Adenosine-sensitised Photolysis of Alanine

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Photoexcitation of an aqueous solution of adenosine containing alanine with 253.7 nm light, leads to sensitised photodecomposition of the latter to afford NH₃. The presence of O₂, reduces the quantum yield of NH₃ formation, while the presence of N₂O, CO₂ and other quenchers reduces the yield to zero, indicating that the sensitisation occurs via photoionisation of adenosine mediated via the triplet excited state.

The effect of UV radiation on nucleic acids has been extensively investigated¹–³. As DNA intercalates to the proteins in the cells, the effect of UV radiation on the amino acids can be studied by selectively exciting nucleic acids and following subsequent reactions of the excited states of the latter. Electronically excited purines have been shown to react with alcohols⁴, amines⁵,⁶ and aliphatic amino acids⁷, to give substituted purines. In several cases the functional groups of the substrates do not appear in the final product, suggesting that the substrates are cleaved by interaction with the excited purines. With amino acids as substrates, loss of amino groups has also been observed⁸. The effect of UV irradiation on aliphatic amino acids (e.g. alanine) in the presence of adenosine should serve as a useful model for photosensitisation in biological systems⁹,¹⁰, since light is almost exclusively absorbed by adenosine, producing excited adenosine, which then interacts with the amino acid. In this paper, estimation of ammonia formed and luminescence studies have been used to elucidate the mechanism of photosensitisation.

Materials and Methods

Alanine (SISCO Research Laboratories, India) was recrystallised seven times from water. Adenosine (Sigma) was used as received. Triply distilled water was de-ionised by passing it in succession through cation and anion exchanger columns. High purity oxygen and purified N₂O (Indian Oxygen Ltd) were bubbled through the solution when necessary. Carbon dioxide used was generated by reacting CaCO₃ with HClO₄ in situ.

Ammonia was estimated with an Orion 95-10 gas-sensing electrode fitted with improved multilayer gas diffusion membranes, coupled with an Orion 407A specific ion meter (sensitivity better than 0.1 mV).

Degassing was done by freeze-pump-thaw method at 77K, using a vacuum line. Photolysis was done in quartz tubes (int. diam. 15 mm), using a Rayonet RPR-100 photochemical reactor (Southern New England Ultraviolet Co., USA), fitted with 16 low pressure mercury resonance lamps (253.7 nm) in a cylindrical symmetry and a merry-go-round rotating at 4 rpm for uniform irradiation.

Absorption spectra were recorded on a Hitachi model 200-10 spectrophotometer. Fluorescence at room temperature and phosphorescence at 77K were studied using an Aminco Bowman spectrophotofluorometer (model 4-8202 B) fitted with an Aminco Keirs phosphoroscope. The photon flux was determined using a uranyl oxalate actinometer¹⁰. Typical photon flux used was 1.65 × 10¹⁹ dm⁻³ s⁻¹ for a 90-min irradiation.

The quantum yield (φ) for the sensitised photodecomposition was defined as:-

φ = (Number of NH₃ molecules produced due to sensitisation)/(Number of photons absorbed by sensitisier molecules)

Results and Discussion

In solutions containing 5 × 10⁻⁵ mol dm⁻³ adenosine and 1 mol dm⁻³ alanine, 253.7 nm UV light was absorbed exclusively by adenosine (ε²₅₃.₇ = 1.7 × 10⁴ dm³ mol⁻¹ cm⁻¹ for adenosine and ε₀.₀₁₄ dm⁻³ mol⁻¹ cm⁻¹ for alanine). Hence (Ce)ₐdenosine/(Ce)ₐlanine = 61. Ammonia was found to be a product formed by the photosensitisation of alanine by excited adenosine. Typical quantum yield in degassed solution was 5.6 × 10⁻⁴. Increase in O₂ concentration in solution decreased the quantum yield of ammonia to 2.6 × 10⁻⁴ and 2.4 × 10⁻⁵ in air and oxygen saturated solutions respectively, indicating that triplets of adenosine were probably involved.

At room temperature, an aqueous solution of adenosine (pH 7) was devoid of any fluorescence. At pH 1, a very weak fluorescence was observed with λ_max at ~ 395 nm (λ_exc ~ 285 nm). Addition of alanine had no
effect on this fluorescence, indicating that adenosine singlet states do not possibly interact with alanine. Steele and Szent-Gyorgyi\textsuperscript{11} also did not observe any fluorescence from adenosine at any pH at room temperature. However, they reported phosphorescence from a 10\textsuperscript{-3}mol dm\textsuperscript{-3} adenosine solution frozen at 77K with $\lambda_{\text{max}} \sim 420$ nm and life-time ($\tau$) of $\sim 2.5$ s. Due to such a long-life-time of T\textsubscript{1} state, further photon absorption to T\textsubscript{n} state becomes very probable. Hélène et al\textsuperscript{12} and Rosenthal et al\textsuperscript{8} have actually established that even in steady state photolysis, biphotonic excitation from T\textsubscript{1} to higher triplet states occurs in adenosine, followed by auto-ionisation. The $\pi$-electron ionisation energy of adenosine has been calculated quantummechanically as $\sim 8$ eV\textsuperscript{13}, but 253.7 nm corresponds only to $\sim 5$ eV, while $E(T_1) \sim 3.4$ eV. Evidently, direct photoionisation from T\textsubscript{1} state is not possible, while biphotonic absorption via T\textsubscript{1} state to T\textsubscript{n} $> 8$ eV followed by ionisation becomes feasible. Hélène and coworkers\textsuperscript{12,14} also suggested that any subsequent chemical transformation in the adenosine-sensitised reactions is due to interaction between the photojected electrons and the substrate.

Presently it has been observed that an increase in O\textsubscript{2} concentration decreases the quantum yield of NH\textsubscript{3} production via sensitisation, indicating that triplets of adenosine are probably involved. However, the autoionisation process must be competing with the reaction with O\textsubscript{2}, since, otherwise O\textsubscript{2} should have reacted completely with such a long-lived triplet, giving no NH\textsubscript{3} as the product. In the presence of metal ions (Cu\textsuperscript{2+}), no NH\textsubscript{3} could be detected, indicating complete inhibition due to triplet quenching\textsuperscript{15}.

At lower pH ($< 4$) no sensitisation was observed. This is possibly due to the lowering of triplet yield of adenosine, since it is found that the phosphorescence from adenosine is quenched by protonation ($pK_a = 4$). Acid inhibition could also be due to scavenging of photojected electrons by acid protons (see Eq. 1)

$$e_{aq}^- + H^+ \rightarrow H $$ \hspace{1cm} (1)

When N\textsubscript{2}O, an efficient electron scavenger\textsuperscript{16}, was bubbled through the mixture before photolysis, the quantum yield for sensitised photodecomposition decreased to zero, indicating the intermediary of hydrated electrons in the sensitised photodecomposition in the absence of N\textsubscript{2}O. Since the NH\textsubscript{3} yield actually decreased, involvement of OH produced by the reaction between e\textsubscript{aq} and N\textsubscript{2}O was also ruled out. Similar results were observed when N\textsubscript{2}O was replaced by CO\textsubscript{2}, another efficient electron scavenger.

The mechanism involving the generation of triplet adenosine, followed by ionisation from higher T\textsubscript{n} state, explains our observations (see Scheme 1)