PHOTOLYSIS OF L-TYROSINE AND PHOTOMODIFICATION OF PEPTIDES[1]

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It is well known that light may inactivate enzymes and denature proteins (Luse and McLaren, 1963; McLaren, Gentile, Kirk and Levin, 1953), but what is much less understood is the mode of action of the incident radiation. The prevailing view, accounting for the chemical change induced by ultraviolet light, is that the preferential breakage of S-S, S-H and C-S bonds occurs, followed by modification of aromatic residues in an, as yet, unestablished manner (Luse and McLaren, 1963). Previous work in our Laboratory has shown that L-histidine, which contains an heteroaromatic ring nucleus, on photolysis in solution, yields a variety of end products (Jaskewycz and Johns, 1965). Subsequent quantitative studies have shown that histidine cleaves readily and rapidly. Within 5 minutes of u.v. irradiation the aliphatic acids, aspartic, glutamic, γ-hydroxyglutamic as well as citrulline are formed (Jaskewycz and Johns). This report details our findings on the photolysis of L-Tyrosine which may be taken as a representative aromatic amino acid, and the conclusion reached is that under the

experimental conditions, the phenolic ring opens to give aliphatic amino acids. An extension of these findings is that when aromatic and heterocyclic amino acid residues are incorporated in peptides and proteins, they might be expected to be transformed by light to aliphatic amino acids to give, where the peptide bond remains intact, profoundly modified and new peptides or proteins. We have tested our hypothesis with the tripeptides glycylhistidylglycine and glycyltyrosylglycine, and also with glycyltyrosine. Our results, reported here, show that just such a photomodificatio does in fact occur.

Incomplete and conflicting reports have appeared on the products derived from L-tyrosine in aqueous solution when irradiated with u.v. light (Luse and McLaren, 1963 summarise these data). From a series of photolyses of L-tyrosine (2.9×10^{-4}M. pH 1.5, 2.7, 4 and 5.8; 17°C) using a medium pressure Hg lamp with main output between 3340 and 2480 A° and at varying lengths of time from 2 to 15 minutes, the following were identified: p-hydroxyphenyl-lactic and acetic acids; dopa, glycine, alanine, serine and aspartic acid. The products were identified by their paper chromatographic behaviour in neutral, acidic and basic solvents, together with a combination of colour tests. The concentration of dopa reaches a maximum at low pH in the initial radiation period and is then rapidly transformed. At alkaline pH, its transient formation could not be confirmed. It is probably a true intermediate in the formation of the coloured product derived from photolysed tyrosine. Tyramine, formed by decarboxylation has been identified at alkaline pH only. Aspartic acid is derived by photolytic oxidative cleavage

of the phenolic ring. Quantitative studies under way may lead to a clarification of the mechanistic pathway (Gordon and Johns).

When glycyltyrosine was photolysed at pH 5.8 (45 minutes; 17°C; 4MHAc filter), glycylaspartic acid and glycylalanine as well as free glycine and aspartic acid were identified by conventional paper chromatographic procedures. Peptide bond cleavage is a major reaction. An unphotolysed control showed negligible quantities of free amino acids. The aspartic acid may be derived from hydrolysis of the glycylaspartic acid or from the tyrosine liberated from the original dipeptide.

Photolysis of the tripeptide glycylhistidylglycine (pH 5.9; 30 minutes; 17°C) yielded the modified tripeptides, triglycine and glycylaspartylglycine among others, as yet unidentified. The former was separated and identified by low voltage electrophoresis procedures; the latter tripeptide by a combination of high voltage electrophoresis at pH4 and ascending paper chromatography. Elution of the tripeptide spot from the paper and subsequent acid hydrolysis yielded only glycine and aspartic acid. No evidence could be obtained for the presence of the dipeptides glycylaspartic acid or aspartylglycine in the photolysis mixture. The identification of glycylaspartylglycine was checked by photolysis of glycylhistidylglycine-C14(u) when glycylaspartylglycine-C14(u) was separated, first by low voltage electrophoresis, and by high voltage electrophoresis found to be homogeneous, identical with an authentic marker and to run separately from the markers of the two possible dipeptides. Results on hand from the photolysis of

glycyltyrosylglycine suggest that here also, the photolysis follows a pathway involving ring opening to give derived aliphatic tripeptides.

The above results provide good reasons to believe that histidine in particular and also tyrosine, can play a much greater role than has hitherto been ascribed to these residues in photolytic transformations of proteins and peptides. We would suggest that such transformations involving these residues may be understood in terms of conversion of these light sensitive centres to aliphatic amino acid residues, in addition to any initial changes associated with sulphur residues. In a biological context, such profound transformations would be expected to have significant consequences.

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