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THE INTRAMOLECULAR CYCLIZATION OF 2-IODO-3-UREIDOPROPIONIC ACID

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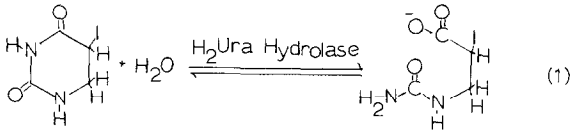
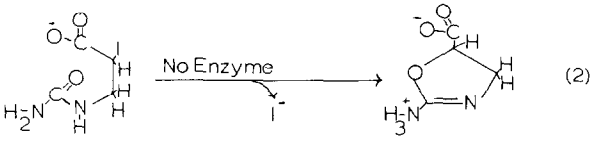
Summary

2-Iodo-3-ureidopropionic acid resulting from the hydrolysis of 5-iodo-5,6-dihydrouracil catalyzed by either dihydrouracil amidohydrolase or hydroxide ion cyclizizes to yield 2-amino-2-oxazoline-3-carboxylic acid. This cyclization involves intramolecular attack of the ureido oxygen atom on carbon two of the ureidoacid to yield iodide ion and the oxazoline as products. The kinetics of this cyclization indicate that from pH 2 to 9 the reaction rate is pH independent. Below pH 2 the rate is diminished due to protonation of the ureido group. Above pH 12 the rate increases dramatically probably due to proton abstraction which would dramatically increase the nucleophilic character of the ureido function. In the pH independent region the reaction is not subject to catalysis by external buffers.

In a recent communication from our laboratory (1) we reported that incubation of 5-iodo-5,6-dihydrouracil (IH_2Ura) with semi-purified rat liver dihydropyrimidine amidohydrolase (EC 3.5.2.2) resulted in iodide ion and 2-amino-2-oxazoline-5-carboxylic acid as products. The reaction was shown to involve two discrete steps. The first reaction (Equation 1) involved



(1)



(2)

dihydropyrimidine amidohydrolase catalyzed hydrolysis of the iododihydrouracil ring system. The second reaction (Equation 2) was shown to be the intramolecular cycliza

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tion of 2-iodo-3-ureidoproprionate to yield iodide ion and the oxazoline as the final products. After enzymatically catalyzed ring hydrolysis of IH_2Ura the rate of iodide ion release resulting from cyclization (Equation 2) was shown to be independent of amidohydrolase concentration (1). In addition, the first-order rate constants for iodide ion release and presumably oxazoline formation were essentially equal when measured after either enzymatically or NaOH catalyzed IH_2Ura ring hydrolysis (1). This evidence coupled with the fact that hydroxide catalyzed dihydropyrimidine ring hydrolysis is a well studied reaction (2) supports the conclusion that 2-iodo-3-ureidoproprionate is an intermediate in the enzymatically promoted formation of iodide ion from IH_2Ura and that its intramolecular cyclization in the absence of enzyme proceeds at a rate sufficient to account for the enzymatically catalyzed process. The objectives of this report are to describe the chemical and kinetic characteristics of this intramolecular cyclization of 2-iodo-3-ureidoproprionate to yield iodide and oxazoline as final products.

Materials and Methods

Deionized, glass distilled water was used to prepare all solutions and reaction mixtures. Inorganic reagents were obtained from Fisher Chemical Company and used as received. Tris (hydroxymethyl) aminomethane (Tris), 2-(N-morpholino) ethanesulfonic acid (MES) and succinate were from Sigma Chemical Co. IH_2Ura and $[2^{-14}C]IH_2Ura$ were synthesized as previously reported (1). 2-Iodo-3-ureidopropionate (I-UPA) was freshly prepared just prior to use by mixing 1.5 ml of 1.5×10^{-4} M IH_2Ura with 0.02 ml 1.0 M KOH which rapidly caused IH_2Ura ring hydrolysis. The timing of this reaction was carefully established to achieve maximal ring opening with a minimal amount of iodide release.

