced in a hydrogen gas stream at 700 °C (causing conversion to the black doped form containing about 5% rutile), anatase converted to about 70% rutile by heating in air at 1200 °C, and 70% rutile reduced in H_2 at 700 °C (resulting in doping, and conversion to about 90% rutile). The results were essentially independent of the amount of TiO_2 employed in the 0.05–0.2 g range, but were highly dependent upon the placement of the sample tube in the light beam. Control experiments with irradiated solutions in the absence of TiO_2, or TiO_2 containing solutions in the absence of irradiation showed that no or very little oxidation of CN^- occurred under these conditions. When nitrogen was bubbled through the solution during irradiation, the rate of CN^- oxidation decreased to less than 10% of the value found with oxygen bubbling.

The results shown in Figure 1 for high intensity radiation show that the rate of CN^- disappearance was essentially in-

Table I. Percent CN^- Removed by Illumination of Oxygen Saturated Solutions[a]

TiO_2 sample	Light source	Illumination time	% removed
Undoped anatase	450-W xenon	30 min	54
Doped anatase			31
Rutile			9
Doped rutile			9
No TiO_2			<1
Undoped anatase	Sunlight	2 days	>99
Doped anatase			>99
No TiO_2			<1

[a] 10 ml of 1 mM KCN in 0.1 M KOH solutions contained in quartz tube. Essentially the same results are obtained without KOH.