Photoinduced reduction of thymine and uracil derivatives by hypophosphite: unusual high quantum yield of chromophore loss

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The quantum yield of chromophore loss of thymine, uracil and their corresponding nucleosides and nucleoside-5′-monophosphates undergoing irradiation with 254 nm UV light was found to be sharply enhanced by hypophosphite; thymine and uracil were reduced by hypophosphite to give 5,6-dihydrothymine and 5,6-dihydrouracil respectively.

In the photochemistry of nucleic acids, there has been much interest in oxidative damage to DNA and its biological consequences in living cells.[1,2] Here we report the photoinduced reduction (hydrogenation) of thymine (**1a**), uracil (**1b**) and their derivatives by hypophosphite, which was initially found in our tentative simulation of the photochemistry of the primitive sea, by irradiation with a medium pressure mercury lamp.[3] Contrary to the wavelength dependence of the enhancement of the photolysis of nucleic acid monomers by orthophosphates and pyrophosphate on UV light for photoionization of phosphates (< 210 nm),[3,4] no obvious wavelength dependence has been observed in photoinduced reduction by hypophosphite. We present the results obtained by the irradiation with 254 nm UV light.

For actinometry of the low pressure mercury lamp, we repeatedly used the chromophore loss of uridine in areated aqueous solution at pH 6.[5] A 20% $MeCO_2H$ solution of 1 cm pathlength was employed to filter off the photochemically significant quantities of 184.9, 194.2 and 222.4 nm UV light. The quantum yield of chromophore loss (ϕ^{cl}) was determined from the decrease of absorbance at λ_{max} of the substrates. Fig. 1 shows the concentration dependence of ϕ^{cl} of thymine, uracil and their corresponding nucleosides and nucleoside-5′-monophosphates. It is clear from this figure that ϕ^{cl} of thymine, uracil and their derivatives increases when the concentration of sodium hypophosphite is about 3×10^{-3} M or higher. ϕ^{cl} for uracil and thymidine is higher than for the other derivatives. Note that ϕ^{cl} for thymine, thymidine and thymidine-5′-monophosphate increases so sharply that at 1 M sodium hypophosphite ϕ^{cl} for thymine, thymidine and thymidine-5′-monophosphate is 0.016, 0.046 and 0.029, ϕ^{cl} being 0.064, 0.16 and 0.11 for uracil and its derivatives under the same conditions. However, no significant ϕ^{cl} change for uracil and thymine has been observed in the presence of 0.1 M concentrations of other anions (SO_4^{2-}, Cl^-, CO_3^{2-}, citrate, acetate, arsenate, cacodylate, orthophosphates, pyrophosphate), 0.1 M urea and 0.004 M metaphosphate under irradiation with 254 nm UV light. In contrast to the enhancement of the photolysis of nucleic acid monomers by orthophosphates and pyrophosphate,[4] no obvious ϕ^{cl} increase has been observed for cytosine, cytidine and cytidine-5′-monophosphate in the range 10^{-5}–1 M hypophosphite. For adenine, guanine and their derivatives, ϕ^{cl} was observed to increase when the concentration rises to more than 0.01 M, but the relative ϕ^{cl} value is < 5.

Hydrate formation of uracil, uridine and uridine-5-monophosphate was quantified using both HPLC[6] and the method introduced by Moore and Thomson[7] because the latter method has been demonstrated to bring about total dehydration.[6] HPLC elution demonstrates that hydrate formation of uracil and uridine in the concentration range corresponding to photoinduced reduction by hypophosphite ($> 3 \times 10^{-3}$ M) decreases sharply. The low reversibility or irreversibility of the irradiated samples of uracil, uridine and uridine-5-monophosphate in the concentration range corresponding to photoinduced reduction by hypophosphite also indicates that hydrates are not the main products. In dilute solution, dimers of pyrimidine bases and their derivatives arise through the triplet state.[1] ϕ for thymine dimer formation is small (< 0.1), while the reversal quantum yield is about 1.[8] This, combined with the reported limiting concentrations for uracil dimerization (> 0.1 mM)[9] and the aerated aqueous media (O_2 as triplet quencher) in the current system eliminates the possibility of forming the dimers in large quantity.

Fig. 2 indicates that major photoproducts have been formed in aqueous solutions of uracil and thymine containing sodium hypophosphite, which have been purified‡ and characterized.§ Using the authentic samples (Sigma) as internal standard, the retention time of the purified products was found to be the same to 5,6-dihydrothymine (**2a**) and 5,6-dihydrouracil (**2b**). The corresponding peaks were found to disappear by HPLC analysis after incubating the photoproducts in 0.2 M KOH for 1 h at room temperature. This is in agreement with the alkaline lability of dihydrouracil and dihydrothymine.[10] However, after heating the aqueous solution of the photoproducts with or without 0.1 M HCl in a boiling water bath for 10 min, no obvious change was observed *via* HPLC. Both IR and NMR spectra of the purified samples were found to be the same as those of the authentic samples. Overall these data indicate that the major product of

