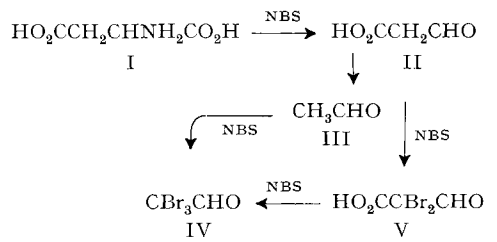
tures. Under these conditions the reaction had gone to completion in 3–5 min at room temperature, with about 75–85% of the final concentration of product present at 1 min. Variation of the pH of the citrate-phosphate buffer showed similar yields of BuOH-extractable antibacterial activity at pH 2.2 and 3.0, about 50% of this amount of activity at pH 4.0, a trace of activity at pH 5.0 and no discernible activity at pH 6.0, 7.0 and 7.9.

Again using the zone of inhibition as an assay, the yield of product from aspartic acid treated with various molar ratios of NBS was estimated. The quantity of NBS was varied from about 1 mole per mole of aspartic acid to about 25 moles per mole. The amount of product was about 34% at 1:1, about 62% at 2.5:1, and about 94% at 10:1, relative to 100% at 25:1.

Since no bioactive product was obtained when glutamic acid was substituted for aspartic acid as the substrate for NBS decarboxylation, this reaction might prove to be of value for the specific assay of aspartic acid without prior purification of an amino acid mixture or protein hydrolyzate.

The formation of bromal, IV, from aspartic acid, I, may follow the route shown below:



The first step, oxidative decarboxylation, is a characteristic reaction of amino acids with NBS[1]. The resulting formylacetic acid, II, would be expected to brominate rapidly at the methylene group to give V, followed by brominative decarboxylation to give bromal, IV. The alternative pathway of decarboxylation of II to acetaldehyde, III, followed by bromination of III to IV, is ruled out by the failure of acetaldehyde to form bromal on treatment with NBS under our conditions.

After the identity of our bioactive substance was ascertained, bromal was evaluated against a variety of microorganisms by a standard twofold tube dilution assay. The results are presented in the Table. Such data for this compound are not tabulated elsewhere, even though the germicidal properties of bromal have been recognized for over 50 years[6].

Zusammenfassung. Bromal wurde als ein Endprodukt der Reaktion zwischen Asparginsäure und N-Bromsuccinimid identifiziert und die antibakterielle Aktivität dieser Verbindung gegenüber einigen Mikroorganismen geprüft.

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The Tryptophans in Flavodoxin and Synthetic Flavinyl Peptides Characterized by Chemical and Photochemical Oxidations

Flavodoxin is a low molecular weight electron transfer flavoprotein first observed in iron-deficient *Clostridium pasteurianum*[1]. The isolated flavoprotein was observed to exhibit a shoulder in the flavin spectrum at 472 nm and was found to function as an electron carrier in the oxidation of pyruvate by the clastic system from the same microorganism with the subsequent formation of acetyl phosphate[2]. Analysis of clostridial flavodoxin revealed 4 tryptophan residues and 1 flavin mononucleotide (FMN) as tightly bound coenzyme with an apparent oxidation-reduction potential more negative than free flavin[3]. A similar FMN-dependent flavodoxin containing 4 tryptophans recently has been isolated from *Peptostreptococcus elsdenii*[4], and the oxidation-reduction properties of this system were examined[5].

The constant presence of tryptophan in flavodoxins suggests a possible role at the FMN-binding site, since of all the common amino acids, tryptophan complexes intermolecularly best with oxidized (quinoid) flavin in aqueous solution. Very tight intramolecular associations occur with synthetic flavinyl tryptophan peptides[6], wherein flavin fluorescence is markedly decreased[7]. Moreover, the generation of a red shoulder in the absorption spectrum of oxidized flavin resultant from association with tryptophan[6,7] is compatible with that seen in oxidized flavodoxins as is the lowering of observed oxidation-reduction potential which can result from selective decrease in the effective concentration of oxidized versus reduced flavin[8].

All of the above considerations led us to the present investigation of the nature of the tryptophan residues in flavodoxin by comparing their chemical and photochemical oxidizability with tryptophan in synthetic flavinyl tryptophan peptides and with tryptophan in the presence and absence of flavin.

