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Evidence for Reductive Radiolytic Dephosphorylation in the Nucleotide Analogue 8,5′-Cycloadenosine 5′-Monophosphate

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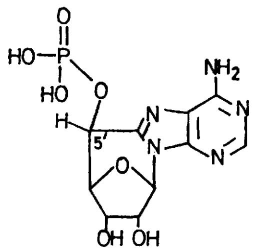
Summary Evidence is presented that hydrated electrons efficiently induce phosphate release from the nucleotide analogue 8,5′-cycloadenosine 5′-monophosphate; hydrogen atoms are ineffective in this process.

REDUCTIVE deamination and dechlorination effected by hydrated electrons (e_{aq}^-) is a well established phenomenon in the radiation chemistry of organic compounds in aqueous solution (*e.g.* refs. 1 and 2). On the other hand, there has been no evidence to suggest that reductive dephosphorylation is a significant process in the radiation chemistry of mono- and di-phosphate esters.[3–6] In keeping with this, the reaction rate constants of e_{aq}^- with these types of esters have been shown to be small ($<10^3\,l\,mol^{-1}\,s^{-1}$).[7] The very efficient release of inorganic phosphate (Pi) in irradiated solutions of 8,5′-cycloadenosine 5′-monophosphate (I, 8,5′-cyclo-AMP)[8] under conditions which clearly implicate e_{aq}^- is therefore interesting.

TABLE. *G* values for phosphate release

Compound	N_2 (e_{aq}^- + OH)	N_2O (2 OH)	N_2 + 10^{-1}M Bu^{t}OH (e_{aq}^-)
8,5′-cyclo AMP[a]	1·90 ± 0·04	0·73 ± 0·05	1·78 ± 0·02
5′-AMP[b]	0·21 (0·17)	0·36 (0·37)	0·02
3′-AMP[b]	0·53	1·02	0·09

[a] *G* values calculated from dose–yield plots except for Bu^{t}OH which is the average of two determinations at 90 krad. *G* values from ref. 5, 160 krad. Values in parentheses from dose–yield plots, this work.



(I)

The results of applying scavenging experiments to the study of the radiation chemistry of 8,5′-cyclo AMP, 5′-AMP, and 3′-AMP are given in the Figure and the Table. Irradiations were performed on solutions of the nucleotides made in triply distilled water at a dose-rate of 11 krad/min (Gammacell 220 ^{60}Co-γ source, Atomic Energy of Canada Limited). Solutions were saturated with N_2 or N_2O ($2{\cdot}5 \times 10^{-2}$M) by bubbling 10 min before and then throughout the time of irradiation. Inorganic phosphate analysis was performed by means of an automated procedure described previously.[4] Somewhat unexpectedly, N_2O was found to inhibit rather than enhance inorganic phosphate release from 8,5′-cyclo AMP (10^{-3}M). This is in contrast to 5′-AMP and 3′-AMP where the presence of N_2O increases the yield of phosphate by a factor of *ca.* 2. Nitrous oxide converts e_{aq}^- formed in the radiolysis of water [reaction (1)][9] to OH [reaction (2)][10] thereby effectively doubling the yield of OH and any radiation chemical change in a solute which is initiated by OH attack. The numbers before each of the species in reaction (1) refer to the number (*G* value) of these species produced per 100 eV of energy absorbed in the solution.

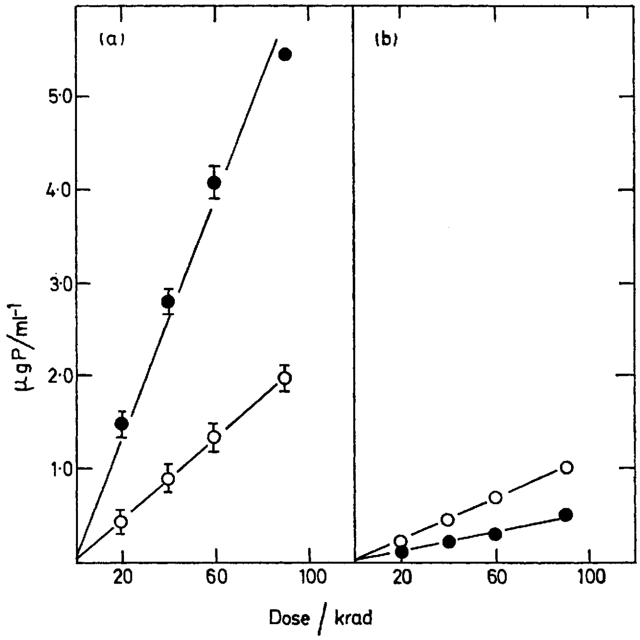


FIGURE. Dose-yield response for inorganic phosphate release from (a) 8,5′-cyclo AMP and (b) 5′-AMP under N_2O (○) and N_2 (●) at pH 7·3. Data points for 8,5′-cyclo AMP are means of 4 determinations and for 5′-AMP, the average of two determinations. The yield is given as μg phosphorus ml^{-1}.

Inhibition of phosphate release by N_2O clearly implicates e^-_{aq} as the reactive species initiating phosphate ester cleavage in 8,5′-cyclo AMP. In confirmation, the addition of the OH scavenger, t-butyl alcohol [$k(\text{Bu}^t\text{OH} + \text{OH}) = 5{\cdot}2 \times 10^8\ \text{l mol}^{-1}\ \text{s}^{-1}$],[11] was found to have little effect on *G*(Pi) under conditions where 90% of OH is scavenged. Under the same conditions, *G*(Pi) was decreased for both 5′-AMP and 3′-AMP by 80—90% (Table).

Taking *G*(Pi) (N_2O) = 0·73 as the yield of phosphate due to OH attack alone, and one half of this, or 0·37, as the contribution of OH attack to *G*(Pi) (N_2), it is possible to estimate that *ca.* 60% [(1·9 − 0·37)/2·6 × 100%] of the e^-_{aq} produced by water radiolysis leads to phosphate release in 8,5′-cyclo AMP. This efficiency of e^-_{aq}-initiated phosphate release coupled with the low reaction rate constant of e^-_{aq} with phosphate ester groups suggests that e^-_{aq} attachment to the purine ring precedes phosphate release. As such, the process of ester cleavage represents an intramolecular transfer of radiation damage from base to sugar unit in the nucleotide analogue.

$$H_2O \rightsquigarrow 2{\cdot}63\,e^-_{aq} + 0{\cdot}55\,H + 2{\cdot}72\,OH + 0{\cdot}68\,H_2O_2 + 0{\cdot}45\,H_2 \qquad (1)$$

$$N_2O + e^-_{aq} \rightarrow OH + OH^- + N_2 \qquad (2)$$

At pH < 4 in N_2-saturated solution, *G*(Pi) for 8,5′-cyclo AMP decreases sharply, reaching a minimum of 0·5 at pH 1. The pH response is consistent with competitive e^-_{aq} scavenging by H^+ and 8,5′-cyclo AMP ($e^-_{aq} + H^+ \rightarrow H$, $k = 2{\cdot}3 \times 10^{10}\ \text{l mol}^{-1}\ \text{s}^{-1}$).[12] Furthermore, this result shows that a clear distinction between e^-_{aq} and H atom activity exists in the reductive elimination of phosphate from 8,5′-cyclo AMP. Pulse radiolysis experiments show that both e^-_{aq} and H react readily with purine bases.[11] The difference in e^-_{aq} and H reactivity with respect to phosphate ester cleavage presumably reflects a difference in the site of unpaired electron density on the adenine ring in 8,5′-cyclo AMP, following the respective addition of these two species.

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