Pulse Radiolysis of Aqueous Solutions of Deoxyribonucleotides and of DNA: Reaction with Hydroxy-radicals

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THE chemical and physicochemical aspects of the radiolysis of dilute aqueous solutions of nucleic acids and related compounds have been extensively studied.[1] The recent advent of the pulse-radiolysis technique has enabled some of the primary radiolytic events which occur in these systems to be studied directly; some findings in this field have already been reported.[3] We have investigated the spectral and decay characteristics of the transients formed from reaction of hydroxy-radicals with these solutes, and present results from solutions of purine and pyrimidine deoxyribonucleotides and of calf thymus DNA. The experiments were carried out with the electron pulse generators at Mount Vernon Hospital, Northwood, Middlesex, and at the Christie Hospital, Manchester. Single 0·2 μsec. pulses of 1·8 or 4 Mev electrons were used, the dose being *ca.* 1·5 krads/pulse. Optical measurements of the pulsed solutions were confined to wavelengths greater than 300 nm., because of the strong optical absorption of the solutions themselves below this wavelength.

The transients formed from reaction with hydroxy-radicals were studied in dinitrogen oxide-saturated solutions (1 atmos.). Nitrous oxide removes the solvated electrons,

$$N_2O + e^-_{aq} \rightarrow OH + OH^- + N_2$$

thus approximately doubling the yield of available hydroxy-radicals (Although reactions of hydrogen atoms with the solutes may also occur, effects due to these will be relatively small since the yield of hydrogen atoms under these conditions is less than 10% of that of the oxidising species.)

The optical absorption spectra of the transients from pulsed nitrous oxide-saturated solutions of the 5′-deoxyribonucleotides of adenine, guanine, thymine, and cytosine, and also of deoxyribose-5′-phosphate are given in the Figure (a and b). All solutions were at a concentration of 6×10^{-4}M and were adjusted to neutral pH. The spectra were recorded immediately after the pulse. The results show that the absorptions in the nucleotide solutions at wavelengths >300 nm. are mainly due to radical reactions with the heterocyclic base components. This is consistent with results from stationary state experiments,[4] which have indicated that in the pyrimidine nucleotides only 15—20% of the radiation-produced hydroxy-radicals react with the sugar, and that in the purine nucleotides just 25—30% react in this way; therefore, the contribution to the transient absorptions by reaction with the sugar component must be small. The absorptions in the thymidylic and cytidylic acid solutions decay in a few msec. and can be adequately described by a second-order rate law. Here, the bimolecular reactions presumably involve nucleotide radicals which are predominantly hydroxy-adducts to the 5,6-pyrimidine double bond.[4] The decay characteristics of the transients in the irradiated purine nucleotide solutions are more complex. An initial first-order process (half life *ca.* 30 μsec.) which is followed by a second-order disappearance of the transient absorption suggests that the purine hydroxy-adducts can

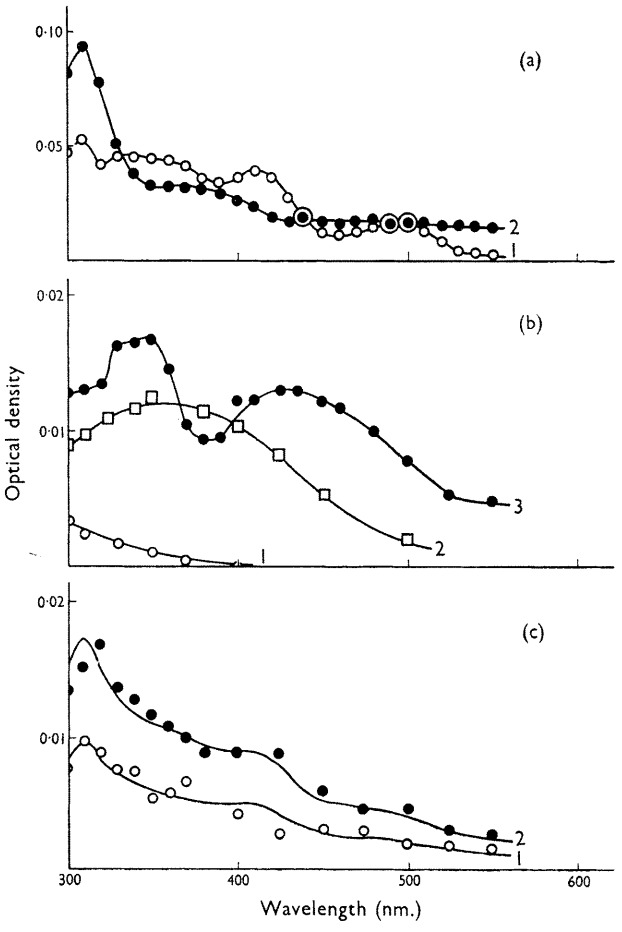

FIGURE. *Transient optical absorption spectra in pulsed* N_2O*-saturated solutions.* (a) *Purine nucleotides* (6×10^{-4}M; pH 7·7). *Spectra taken immediately after pulse; dose* 1·5 *krad;* 1, *deoxyadenylic acid;* 2, *deoxyguanylic acid.*

(b) *Pyrimidine nucleotides and deoxyribose phosphate* (6×10^{-4}M); *spectra taken immediately after pulse; dose* 1·5 *krad;* 1, *deoxyribose-5′-phosphate,* pH ca. 6; 2, *thymidylic acid,* pH 7·5; 3, *cytidylic acid,* pH 8·0.

