Photosensitised Conversion of Cytosine into Uracil Derivatives in the Presence of Mercaptans

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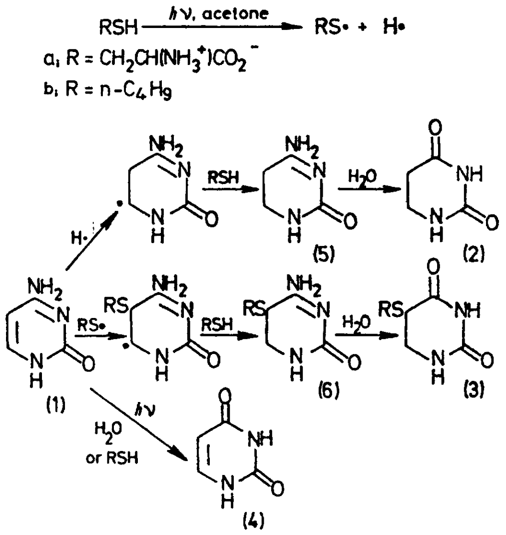
Summary Acetone sensitized photochemical reaction between cytosine and cysteine in water yields 5,6-dihydrouracil (72%), 5-*S*-cysteinyl-5,6-dihydrouracil (24%), and uracil (2·2%), suggesting that radiation may cause not only crosslinking of proteins to nucleic acids but also base modifications.

STUDIES on the mechanism of action of u.v. irradiation on bacterial cells have indicated that the crosslinking of proteins to DNA might be an important biological lesion in addition to pyrimidine dimer formation and photohydration.[1] Chemically, u.v. light may induce crosslinking of cysteine to uracil or thymine.[2] We report the photosensitized conversion of cytosine into uracil derivatives in the presence of cysteine or butane-1-thiol. The results suggest that radiation may cause not only cross-linking of proteins to nucleic acids but also base modifications in the presence of cysteine and its derivatives.



SCHEME

A nitrogen saturated solution of cytosine [(**1**) $1\cdot9 \times 10^{-3}$ M] and cysteine ($6\cdot6 \times 10^{-3}$ M) in acetone–water (1v:2v) was irradiated with a Hanovia Hg-lamp until 18% of (**1**) was consumed. After removal of cystine by filtration from the acidified solution, three products were isolated by a combination of ion-exchange chromatography on Bio-rad AG50WX8 resin, cuprous salt precipitation, and partition chromatography on cellulose. They were identified by comparison with respective authentic samples as 5,6-dihydrouracil [(**2**) 72%][3]† 5-*S*-cysteinyl-5,6-dihydrouracil [(**3a**) 24%],[2] and uracil [(**4**) 2·2%]. Prolonged irradiation of the solution until most of the cytosine had been consumed gave (**4**) (13%), (**3a**) (trace), and (**2**) (72%). In addition, alanine and several other unidentified minor products were also formed. Therefore, (**3a**) is apparently unstable under our experimental conditions. The use of a Corex filter to cut off light below 260 nm had no effect on the product composition. Cytosine reacted with butane-1-thiol under similar conditions to give (**2**) (27%), (**3b**) (46%), and (**4**) (3%).‡

Dihydrocytosines may be the intermediates in the formation of uracil derivatives from cytosine[4] which undergo rapid hydrolysis *in situ*.[5] When the progress of these photochemical reactions was followed by t.l.c., 5,6-dihydrocytosine (**5**) was detected initially, identified by comparison with an authentic sample and its conversion to dihydrouracil (**2**),[5] suggesting that (**5**) and (**6**) are intermediates in the formation of (**2**) and (**3**).

We, and others,[2] have found that the cytosine–cysteine system is relatively photostable in the absence of a sensitizer. The role of acetone as a sensitizer in photochemical reactions of thiols is well known.[6] The reaction is initiated by the cleavage of the thiol to a RS· radical and a hydrogen atom (Scheme). Although uracil (**4**) is a photoproduct from cytosine in an aerated solution,[7] we have found that its formation is unimportant in a deaerated solution. Therefore, (**4**) is probably formed *via* cytosine hydrate[8] or the *S*-mercapto-adducts of cytosine.

The formation of (**3**) or (**6**) in these reactions suggests that cysteine may also be linked to cytosine as well as to uracil or thymine. In addition, a major product formed in these reactions is 5,6-dihydrocytosine (**5**) which may be subsequently hydrolysed to 5,6-dihydrouracil (**2**). Although (**2**) is apparently inactive in template activity,[9] (**5**) is recognized in a RNA polymerase system as uracil or thymine.[10] Preliminary investigation on the radiation chemistry of cytosine and cysteine in water with a ^{60}Co-source indicated that (**5**) and (**2**) were also formed in this reaction. Our results imply that in the presence of cysteine and its derivatives radiation may effect base modification in nucleic acids in addition to crosslinking.

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† The yields given are based on the amount of cytosine consumed.

‡ Satisfactory elemental analysis and spectral data have been obtained for (**3b**).

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