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RADIATION-INDUCED RELEASE OF UNDAMAGED URACIL FROM POLYURIDYLIC ACID AND THE FORMATION OF PHOSPHOMONOESTER END GROUPS IN IRRADIATED POLYNUCLEOTIDES

DAVID J. DEEBLE

The North Wales Institute, Kelsterton College, Connah's Quay, Deeside Clwyd CH5 4BR, UK

CLEMENS VON SONNTAG

Max-Planck-Institut für Strahlenchemie, Stiftstrasse 34–36, D-4330 Mülheim a.d. Ruhr, FRG

The radiation chemistry of free pyrimidines in aqueous solution has been extensively studied and is now in many respects, well understood. In DNA the bases are fixed in a polymer chain and this can cause drastic changes in the types of reactions undergone by the base radicals. Polyuridylic acid (poly(U)) was chosen as a model system for study since it contains only one base, uracil, whose radiation chemistry is well documented and is a single stranded polynucleotide existing in solution as a random coil.

Product identification and quantitisation following irradiation of nucleic acids is often difficult since the products usually remain bound to the polymer chain. However, easily separable and quantifiable products of nucleic acid radiolysis are the free bases and the initial part of this paper will deal chiefly with the radiolytically induced release of undamaged uracil from poly(U).

Attack by both OH radicals and H atoms on poly(U) results in the formation of 'labile' uracil in relatively high yields, OH radicals leading to uracil release with an efficiency of 51% and H atoms with an efficiency of 28%.[1] Measurement of molecular hydrogen yields showed that H atoms react with poly(U) almost exclusively ($> 95\%$) with the uracil moiety. Since uracil release must occur as the result of sugar damage, the initially formed base radical must be converted to a ribose radical, a similar conclusion can be drawn from OH radical induced uracil release. Previous studies on radiolytically induced strand breakage in poly(U) also lead to the proposal of base radical attack (H abstraction) on the ribose.[2] The fact that undamaged uracil is released shows that the base radical does not abstract an H atom from the ribose to which it is bound but from the ribose of a different nucleotide. The oxidant tetranitromethane (TNM) can be used to measure the radiolytic yields of reducing radicals[3] and following attack by OH radicals and H atoms 81% and 60% respectively of the poly(U) radicals formed are reducing.[4] The presence of TNM during irradiation caused a major reduction in OH radical induced uracil release, (in N_2O saturated 1×10^{-3} M poly(U) G(uracil) fell from 0.3 to 0.02 μmol J^{-1}). From this data it can be calculated that some 7% of OH radicals directly abstract H atoms from the ribose, 23% add to the uracil moiety at C-6 while 70% add at C-5, the latter being the main initial precursor of free uracil.[5]

On investigating the kinetics of uracil release from poly(U) irradiated in N_2O-saturated aqueous solution at least three separate processes can be distinguished.[5] These have been designated according to their rates as, immediate (20%), fast (50%) and slow (30%), the latter two can only be readily followed at elevated temperatures (e.g. at 50°C their half lives are 83 minutes and 26 hours). Hence it would seem that at least three different sugar lesions release uracil.

Radiolysis of N_2O/O_2 (4/1) saturated solutions of poly(U) leads to immediate uracil release with a G value of $0.16\,\mu$mol J^{-1}.[6] Other major products are carbon dioxide ($0.28\,\mu$mol J^{-1}) and osazone forming material ($0.28\,\mu$mol J^{-1}). The high yields of labile uracil are again indicative of base radical mediated sugar damage, in this instance the base peroxyl radical being the species involved.

Strand breakage in a polynucleotide must result in the formation of phosphomonoester end groups. Radiolytic yields of phosphomonoester end groups have been determined by measuring inorganic phosphate released from irradiated polynucleotides by alkaline phosphatase. In N_2O-saturated solutions of poly(U), poly(C), poly(A) and poly(G) radiolytic yields of phosphomonoester end groups were 0.52, 0.17, 0.04 and $< 0.04\,\mu$mol J^{-1} respectively.[7] Although the value for poly(C) agrees with the yield of strand breaks ($0.18\,\mu$mol J^{-1}) measured by light scattering, in poly(U) there are twice as many phosphomonoester end groups produced as strand breaks ($0.23\,\mu$mol J^{-1}).[8] The reason for this is not yet known. In the poly(C) system these high yields again suggest base radical mediated ribose damage. The purine polynucleotides have very low yields of strand breakage and phosphomonoester end group formation.

In the presence of oxygen (N_2O/O_2) initial radiolytic yields of phosphomonoester end groups for poly(U), poly(C), poly(A) and poly(G), were 0.43, 0.21, 0.05 and $< 0.04\,\mu$mol J^{-1}, after post-irradiation heating (95°C, 1 hour) these yields increased to 0.75, 0.51, 0.12 and 0.06 respectively. The marked increases in phosphomonoester end groups induced in irradiated poly(U) and poly(C) by heating could well be associated with the thermal decomposition of polynucleotide hydroperoxides which are present in high yields in both systems.

Although our understanding of the free-radical chemistry of nucleic acids systems continues to increase it is apparent that the processes occurring are complex and many more problems await solution.

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