PHOTOCHEMICAL DEAMINATION OF CYTOSINE AT 2537°A

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Received June 4, 1964

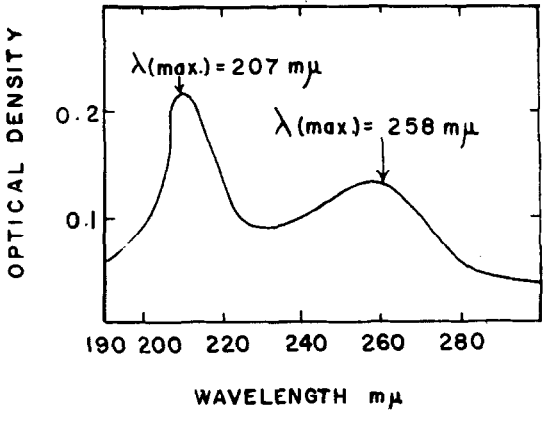
The photochemistry of aqueous solutions of cytosine has largely been discussed in terms of reversible formation of a water adduct (not isolated or characterized) (Shugar, 1960, Wierchowski and Shugar, 1961). An absorption spectrum with λ max = 2400°A has been reported for this photoproduct (Wierchowsky and Shugar, 1961). This interpretation has been challenged by Wang (Personal Communication) on the grounds that a synthetic dihydrocytosine has no absorption at a wavelength longer than 2200°A, and that facile deamination of 5-6 saturated cytosine derivatives renders it uncertain whether cytosine or uracil derivatives are in fact being studied.

However, it seemed to us that undue attention has been paid to hydration (which has no obvious biological importance) whereas there was a good possibility that deamination would be a direct consequence of photochemical excitation. Accordingly, we have carried out experiments designed to investigate this point.

Cytosine, in fresh, air-saturated solution, was irradiated at 2537°A by the output of a low pressure mercury lamp, filtered through 10mm of 1% acetic acid to remove 1849°A radiation. The liberation of ammonia was observed using Nessler's Reagent; hydroxylamine was searched for by oxidation with I_2 and testing for nitrite by the sensitive diazo-coupling technique. None could be detected. Control experiments showed the absence of urea at the doses utilised. The rate of ammonia formation was in all cases linear with absorbed dose, with a quantum yield of 5×10^{-3} at a cytosine concentration of 5×10^{-4}M. It is evident that ammonia is a

major product of the photolysis. Over the same dose range the apparent loss of cytosine as calculated from the loss in optical density at 2640°A (the near u.v. absorption maximum for cytosine) has only a quantum yield of 2.8×10^{-3}.

The nature of the photoproducts was studied by fractionation of irradiated solutions on a cation exchange column (Cohn, 1949), and measuring the absorption spectra of the fractions from 1800°A to 3500°A. In control experiments in 1NHCl, cytosine and uracil (the most obvious deamination product) were clearly separated, cytosine appearing in tubes 13 to 21, and uracil in tubes 4 to 9. Under various conditions of treating the irradiated solution before fractionation (such as standing in neutral solution, standing in acid) or on immediate fractionation, fractions eluted in the positions corresponding to uracil (tubes 4-9) had the characteristic near u.v. absorption spectrum of uracil. A typical spectrum (tube 7) is shown in Fig. 1 and it can be seen to have an absorption band



in the near u.v. λmax = 258 mμ. In neutral solution this shifts slightly to 260 mμ, and the O.D. (max) decreased by 5%. This behavior is characteristic of uracil. By comparison, cytosine at pH 1 has an absorption band with λmax at 273 mμ, shifting to 268 mμ and decreasing by 40% in neutral solution (Beavan, Holiday and Johnson, 1960). The eluted solution

was then run on ascending paper chromatograms in isopropanol: HCl (4:1) against a sample of synthetic uracil; Rf's were identical within experimental error at 0.4.

We accordingly conclude that uracil is a product of the photolysis of cytosine. The determination by these means of the amount of cytosine converted to uracil is not at all accurate, but assuming that the near u.v. band in the tubes 4-9 is due only to uracil, then we estimate that $\frac{\Delta(\text{uracil})}{\Delta(NH_3)} \simeq 1$

Two other photoproducts absorbing in the further u.v. were absorbed on immediate fractionation, one with maximum absorption at 2300°A, and one with a maximum absorption at 2100°A. These products elute approximately with uracil, though with a broader separation, the 2300°A product disappearing after standing for 24 hours in acid or at neutral pH.

We hence conclude that a deamination reaction of the excited state of cytosine is occurring. Such a deamination results in the transformation of cytosine into uracil. It is worth noting that this change is detectable only with difficulty by spectrophotometric measurements at 2600°-2700°A, since uracil and cytosine have very similar extinction coefficients in this region (at pH6). Accordingly we conclude that previous determinations (Shugar, 1960) of the quantum yield for photochemical conversion of cytosine may be low by a factor of 2.

If reactions such as these occur when the base is incorporated in a D.N.A. molecule, then these findingshave considerable genetic significance. For when replication of an irradiated D.N.A. strand occurs, then the 'changed cytosine' (uracil) will lead to the appearance of adenine in the replicated strand, instead of the guanine which would otherwise have been expected.

A natural extension of this is the possibility that analogous reactions might be found for adenine and guanine to give respectively hypoxanthine and xanthine. Preliminary experiments in this laboratory have indeed

demonstrated a significant photodeamination of adenine, and this work is continuing.

While this work was in progress, a review appeared (Wacker, 1963) quoting results from an unpublished doctoral thesis. These indicated that upon u.v. irradiation in frozen aqueous solution cytosine forms a product very similar to uracil dimer. Neither ammonia nor uracil formation was reported, and the results may not be pertinent to liquid solutions.

Acknowledgements

This work is part of a research program supported by a grant from the National Institutes of Health of the U.S. Public Health Service, AM-06420.

The authors wish to express their thanks to Mrs. Awilda R. Sandoval for her experimental assistance.

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