Spectra were measured with either a Cary 14 at room temperature or with a Zeiss PMQII spectrophotometer equipped with a thermostatted cell compartment.

The kinetics of iodide release were monitored spectrophotometrical at 225-235nm taking advantage of the absorbance of iodide ion which has a λ_{max} at 225 nm. Reaction mixtures containing the appropriate amount of buffer were preincubated at 25° to achieve thermal equilibrium prior to reaction iniation with 0.20 ml of 1.43×10^{-4} M freshly prepared I-UPA. In studies of the effect of pH on reaction rate, all buffers were at a final concentration of 0.033 M. Ionic strength (μ) was maintained constant at 1.0 M by the addition of 3.0 M KCl. The first-order rate constants (k_{obsd}) for iodide release were graphically determined from semilogarithmic plots of extent reaction against time using the relationship $k_{obsd} = 0.693/t_{1/2}$.

The reaction product resulting from the alkaline hydrolysis of the ring structure of IH_2Ura was isolated from reaction mixtures which contained 0.3 ug $[2^{-14}]IH_2Ura$ (specific activity, 5.8 mCi $mmole^{-1}$) and 0.47 gm IH_2Ura in 100 ml of 0.10 M KOH. After 48 hours at room temperature to insure complete reaction, the solution was carefully neutralized to pH 8.0 with formic acid and placed on a Dowex-1-formate column. (4.5 x 30 cm). Elution was accomplished with a linear 250 ml, 0 to 1.0 M formic acid gradient Radioactive fractions were pooled and lyopholized yielding a white crystalline powder.

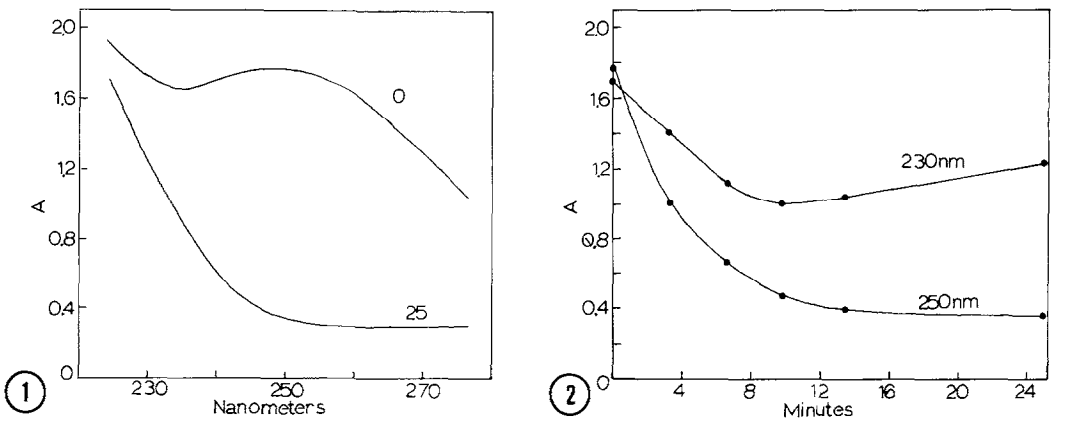


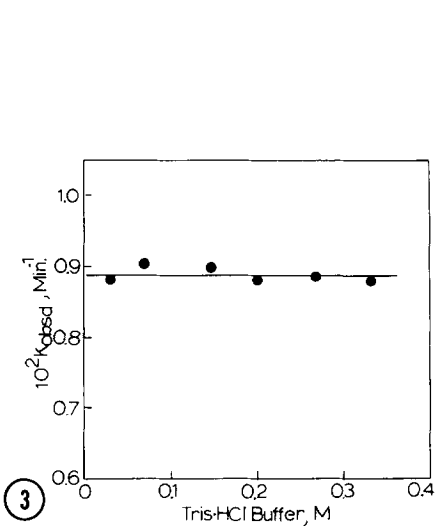
Figure 1. Ultraviolet absorption spectra of 1.43×10^{-4} M IH_2Ura in 0.013 M KOH. Spectra were recorded at room temperature. Numbers refer to the time in minutes that the spectra were recorded.