(c) *DNA* [0·01% (*w/v*) *in* 3×10^{-3}M-*Sørensen phosphate buffer,* Ph 7·5]; *spectra taken* 15 μsec. *after pulse; curves are computed* (*see text*); 1, *single pulse of* 1·5 *krads;* 2, *solution pre-irradiated by pulsing* (18·5 *krad*) *followed by single pulse of* 1·5 *krads.*

undergo intramolecular changes, or other first-order processes (*e.g.* hydrolysis), prior to their disappearance by radical–radical interaction.

Pulse-radiolysis studies of DNA solutions (0·01% w/v Sigma Chemical Co. calf thymus DNA) were carried out in the presence of phosphate buffer (10^{-3}M) in order to prevent denaturation. At this concentration the rate of transient formation could be followed and was found to be exponential (half-life 2·4 μsec., both at 310 and at 420 nm.). This corresponded to an overall bimolecular rate constant (k_{OH+DNA}) of $1{\cdot}3 \times 10^{13}$M^{-1} sec.$^{-1}$, with a weight average molecular weight for DNA of 5×10^6. (Alternatively, the data imply an average rate constant per nucleotide base-group of DNA of 8×10^8M^{-1} sec.$^{-1}$.) Previous pulse studies[2] have shown that a 0·01% DNA solution has an overall reactivity towards hydroxy-radicals equal to that of a 10^{-5}M-thiocyanate solution. Recent experiments[5] indicate that $k_{OH+CNS^-} = 1 \times 10^{10}$—$2 \times 10^{10}$M^{-1} sec.$^{-1}$, which gives $k_{OH+DNA} = 0{\cdot}5 \times 10^{13}$—$1 \times 10^{13}$M^{-1} sec.$^{-1}$, a value in fairly good agreement with that obtained above. The transient optical absorption spectrum in these DNA solutions, taken 15 μsec. after the end of the pulse to allow for the build-up, is shown in the Figure (c). Given that sugar radicals do not absorb at wavelengths >400 nm., it seems that again one is dealing mainly with transients arising from attack on the base components of the polynucleotide. The absorption increased with further pulsing of the solution (allowing the radicals to decay between each pulse), eventually becoming *ca.* 80% greater after a total dose of 20 krads [Figure (c)]. At the same time the rate of build-up of transient increased (half life 1·7 μsec. after 20 krads) indicating an enhanced reactivity of the denatured DNA towards hydroxy-radicals.

We compared the spectra from the pulsed polynucleotide solutions with a weighted-average spectrum of the separate mononucleotides. The latter was calculated for a 6×10^{-4}M (total) nucleotide solution, assuming equal reactivities of the solutes and taking into account an AT : GC ratio of 58 : 42 in the DNA sample used. Curves (1) and (2) of the Figure (c) were obtained empirically by dividing the computed optical densities by 4·4 and 2·5 respectively. There is fair agreement with experiment, confirming that the observed transient absorption is essentially associated with attack on the purine and pyrimidine bases of the polynucleotide. The small initial yield of transients in these irradiated DNA solutions is not surprising in view of the low effective scavenger concentration. Of more interest is the observed effect of multiple pulsing. Some increase in the yield of transient with dose would result if the effective scavenging power of the system increased, owing, *e.g.*, to denaturation (strand separation) or fragmentation of the polynucleotide chains. Indeed, a 30% increase in reactivity was noted. In addition, one cannot exclude the existence of some hypochromicity in the base free-radicals when these are incorporated in the double helix; a radiation-induced hyperchromicity would then result on destruction of the secondary helical structure. The magnitude of the increase in the transient yield with dose suggests the possibility that the more organised helical configuration may reduce the extent of reaction of hydroxy-radicals with the bases, as has already been proposed.[6] The decay of the transient absorption in the DNA solutions is quite complex. Although some partial decay is observed over the first few hundred μsec. (particularly noticeable at 300 and 550 nm.) a substantial fraction of the transients appear to be long-lived. No appreciable change in the optical absorption over the time interval of 300—900 μsec. was observed. In view of the low mobility of the macromolecules in solution and also of the fact that free radicals are formed inside the double helix, it is not surprising that the transients from DNA have a relatively long life-time.

This work was supported by a Research Grant from the Medical Research Council. We thank Professor J. J. Weiss for his interest.

(*Received, September 23rd*, 1968; *Com.* 1287.)

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