Reversible photolysis of pyrimidine derivatives, including trials with nucleic acids

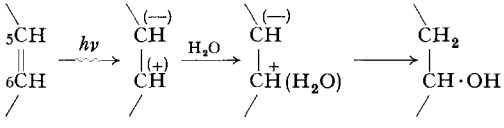
The observation of SINSHEIMER AND HASTINGS that the ultraviolet photolysis of uridylic[1,2] and cytidylic[1] acids may be reversed under suitable conditions is of considerable significance, not only generally as regards the photochemistry of nucleic acids and their derivatives, but also from the point of view of the phenomenon of photoreactivation.

We have been investigating the photochemistry of a wide variety of natural and synthetic pyrimidine derivatives and, in particular, the possibility of reversing the photolysis of various cytosine derivatives.

For cytosine and 1-methylcytosine the course of the photochemical reaction is characterized by a gradual decrease in absorption over the entire spectral range (Fig. 1). However, in the case of cytosine nucleosides and nucleotides, the decrease in absorption of the principal maximum at about 2700 A is accompanied by the simultaneous appearance of a new maximum at 2360 A (Fig. 2). For all the above compounds the reaction is reversible by acid or heat in the pH range in which none of the potentially dissociable groups is dissociated. Dissociation of the carbohydrate hydroxyls (pH >12) results, on the other hand, in a marked change in quantum yield and the reaction is no longer reversible. Under the conditions of irradiation used, the sugar component itself is not affected as judged by its reaction with orcinol.

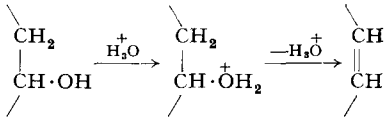
The quantum yields for photolysis of cytosine and 1-methylcytosine in neutral solution are of the same order of magnitude ($\Phi \approx 1.6 \cdot 10^{-3}$). On the other hand the quantum yields for cytosine nucleosides and nucleotides under the same conditions are approximately one order of magnitude greater (*cf.* corresponding curves in Figs. 1 and 2). It therefore follows that substitution on the number 1 nitrogen of the pyrimidine ring does not directly affect the course of the reaction; consequently the increase in quantum yield in the case of nucleosides and nucleotides is due to some kind of interaction between the pyrimidine ring and the sugar hydroxyls. A somewhat similar situation exists with regard to uracil derivatives, although it is less marked. These results therefore have some bearing on the structure of cytidine proposed by FURBERG[3], as well as other nucleosides. Spectral evidence in favour of some such type of interaction has been previously presented[4].

Fairly good evidence has been presented by MOORE AND THOMSON[5] that the photolysis of 1,3-dimethyluracil involves addition of a water molecule at the 5:6 double bond. We have tested this hypothesis by studying the rates of the forward and backward reactions in water and deuterium oxide for 1-methyluracil, cytosine and cytidine. For all three compounds the rate of photolysis in heavy water is about one-half that in ordinary water, while the reaction is first order in both light and heavy water. It therefore appears to us that the reaction involved is as follows:



If such is the case then the reaction in heavy water, which requires a greater energy of activation, should be slower, as is actually observed.

The reverse reaction, involving elimination of a molecule of water, as a catalyzed acid–base reaction should proceed in the following manner:



Deuterium oxide is less basic than water, since its autoproteolysis factor is only 20% that of water[6]. Hence the photolysis product should compete with the solvent for the deuteron in D_2O more effectively than for the proton in H_2O. The concentration of the conjugate acid of the photolysis product will then be higher in heavy water and the rate of the elimination reaction (of a water molecule) will then be greater in heavy than in light water. This is, in fact, what we have found: the elimination reaction in D_2O for those cases studied is 2–3 times as fast as in H_2O.

Further evidence for the above is provided by the recent findings of WANG, APICELLA AND STONE[7] which confirm the original suggestion of MOORE AND THOMSON[5].

We have been investigating also the possibility of reversal of the photolysis of nucleic acids, because of its obvious *in vivo* importance. From the above, and from results that we have obtained with other pyrimidine derivatives, we would expect this to be dependent on the nature

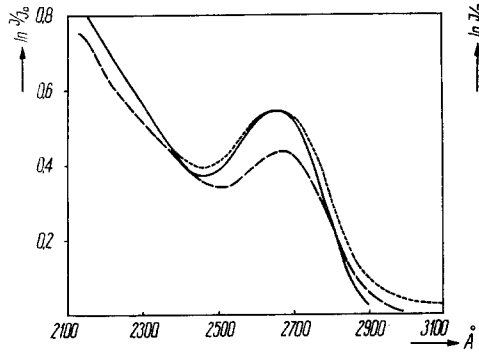


Fig. 1. Cytosine at pH 7.2, non-irradiated (——), after 85 minutes irradiation (– – – –) and after heating irradiated solution 10 min at 80° (----).

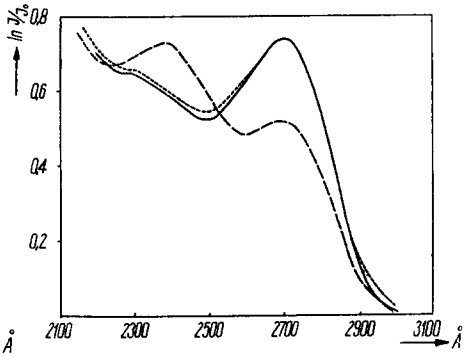


Fig. 2. Cytidine at pH 7.2, non-irradiated (——), after 20 minutes irradiation (– – – –) and after heating irradiated solution 5 min at 80° (----).

of the secondary linkages involving the pyrimidine rings. To date we have been able to obtain only indirect evidence for about 10–15% reversibility by heat with RNA in buffered medium, if the initial photolysis is performed with unfiltered radiation from a mercury resonance lamp (*i.e.* $\lambda \leqslant$ 2537 A). On the other hand a sample of apurinic acid (in which we have shown that the potentially dissociable amino and keto groups are essentially free[8]) exhibits considerable reversibility, which we attribute to the desoxycytidylic acid component. By direct hydrolysis and paper chromatography we have found that the quantum yields for photolysis of the pyrimidine nucleotide components in RNA are of the same order of magnitude as the quantum yields for the free nucleotides. In agreement with other observers we noted that the purine derivatives are, by comparison, little affected.

We should like to thank Prof. A. SOLTAN for a gift of heavy water.

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Received December 14th, 1956

Electrolytic reduction of mercaptides
A new method for the isolation of thiol amino acids and peptides

Peptides which contain a sulfhydryl group are usually isolated by precipitation as an insoluble metal mercaptide with, for example, copper or mercury. This is followed by double decomposition with H_2S, removal of the metal sulfide and recovery of the free peptide from the filtrate. This classical method is tedious and time-consuming and among its disadvantages are:

(1) Adsorption of the peptide on the metal sulfide precipitate.
(2) Dilution of the product through repeated washing of this precipitate.
(3) Decomposition during the subsequent concentration.
(4) It is frequently difficult to remove salts completely by washing of the metal mercaptide.
(5) All steps after the H_2S decomposition must be carried out in the absence of oxygen.
(6) Formation of colloidal sulfur from H_2S is often troublesome.

We have now found that mercury mercaptides of thiolamino acids and peptides can be