Figure 2. Time course for the alkaline hydrolysis of IH_2Ura observed at both 230 and 250 nm. Reaction mixtures were 1.43×10^{-4} M IH_2Ura and 0.013 M KOH, room temperature

Melting points were determined on a Fisher Hot Stage. Infra-red spectra were measured in KBr pellets. Nuclear Magnetic Resonance spectra were measured in D_2O with a Varian A60A using sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as an internal standard. All pH measurements were made with a Radiometer PHM-26 pH meter equipped with a Radiometer GK2321C combination electrode.

Results and Discussion

Ultraviolet absorption spectra of IH_2Ura in dilute hydroxide (Figure 1) indicates the presence of a dihydropyrimidine anion with a broad peak at about 245 nm. Since dihydropyrimidines are subject to ring hydrolysis via a multistep reaction mechanism which involves the formation of a tetrahedral addition intermediate (2), it was expected that spectra of such reaction mixtures would show a loss of the IH_2Ura anion peak indicative of the formation of I-UPA. Since the objective of this research was to understand the intramolecular cyclization of I-UPA, it was necessary to optimize the conditions and timing of the hydroxide catalyzed ring hydrolysis to insure maximum $I\text{-}H_2Ura$ hydrolysis with minimal dehalogenation of the resulting I-UPA. Consequently, complete spectra of 1.43×10^{-4} IH_2Ura in varying concentrations of KOH were recorded. The data at 230 and 250 nm were then plotted as a function of time. Since these two wavelengths are indicative of iodide ion and IH_2Ura anion absorbance



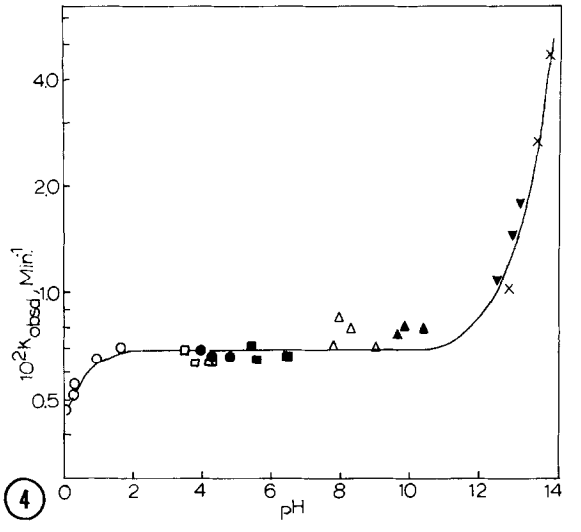
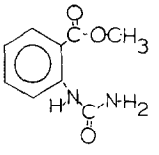


Figure 3. Lack of catalysis by increasing Tris · HCl buffer concentration on iodide ion formation from I-UPA, 25°, pH 8.0, μ = 1.0 M.

Figure 4. Relationship between k_{obsd} and pH for iodide ion formation from I-UPA, 25°, μ = 1.0 M. 0, HCl; acetate buffer; □, succinate buffer; ■, MES buffer; △, Tris buffer; ▲, bicarbonate buffer; ▼, tribasic sodium phosphate buffer and X, NaOH.

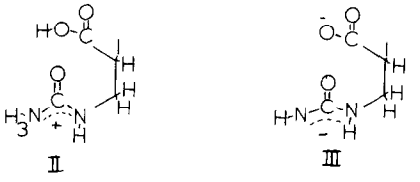
respectively, Figure 2 shows that a 12 - 15 minute room temperature incubation of I-DHU with 0.013 M KOH results in maximum I-UPA formation with minimal iodide ion formation. These conditions were selected for the preparation of I-UPA for all subsequent kinetic studies.