Materials and method. Flavodoxin was purified from iron-deficient *C. pasteurianum* and the phosphoroclastic assay used[2]. Flavinyl tryptophans with chains of 1 and 5 methylene groups separating amide-linked tryptophan methyl ester from the N-10 position of the flavin nucleus were synthetized as described previously[6]. Chemical oxidations with N-bromosuccinimide (NBS) were carried out

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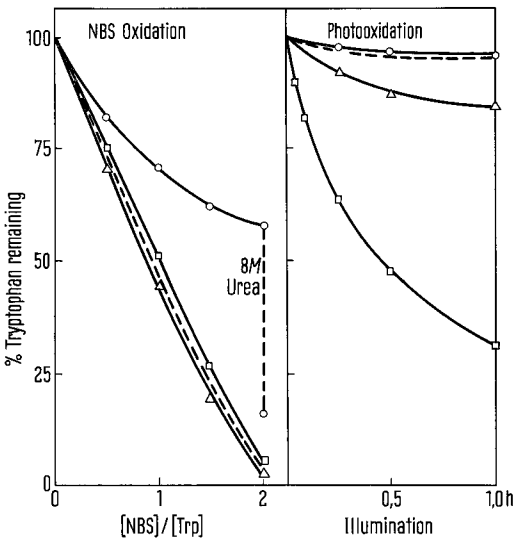
in 0.1 *M* sodium acetate buffer, pH 4, at 25 °C for 30 min essentially according to SPANDE and WITKOP[9]. Loss of FMN from NBS-treated flavodoxin was noted by spectral measurement of the protein after dialysis. Photochemical oxidations with 450 nm light from a xenon lamp monochromatized by an Aminco-Bowman spectrophotofluorometer were carried out in aerobic solutions of 0.1 *M* sodium phosphate buffer, pH 7.5, at 25 °C. These conditions have been found satisfactory for photooxidation of tryptophan in the presence of FMN[10]. Tryptophan was assessed by the colorimetric method of SPIES and CHAMBERS[11], which is insensitive to the oxidation products of this amino acid.

Results and discussion. Results obtained upon treatments of tryptophan alone, flavodoxin, flavinyl tryptophan with 5 methylene groups between chromophores, and tryptophan in the presence of FMN in the ratio known for flavodoxin is shown in the Figure. Under the conditions used for the oxidation with NBS, tryptophan alone, with FMN, and in the flavinyl peptide is extensively and almost equally oxidized. The same behavior was also found for the flavinyl tryptophan with only 1 methylene group between chromophores. Hence, such tight complexing as occurs between flavin and tryptophan portions of the synthetic peptides does not prevent the ready and complete chemical oxidation of the amino acid. On the other hand, only about half of the tryptophan residues of flavodoxin are oxidized by similar treatment, whereupon FMN is released and activity lost. Most of the remaining tryptophan in this flavoprotein becomes susceptible to oxidation by NBS in 8 *M* urea, as indicated. Apparently 2 of the 4 tryptophans are much less exposed. As shown by the data for photooxidation in the figure, the flavin coenzyme in flavodoxin cannot act as photosensitizer for oxidation of tryptophan residues in flavodoxin, as is also true for the flavinyl tryptophan with 1 methylene group between chromophores, under conditions where ready photooxidation of tryptophan in the presence, but not absence of FMN occurs. Even the flavinyl tryptophan with 5 methylene groups between chromophores loses but little of the tryptophan portion. Hence, tight complexing between flavin and tryptophan does prevent ready and complete photochemical oxidation of the amino acid.



Oxidation by N-bromosuccinimide (left) and aerobic illumination at 450 nm (right) of tryptophan alone (dashed lines), within flavodoxin (○), in a flavinyl-(5-methylenes)-tryptophan peptide (△), and together with FMN in solution (□). All solutions were initially equimolar with respect to tryptophan.

Overall, the present results indicate that 2 of the 4 tryptophan residues of flavodoxin are relatively buried and that at least one of these could be tightly complexed with FMN. It should also be recalled that a cysteine sulfhydryl has been implicated in the binding of the coenzyme[3]. NBS treatment might also oxidize this function and lead to a loss of activity, and photochemical treatment would not necessarily involve tryptophan[12].

Zusammenfassung. Zwei der vier Tryptophan-Reste in Flavodoxin sind relativ gut abgeschirmt, wie das Ausmass der Oxidation durch N-Bromsuccinimid zeigt. Ausserdem kann wahrscheinlich mindestens einer dieser Tryptophan-Reste mit FMN einen stabilen Komplex bilden, wie die Unempfindlichkeit gegenüber Photooxidation nahelegt.

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Graduate School of Nutrition and Biochemistry Section, Cornell University, Ithaca (N.Y. 14850, USA), 15 September 1969.

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Turnover of Hepatic Collagen in Reversible and Irreversible Fibrosis

Diffuse hepatic fibrosis in the rat produced by ethionine intoxication is characterized by a parallel increase in the amount of collagen and in the number of cells. Therefore, there is a constant hydroxyproline (OHPr)/DNA ratio[1]. The fibrosis is reversible upon replacement of ethionine by methionine when 5 times the normal collagen content of the liver is catabolized within 14 days. During the period of rapid fiber resorption, the half-life of hepatic collagen is only 10 days[2].

In early carbontetrachloride (CCl_4) induced hepatic fibrosis, the OHPr/DNA ratio similarly remains constant. Later the ratio increases[3]. These observations pose the following questions: (a) Does hepatic fibrosis remain reversible when the OHPr/DNA ratio is increased? (b) Is

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