4a,5-Cycloaddition Reactions of Acetylenic Compounds at the Flavoquinone Nucleus as Mechanisms of Flavoprotein Inhibitions

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(Z. Naturforsch. 27 b, 1050—1052 [1972] ; received May 10, 1972)

4a,5-Cycloaddition at the flavoquinone, Mechanism of flavoprotein inhibitions

Photochemically, covalent cycloaddition products of flavoquinone and acetylenic enzyme inhibitors have been prepared and elucidated, which appear analogous to flavoprotein-inhibitor complexes (monoamine and lactate oxidase). The additions occur with inhibitors of general type \( \text{HC} = \text{C} - \text{CH} < \) at the \( \text{C}(4a)=\text{N}(5) \) azomethine grouping of oxidized flavin. The spectral properties of adducts and their chemical reactivity is described.

The well known monoamine oxidase (MAO) inhibitors $^1$ of Pargyline$^\circ$ type have been found to induce irreversible photoreduction of flavoquinones (\( \text{Fl}_{\text{ox}} \)), yielding a number of covalent flavin-inhibitor adducts, two of which (1 and 2) have been isolated in the crystalline state. Analogous addition reactions have been found with the lactate oxidase inhibitor, \( \text{CH} = \text{C} - \text{CH(OH)} - \text{COOH} $\footnote{2}. In both cases, the dark reaction at the enzyme active site $^3$ seems to parallel the photoreaction of the chemical model, though the latter is, of course, less specific and yields a greater variety of products. However, it is clear, that the inhibitions observed with both enzymes are due to the irreversible cycloaddition to

![Diagram](image-url)

Fig. 1. $^1$H-NMR-spectrum of compounds 1 (full line in \( \text{CF}_3\text{COOH} \); dotted line in \( \text{CF}_3\text{COOD} \)) and 2 (in \( \text{CDCl}_3 \)). The number of protons as found by integration is indicated at the base of each peak.

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the flavocoenzyme, the apoprotein being unaffected.

From our earlier studies on flavin photoalkylation with e.g. phenylacetic acid\(^4,5\) it can be concluded that the primary intermediates in the above reaction are N(5)- and C(4a) - CH(NMe\(_2\)) - C = CH isomers, which then undergo rapid ring closure to yield 2 and 1, respectively (cf. Scheme). Type 1 contains two stable new centers of chirality and will therefore appear as a mixture of diastereoisomers. However, in type 2 the 5α-center is a hemiaminal, which causes protolytic loss of dimethylamine and selective reclosure of the ring to yield only one diastereoisomer of highest thermodynamic stability.

In compound 1 the olefinic group is part of an enamine system, which undergoes deuterium exchange through C(5β)-deuteration in deuterotri-fluoroacetic acid, as demonstrated by NMR (Fig. 1). The resonance of the olefinic group explains, moreover, the absorption properties of the new chromophore with its very high extinction at 390 nm (Fig. 2). In contrast, compound 2, which has a non-conjugated olefin, shows a typical HC - OH-double dublett in the NMR (Fig. 1), the OH-part of which disappears with \(\text{D}_2\text{O}\) alone while the neighbouring CH-splitting collapses. Compounds 1 and 2 differ characteristically by their reconversion to yield \(\text{Fl}_{\text{ox}}\): Compound 1 is easily reconverted, e.g. via 1a, by alkaline treatment followed by photooxidation, or directly and more specifically, with any non-tertiary amine base at \(\text{pH} \geq 8\) (cf. Scheme). This explains why the formation of type 1 compounds and, therefore, the inhibitory action, is only found with tertiary propargylamines. Com-

![Scheme](image_url)
4 of the flavin nucleus. These findings support the general idea that (4a)C = N(5) is the main redox reaction center of the flavin nucleus. Furthermore, it is obvious that the minimum requirement for irreversible additions of the above type is the "substrate" grouping HC≡C—CH< and that such inhibitions might be a general principle in flavoprotein chemistry.

As the result of a search for compounds of simple structure which might interact with enzyme-bound flavin, we have found that nitroalkanes are general reductive substrates for the flavoprotein oxidases. We describe here the more salient features of their properties as substrates for D-amino acid oxidase, glucose oxidase and L-amino acid oxidase. Each enzyme differs somewhat in its specificity for nitroalkanes. At pH 8.1, with substrates in their equilibrium states of ionization, the bimolecular constant for flavin reduction is in the order nitromethane < nitroethane < 1-nitropropane < 2-nitropropane for D-amino acid oxidase. This order of reactivity is also approximately true for L-amino acid oxidase, whereas with glucose oxidase the order is almost completely reversed. The nitroalkanes reduce free FAD photochemically, but we have observed no reactivity in the dark. The overall stoichiometry of nitroalkane oxidation is analogous to that for the natural substrates. The D-amino acid oxidase reaction is accurately described by Eqn. 1.

\[
R\text{CH}_2\text{NO}_2 + \text{OH}^- + \text{O}_2 \rightarrow \text{RCHO} + \text{NO}_2^- + \text{H}_2\text{O}_2.
\]

In the case of glucose oxidase at pH 5.0, Eqn. 1 does not entirely account for the stoichiometry since only about 0.5 moles each of H_2O_2 and NO_2^- are formed. The remaining nitrogen is largely accounted for as NO_3^−, with a small fraction appearing as 1-dinitroalkane.