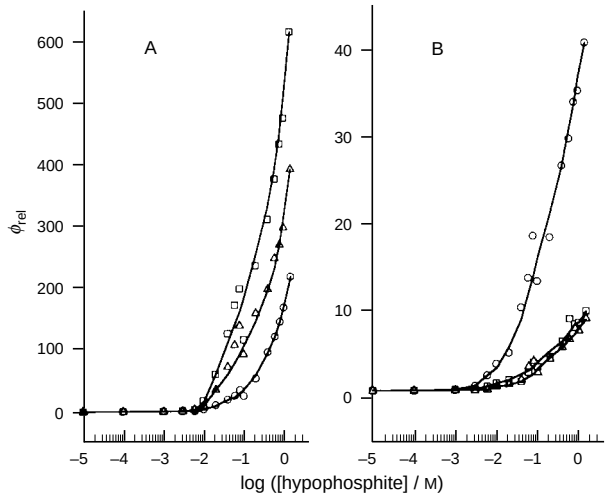


Fig. 1 ϕ^{cl} for thymine (A), uracil (B) and their derivatives as a function of the molar concentration of sodium hypophosphite undergoing the irradiation with 254 nm UV light (6.05×10^{-4} Einstein min^{-1}): (○) the base, (□) the corresponding nucleoside and (△) nucleoside-5′-monophosphate. Generally, 1×10^{-4} M aerated pyrimidines or their mixed solutions with sodium hypophosphite (pH 7 ± 0.3) were irradiated so as to facilitate UV absorbance and spectrum assay. ϕ^{cl} for thymine, thymidine and thymidine-5'-monophosphate in the absence of hypophosphite is 9.7×10^{-5}, ϕ^{cl} for uracil, uridine and uridine-5′-monophosphate in the absence of hypophosphite is 1.8×10^{-3}, 0.018 and 0.014. ϕ^{cl} in the presence of hypophosphite has been normalized to ϕ^{cl} in the absence of hypophosphite.

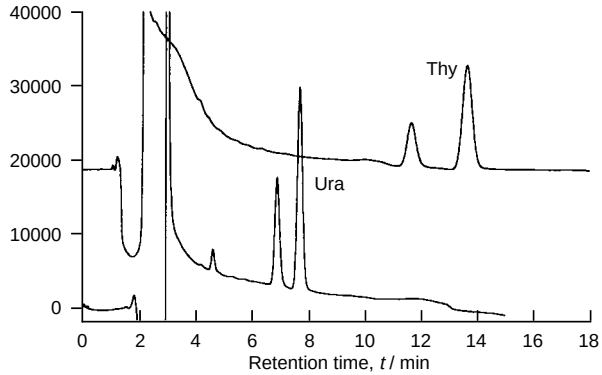
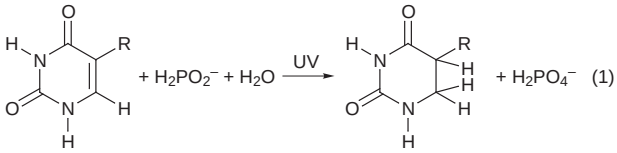


Fig. 2. HPLC elution profiles of products from irradiated 1×10^{-4} M thymine (Thy) and uracil (Ura) containing 0.2 M hypophosphite. The products were eluted from the μBondapak C_{18} column (Waters, 3.9×300 mm) at a flow rate of 1.4 ml min^{-1} (Thy) and 1 ml min^{-1} (Ura). The chromatograms were monitored at 210 nm.

thymine and uracil containing hypophosphite is 5,6-dihydrothymine (**2a**) and 5,6-dihydrouracil (**2b**). UV absorbance at 254 nm of 1 M sodium hypophosphite is 0.009, eliminating complications in the reaction mechanism owing to the competitive UV absorbance of hypophosphite. This, combined with detection of the quantitative oxidated hypophosphite (600 MHz, ^{13}P NMR spectrum), indicates that the enhancement of the chromophore loss by hypophosphite is indeed the photoinduced reduction by hypophosphite [eqn. (1)].



The fact that no obvious difference in the quantum yield has been found between the aerated and nitrogen-saturated samples favors the singlet pathway as the reaction mechanism.

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Notes and References

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‡ The major photoproducts were prepared by 254 nm irradiation of 1×10^{-3} M aerated thymine or uracil aqueous solution containing 1 M sodium hypophosphite (pH 7 ± 0.3). The photoproducts were extracted using ethyl acetate (for photoproducts of thymine) and a mixed solution of butanone and ethyl acetate (80 : 20, v/v) (for photoproducts of uracil). The collected samples were concentrated on a rotary evaporator. The residue was taken up in water, the products were purified by reversed-phase HPLC (Waters 600, Waters μBondapak C_{18} 19 × 300) with aqueous solution (uracil) or 2% MeOH aqueous solution (thymine). The collected samples were lyophilized to dryness.

§ *Selected spectroscopic data*: **1**, UV spectrum of **2a** and **2b** shows the disappearance of the characteristic absorption (> 230 nm) of uracil and thymine. **1**, **2a** ($C_5H_8N_2O_2$), Anal. Calc. for $C_5H_8N_2O_2$: C, 46.88; H, 6.25; N, 21.88; O, 25. Found: C, 47.1; H, 7.1; N, 22.1; O, 23.8%. FTIR (ν/cm^{-1}) 3236m, 3088m, 2892w, 1736vs, 1715vs, 1496m, 1392w, 1240s; 819w, 696w, 448w; δ_H(DSS, 600 MHz) 1.079 and 1.096 (d, Me), 2.647–2.723 (m, 5 H), 3.043–3.099 (m, 6 H), 3.036–3.045 (m, 6 H). *m/z* (atmosphere pressure chemical ionization), 129 $(M + 1)^+$. **2, 2b** ($C_4H_6N_2O_2$), Anal. Calc. for $C_4H_6N_2O_2$: C, 42.1; H, 5.26; N, 24.56; O, 28.07. Found: C, 42; H, 5.1, N, 24.6, O, 28.54%. FTIR (ν/cm^{-1}) 3236m, 3087m, 2892w, 1736vs, 1715vs, 1678m, 1496m, 1391w, 1239s, 820w, 756w, 453w; δ_H(DSS, 600 MHz) 2.554–2.589 (t, 5 H), 3.324–3.405 (t, 6 H); *m/z* 115 $(M + 1)^+$.

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