RADIATION CHEMISTRY OF THYMINE IN
AQUEOUS ALKALINE SOLUTION*

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Introduction

Interest in irradiation effects on thymine in aqueous alkaline solutions is derived from two considerations: a) the changes in structure which take place when thymine ionizes and which might have interesting consequences with respect to free radical reactions and b) uncertainty as to which ionization state of thymine serves best as a model for the hydrogen bonded thymine in nucleoproteins. Accordingly the radiation induced changes in thymine have been investigated over a wide pH range using chiefly the techniques of ultra-violet spectroscopy and two dimensional paper chromatography combined with radioautography.

Experimental

Thymine (California Biochemical Corp. Grade A) solutions, usually $1-2 \times 10^{-4}M$ were exposed to cobalt-60 γ -rays at a dose rate of approximately 10^{20} e.v./l-min. Samples were contained in loosely stoppered test tubes.

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Measurements of ultra-violet absorption by the solutions were made for the most part on a Cary Model 11 recording spectrophotometer. In some cases measurements at a particular wavelength were made on a Beckman Model U.V. spectrophotometer.

Two dimensional paper chromatograms and autoradiographs were made by the Denon technique. In early experiments development of the chromatogram was with phenol/water (100g / 39g) followed by m-butanol/propionic acid/water (919ml/469ml/612ml). The phenol solvent, however, caused secondary reaction in some of the thymine radiation products and its use was discontinued. Subsequent work was done with the butanol solvent alone, its use in two dimensions giving improved separations and indicating clearly when interactions during chromatography occurred.

For the autoradiographs, carbon-14 labeled thymine, either the 2-C¹⁴ (Cal Biochem) or the 5-methyl C¹⁴ (New England Nuclear) compound, was added to the solutions prior to irradiation.

In the very alkaline region it was necessary to remove the NaOH from solutions to get good resolution on the paper chromatograms. After irradiation the solutions were acidified to a pH of 1 with HCl and shaken with activated charcoal (Norite A, 100 mg/200 μ M thymine) for an hour at room temperature. The mixture was then centrifuged, the charcoal was washed once with

water, and thymine and its irradiation products were eluted with EtOH/H₂O/NH₃ (50/45/5). The solution was evaporated to dryness and dissolved in water for chromatography. With irradiated 1 N NaOH solutions, the procedure yielded about 70% of the original activity or 75% of the activity which sorbed onto the charcoal. Losses due to incomplete absorption are shown in Fig. 6.

Hydrogen peroxide yields were determined by the titanium sulfate method and total peroxides by the potassium iodidemolybdate method is modified by Hochanadel and Ghormly. Organic peroxides yields were obtained by difference.

Results and Discussion

Changes in the ultra-violet absorption spectra of irradiated air-saturated thymine solutions are shown in Fig. 1 for solutions of pH 9, 11.8, and 14. As the pH is increased the change in the spectrum for a given dose decreases, and thus the chromophoric group is less sensitive to radiation at the higher pH values. At pH 9 and at 11.8 no significant changes in spectrum shape occur with radiation, but there is a slight change in shape at pH 14. The change in the wavelength of the maximum with pH is due to the change in thymine structure (Fig. 2 - calculated from the results of Shugar and Fox). The chromophoric group involves the 5, 6 double bond.

Typical dose curves for the change in peak optical density with radiation dose are given in Fig. 3. The dose curve in

strongly acid solutions is linear to near completion of the reaction. At higher pH values the instantaneous yield decreases with increasing dose, indicating that more than one radiation induced reaction is occurring. The curve for pH 14 gives further evidence of the low disappearance yield of the chromophoric group in alkaline solutions, and incidentally illustrates a difficulty encountered in obtaining reproducible results.

Figure 4 gives the initial yields for the disappearance of the chromophore as a function of pH. Noteworthy features are 1) the short near plateau between pH 5 and 7, and the rapid drop with increase in pH beyond neutrality.