Since it was necessary to control pH by the use of various buffers, the rate constants for iodide ion formation from I-UPA were measured as a function of increasing concentration (0.033 M to 0.33 M) of acetate (pH 4.50), Tris (pH 8.0), bicarbonate (pH 9.50), and tribasic sodium phosphate (pH 12.50) buffers; μ = 1.0 M. The results shown in Figure 3 for the Tris buffers at pH 8.0 illustrate the results obtained for all the buffers listed above. At constant pH, the rate constants do not increase as a function of increasing buffer concentration and hence the reaction is not subject to general acid/base catalysis of proton transfer. This result is compatible with the data of Hegarty and Bruice (3) who showed that increasing glycine buffer concentration has no effect on the rate of cyclization of methyl-2-ureidobenzoate (I).



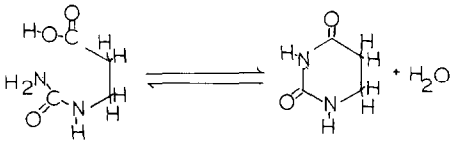
I

The kinetics of iodide formation from I-UPA were studied as a function of pH in either dilute buffers (0.033M), HCl or KOH, μ = 1.0 M, 25°. The results of these studies shown in Figure 4 confirm and extend our preliminary observations (1) that from about pH 2 to 9 the rate of the reaction is not influenced by pH. Below pH 2 the rate constants decrease with increasing hydrogen ion activity most likely because protonation of the ureido group (II), reduces its nucleophilic character. Several kinetic runs in 6.0 M HCl showed essentially no reaction as measured by the formation of free iodide ion. Pojarlieff (4) studying the reversible cyclization of ureidopropionic acid to yield dihydrouracil (Equation 3) demonstrated that this reaction shows



II III

a similar dependence on hydrogen ion activity. In this case the observed rate constants increased and then leveled as a function of the fraction unprotonated ureido

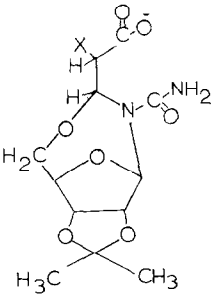


(3)

group and could be fit to a theoretical curve which assumed pKa = -0.26 for the dissociation of the protonated ureido function. This pKa value is somewhat lower than those reported for the conjugate acids of urea, 0.18 and N-methylurea 0.90 (5). In more alkaline solution, Figure 4 shows that the rate constants for I-UPA cyclization increase in almost direct proportion to the increase in pH. This result can be attributed to formation of the ureido anion (III) which would dramatically increase the nucleophilcity

of the attacking ureido function. Based on kinetic data, Hegarty and Bruice (3) estimated a pKa < 14 for the ionization of the ureido function of methyl 2 ureidobenzoate.

Earlier Hegarty and Bruice (3,6,7) in their detailed mechanistic studies dealing with acyl transfer reaction to and from the ureido function reported that depending upon both the reaction conditions and the nature of the leaving group, ureido groups acted as nucleophiles by both O and N attack. Work from Fox's laboratory on the reactions of 2',3'-O-isopropylidene-5-halouridines in strong base indicated (8) that dehalogenation of an intermediate ureido (IV) goes via N attack. Since our earlier work indicated (1) that dihydrouracil amidohydrolase catalyzed hydrolysis of IH_2Ura followed by intramolecular cyclization at near neutral pH yields a product resulting from O attack, we examined the hydrolysis and subsequent cyclization products of $[2^{14}C]IH_2Ura$



IV

reacting in 0.10 M KOH. Lyophilization of the radioactive fractions resulting from Dowex-1-formate chromatography of these reaction mixtures yielded a white crystalline powder which had a melting point (223-225°), infra-red spectrum (KBr pellet) and nuclear magnetic resonance spectrum in D_2O identical with the 2-amino-2-oxazoline-5-carboxylic acid product isolated previously from enzymatically hydrolyzed IH_2Ura (1). Thus we conclude that at both neutral and alkaline pH the cyclization of I-UPA goes principally by attack of the ureido oxygen atom on carbon 2 to yield iodide and 2-amino-2-oxazoline-5-carboxylic acid as products.

Acknowledgements

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