FLAVIN-SENSITIZED PHOTOOXIDATIONS 1035


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pH-Dependence, Isotope Effect and Products of Flavin-sensitized Photodecarboxylation and Photodehydrogenation

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Flavin-sensitized photodecarboxylation and photodehydrogenation

The pH-dependence of flavin-sensitized photodehydrogenation and oxidative photodecarboxylation shows a change of reaction rate with an apparent pK of about 5.5. This “photo-pK” cannot be assigned to any known ground state or excited flavin or substrate species, nor is it related to the kind of bond being broken (C—H or C—COO⁻). Therefore, it is attributed to the pK of a covalent flavin-substrate intermediate. Carboxylates R—COO⁻ react by decarboxylation, the nature of R determining only the rate of reaction. The acids R—COOH themselves are unreactive, unless functionally substituted at the α-carbon. Hence X—CH(R)—COOH behaves as does X—CH₂—R,+ for X=OH, OR, NH₂, NR₂, R₃N—CH(R)—COO⁻ is unreactive, while R₃N—CH(R)—COO⁺ is slowly dehydrogenated through its neutral tautomer which is favored in the flavin-substrate exciplex.

Photodeoxidations sensitized by flavoquinone (I) show a change of reaction rate between pH 4 and 7.1—5. This change, observed with numerous substrates, is sigmoid with an apparent pK of about 5.5 (Fig. 1). This “photo-pK” cannot be attributed to a substrate pK nor to the pK of flavin species in any known redox or even photoexcited state. The “photo-pK” cannot be attributed to flavin-substrate π-complexes since it is independent of substrate structure and the kind of bond being broken (C—H or C—COO⁻). Hence we assign the “photo-pK” to a short-lived, covalent flavin-substrate intermediate as could be trapped and characterized in the case of phenylacetate (R=H) under alkaline conditions (2, Scheme I). The reaction pathway via 2 is not obligatory, since some substrates of flavoquinone-sensitized photooxidations show no “photo-pK” (Fig. 2). Furthermore, kinetic and isotope-exchange data demonstrate a second, direct pathway for the formation of 3 (R=H). The substrate residue at position C(8) in the cyclohexa-2,5-dien-1-imine type intermediate 2 readily migrates in an acid-catalyzed rearrangement to position N(5) yielding the well known 5-alkyl-1,5-dihydroflavin (3) and subsequently the thermodynamically more stable 4a-alkyl-4a,5-dihydro-flavin (4) by thermal rearrangement.

In the case of mandelate (R=OH) only the final 1,5-dihydroflavin (5) can be trapped. We propose from the pH-dependence of the reaction rate showing the “photo-pK” = 5.5, that in this case a species of type 2 is also formed but decays very rapidly with liberation of benzaldehyde. The latter has been characterized quantitatively.

From this it seems that flavoquinone has two “photoactive sites”, i.e. 4a,5-C=N and 8,9-C=C bonds. The choice between them depends on the nature of the substrate and on reaction conditions (pH, temperature and solvent polarity) which influence the structure of non-covalent “preequilibrium complexes”. These are typical “exciplexes”...
Fig. 1. pH-dependence of anaerobic flavin photoreduction. Initial rates, measured by the decrease of the long wavelength flavin absorption band, are plotted against pH. Substrate concentration was 0.1 M, 3-methyl-lumiflavin $7 \times 10^{-5}$ M, NaClO$_4$ 0.1 M, buffers 0.01 M (sulfate < pH 3 < acetate < pH 6 < phosphate). Illumination by tungsten lamp with wavelengths 420 < $\lambda$ (nm) < 500 (interference filter Balzer K 2) light intensity $10^5$ Lux, temperature 25 °C. — An analogous pH-dependence of flavin photoreduction between pH 4 and 7 is found with the following substrates:

- $\text{CH}_3\text{S-}-(\text{CH}_3)_2\text{S-}\text{NH}_3^+$,
- $\text{CH}_3\text{S-}-(\text{CH}_2)_2\text{S-}\text{CH(NH}_2\text{)_2-}\text{COOH}$,
- $\text{CH}_3\text{C(}\text{CH}_3\text{)_2OCH}_2\text{-COO}^-$,
- $\text{C}_6\text{H}_5\text{-CH(OH)-COO}^-$,
- $\text{C}_6\text{H}_5\text{-CH(CH}_3\text{)-COO}^-$.

The pK$_a$-values in the Fig. refer to the various substrate acids and are partially overlapping the more constant "photo-pK" of z 5.5.

Fig. 2. pH-dependence of anaerobic flavin photoreduction by different substrates (0.01 M) in 50% aqueous methanol. Other conditions are the same as in Fig. 1.

in the case of photoreactions. In enzymes, the apoprotein will influence the site of substrate attack at the flavin.

Quite generally we can show that carboxylic acid anions react with flavoquinone triplet by decarboxylation, not dehydrogenation. Accordingly, similar to the phenylacetate reaction, no kinetic isotope effect could be found with $\alpha$-D-mandelate, in contrast to the results of Penzer at al. Furthermore, the benzyl groups of the alkylated dihydroflavins and the benzaldehyde formed in these photoreductions show full isotope retention of the starting CH- or CD-equivalents. Carboxylic acid anions without an $\alpha$-CH-group, like 2,2-dimethyl-propionate and 2-phenyl-isobutyrate, react in the same way as carboxylic acid anions with an $\alpha$-CH-group. They also show the "photo-pK" and form 5- and 4a-alkylated dihydro-flavins (3 and 4, see Scheme I). With 2-phenyl-isobutyrate $5 \to 4a$-rearrangement (see Scheme I) proved to be reversible, dependent on pH.

In the case of amino acids the following scheme applies:

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\begin{align*}
\text{H}_2\text{N-CR-COOH} & \rightleftharpoons \text{H}_2\text{N-CR-COOH}^+ & \text{H}_2\text{N-CR-COOH}^+ \rightarrow \text{H}_2\text{N-CR-COOH}^+ \\
\text{R-CO-COOH} + \text{NH}_3, \text{slow} & \rightarrow \text{no reaction} & \text{R-CHO} + \text{CO}_2 + \text{NH}_4^+, \text{fast}
\end{align*}
$$
The amino acid anions react via decarboxylation, similar to carboxylic acid anions. At basic pH values no keto acids are formed. With phenylglycinate no isotope exchange is observed in the benzaldehyde which is formed quantitatively. The neutral species reacts by dehydrogenation, but much slower than the anion. The formation of keto acid could be demonstrated in the case of EDTA. As indicated by the lack of reactivity of betaine, this is due to the fact that only the neutral and not the dipolar species is active and is obviously stabilized in the flavin-amino acid exciplex.

With acids of type $R - CH_2 - COO^-$ we find the following sequence of increasing relative reaction rate with flavoquinone triplet:

1. $R = CN \sim N(CH_3)_2 \sim Cl < OCOCH_3 \sim OH \sim H$
2. $< OCH_3 \sim CH_3 \sim NHCOCH_3 < CH = CH_2 \sim C_6H_5$
3. $< N(CH_3)_2 \sim NH_2$

We find that decarboxylation prevails over dehydrogenation if a COO$^-$-group, but not if a COOH- or COOR-group is present.

It is concluded that flavin-dependent dehydrogenation and oxidative decarboxylation generally occur through covalent flavin-substrate intermediates of type $R - Fl - H$ which, in the case of most "natural" residues $R$, decay very readily with formation of $R^+$ (or solvolyses products thereof) and $Fl_{red}$ (leucoflavin anion).

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