Attempts to relate this curve to changes in primary product yields and composition were completely unsuccessful. No reasonable assumptions predict changes in yield such as those observed. However, the yields can be readily correlated with changes in structure of thymine with pH (cf. Fig. 2). If the yield for the disappearance of the chromophore of the unionized species, H_2T is taken as 2.0, of the singly ionized species, HT^- , as 1.30, and of the doubly ionized species, T^{2-} , as 0.1, then the lower curve of Fig. 5 is readily computed with the aid of the graph at the bottom of Fig. 2. An assumed G of 2.1 for H_2T gives the slightly higher curve. Either of these curves fits the data well above pH 5. The greater yields in acid solutions may be due to the increase in $G(OH)$ in this region, or to the formation of a thymine salt (analogous to a quaternary ammonium compound).

Further evidence of a fundamental change in the radiation reaction as thymine becomes ionized is obtained from determination of $G(H_2O_2)$ and $G(\text{Organic Peroxides})$ (Figs. 6 and 7). At pH 5.5 and 9.7 $G(H_2O_2)$ is 1.1 and 2.7, and $G(\text{Org. Per.})$ 1.8 and 0.4 respectively.

Data of Scholes, Ward, and Weiss at pH 8 fall well in line, with $G(H_2O_2)$ 1.8 and $G(\text{Org. Per.})$ 1.0

Several products are known to result when thymine is irradiated in neutral or acidic solution. Scholes, Ward, and Weiss have reported the presence of a hydroxyhydroperoxide, I, and a glycol, II, and Ekert, working in air-free neutral media has found in addition dihydrothymine, III, hydroxymethyl uracil (IV), and one other compound.

Paper chromatographic autoradiographic techniques have confirmed that several compounds are formed in air-saturated neutral solutions.

These appear to be the cis- and trans-glycols, a compound which behaves chromatographically like dihydrothymine, a compound which gives a test for ureido-butyric acid, and an unidentified component. (The hydroxy-hydroperoxide is lost during chromatography and contributes to the glycol yield).

In strongly alkaline solution the appearance of the autoradiographs is entirely different. Only one product can be observed in any appreciable yield. This product has been eluted from the paper chromatograms and co-chromatographed with hydroxymethyl uracil and found to travel precisely with it in the following solvents: propanol/H₂O (172/28) (V/V), butanol/propionic acid/water (92/47/61) and butanol/ammonia/water (172/18/10). Its yield at pH 14 is 1.6 or possibly slightly higher (some loss may have occurred in the desalting procedure), and it is certainly among the major product since the procedure used accounts for at least 70% of the total C¹⁴ activity. This is confirmed by the fact that the actual yield for the disappearance of thymine as determined by counting the C¹⁴ in the thymine eluted from the developed chromatogram is no more than 1.9. Together these yield values suggest that two OH radicals are required for the formation of hydroxymethyluracil.

It is evident from these results that the principle reactions of the unionized form of thymine are by addition to the double bond; and that as the molecule becomes ionized and the ring more aromatic in character, the double bonds are inactivated and the hydrogens on the methyl group are activated with respect to free radical reactions.

ABSORPTION SPECTRA OF IRRADIATED AQUEOUS SOLUTIONS OF THYMINE

OPTICAL DENSITY

Fig. 1b
(Place under 1a)

THYMINE STRUCTURES

THE RATE OF RADIOLYSIS OF THYMINE IN 10^{-4} M AQUEOUS SOLUTIONS

Fig. 4

Yields for Disappearance of
the Chromophoric Group
as a Function of pH

Fig. 5.

CORRELATION BETWEEN THE RADIATION REACTION
AND IONIC FORMS OF THYMINE

Fig. 6

IRR. OF $10^{-3}M$ rhy-oxg.
YIELDS OF PEROXIDE
pH-5.5

mm / 100 ml

400

300

200

100

Total PEROXIDES

ORG. PEROXIDES

H_2O_2

DOSE $\times 10^{17}$ eu/ml

10X10 TO THE CM 35N14
KODAK SAFETY FILM

Fig. 7

YIELD OF ^{131}I -THY-ORX.
YIELDS OF PEROXIDES
pH 9.7

TRACED ION OF ^{131}I -THY-ORX.
YIELDS OF PEROXIDES
pH 9.7

TOTAL PEROXIDES

4.0

0.3 PEROXIDES