

PHOTOCHEMICAL ADDITION OF AMINO ACIDS TO  $^{14}\text{C}$ -URACIL

Kendric C. Smith

Department of Radiology, Stanford University  
School of Medicine, Stanford, California 94305

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Summary. A survey was performed of the ability of the 22 common amino acids to add photochemically (2537Å) to  $^{14}\text{C}$ -uracil. The 11 reactive amino acids were glycine, serine, phenylalanine, tyrosine, tryptophan, cystine, cysteine, methionine, histidine, arginine and lysine. The most reactive amino acids were phenylalanine, tyrosine and cysteine. The relevance of these reactions to the mechanism by which DNA and protein are cross-linked in vivo by ultraviolet light is discussed

When bacterial cells are irradiated with ultraviolet (U.V.) light, DNA and protein become photochemically cross-linked (Smith, 1962; 1964). This photochemical lesion has been shown to be of major biological importance in irradiated bacteria under certain experimental conditions (Smith and O'Leary, 1967; Smith, Hodgkins and O'Leary, 1966). The chemical mechanism by which DNA and protein are photochemically cross-linked in vivo is not known. However, a model compound, 5-S-cysteine, 6-hydrouracil, was produced when a solution of cysteine and uracil was irradiated at 2537Å<sup>0</sup> (Smith and Aplin, 1968). More recently, the thymine analog, 5-S-cysteine, 6-hydrothymine, has been prepared photochemically (Smith, 1969).

Several lines of evidence have suggested that cysteine is probably not the only amino acid capable of combining photochemically with the nucleic acids. The first line of evidence was the observation that gelatin, which contains no cysteine does photochemically cross-link with DNA in vitro albeit at a much reduced efficiency compared with bovine serum albumin which does contain cyst(e)ine (Smith, 1967). The second indication was that in preliminary experiments using  $^{35}\text{S}$  labeled E. coli cells no increase was observed in the amount of radioactivity remaining on the DNA with increasing doses of U.V. after pronase digestion (Smith,

1968). This could suggest that either the number of sulfur atoms attached to the DNA was below detection or that amino acids devoid of sulfur were responsible for the cross-linking *in vivo*. The third was the direct observation that serine and tyrosine photochemically add to DNA (Smith and Meun, 1968), and that amines add to uracil (Yang and Gorelic, 1968). In order to know the scope of the reactivity of amino acids with the nucleic acids, a survey of the ability of all of the common amino acids to photochemically add to  $^{14}\text{C}$ -uracil was undertaken.

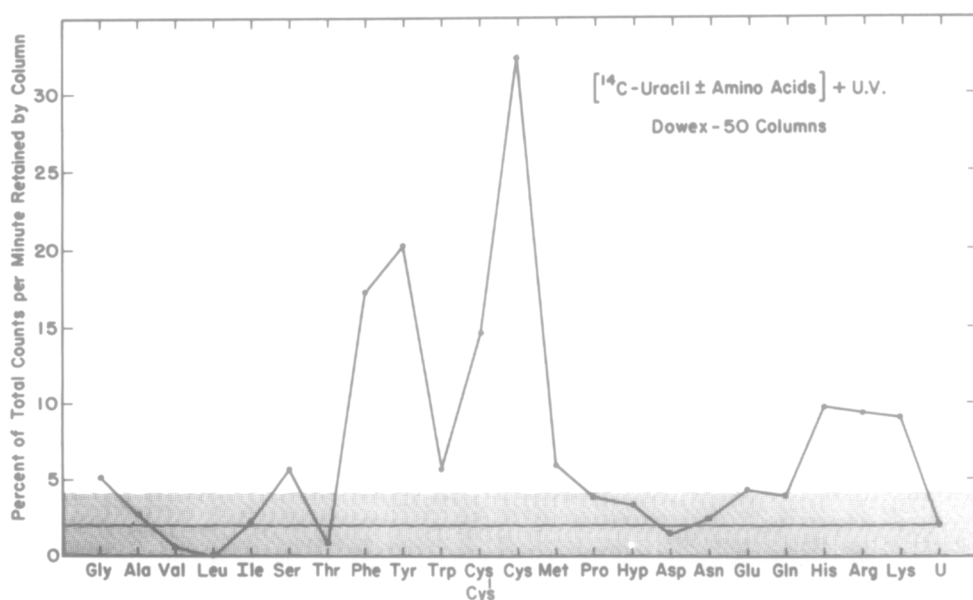


Figure 1. The Photochemical Addition of Amino Acids to  $^{14}\text{C}$ -Uracil. A 0.2 ml aliquot of amino acid solution (0.01M; except tyrosine at 0.003M) was mixed with 0.05 ml of  $^{14}\text{C}$ -2-uracil (0.001M; 25  $\mu\text{Ci/ml}$ ). The molar ratio of amino acid to uracil was thus ~36:1 (except for tyrosine at ~11:1). The solution was irradiated for 200 minutes in a Pyrex spot plate in contact with the rim of a Mineralight U.V. lamp<sub>0</sub> (Model UVS-11, Ultra-Violet Products, Inc.) whose output is mainly at 2537Å. An aliquot was then assayed for content of radioactivity (liquid scintillation counter) and 0.05 ml was introduced to a 4 ml column of Dowex-50-HCl in a plastic 5 ml syringe. The column was rinsed with 25-one ml portions of water (into a volumetric flask) and an aliquot of the combined effluent was counted for radioactivity. The 100% sample minus the material that did not stick to the column gives the amount retained by the column. Most of these counts could be recovered by eluting with 6N HCl. The results plotted here are the average of 2-3 experiments (and 9 for  $^{14}\text{C}$ -uracil (U) alone). The hatched area indicates the spread of the data for  $^{14}\text{C}$ -uracil irradiated in the absence of amino acids.

The technique used for the bulk isolation of the uracil-cysteine compound made use of the fact that while uracil is not normally retained by a Dowex-50 column, it is retained when combined with an amino acid containing a free amino group (Smith and Aplin, 1966). Thus, if  $^{14}\text{C}$ -uracil becomes attached to an amino acid, the complex should be retained by the column and should be assayed as a loss of  $^{14}\text{C}$  from the column effluent. Using this simple procedure, we have surveyed the reactivity of the common amino acids for photochemically adding to  $^{14}\text{C}$ -uracil (Figure 1).

The photochemical addition of amino acids to uracil appears to be a fairly general phenomenon. Of the 22 amino acids tested, 11 were significantly reactive. Thus, of the aliphatic amino acids, glycine and serine react; of the aromatic amino acids, phenylalanine, tyrosine and tryptophan react; of the sulfur amino acids, cystine, cysteine and methionine react; and of the basic amino acids, histidine, arginine and lysine react. The most reactive amino acids were cysteine, tyrosine and phenylalanine.

The mechanisms for the addition of these amino acids to  $^{14}\text{C}$ -uracil must await the bulk isolation of the products and their chemical identification. The finding that 11 amino acids react photochemically with uracil suggests many new mechanisms by which DNA and protein can become cross-linked in vivo by ultraviolet radiation.

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