PHOTOREDUCTION OF FLAVOCOENZYMES BY PYRUVIC ACID

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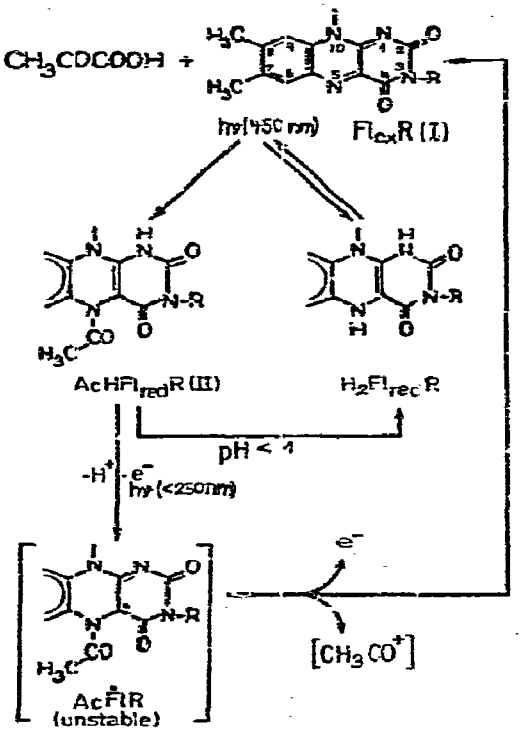
Received 21 August 1968

Recently, De Kok and Veeger [1] described the photoreduction of various flavoproteins and found two different reactions depending on the quantum energy: Under 320 nm irradiation, i.e. the substrate n-π^* transition being excited, an electron transfer from pyruvate to flavoquinone took place, resulting in the formation of flavosemiquinone anion [2], which is stable under anaerobic conditions of fixation to the protein. Under 390 nm irradiation, however, the flavin system is excited which leads to the irreversible formation of an adduct containing flavin and ^{14}C from $^{14}CH_3COCOO^-$. The adduct can be discharged from the protein and be separated chromatographically. While the above mentioned authors found no comparable reaction with *free* flavocoenzymes, we want to show in the present note, that also free flavins (I) undergo irreversible photoreduction by pyruvate under suitable conditions of pH and medium polarity (cf. scheme 1).

As a model flavin we use 3-methyllumiflavin [3] in favor of any natural coenzyme like FMN or FAD, for the following reasons: (i) 3-methyllumiflavin (I, R=CH_3) undergoes *photoreduction* in the same way as the natural coenzymes, but is not amenable to photolysis [4]. (ii) 3-methyllumiflavin is sufficiently soluble in water as well as alcohols or aprotic media (dioxane or DMF). Its apolar solutions reflect the protein-bound flavin better than aqueous solutions of FAD and FMN.

When dilute solutions of 3-methyllumiflavin are irradiated with tungsten light in the presence of pyruvic acid or its salts, reversible photoreduction is the predominant reaction (cf. scheme 1) under any conditions. If this reaction is suppressed by continuous admission of O_2, a somewhat slower irreversible photoreduction becomes marked and finally quantitative. The spectral course of this reaction is shown in fig. 1.



Scheme 1.

The end product of the irreversible reaction has been isolated by evaporating solvent and excess pyruvic acid at high vacuum and reprecipitation of the residual nearly colorless stable leucoflavin derivative from dilute aqueous ammonia by acid. The compound proved to be identical with 3-methyl-5-acetyl-1,5-dihydroflavin by IR-spectra (ν_{CO} = 1642, 1678, 1709 cm^{-1}) [5] and UV-spectra (fig. 2) [6] as well as thin layer chromatography and quantitative reoxidation by HONO [7].

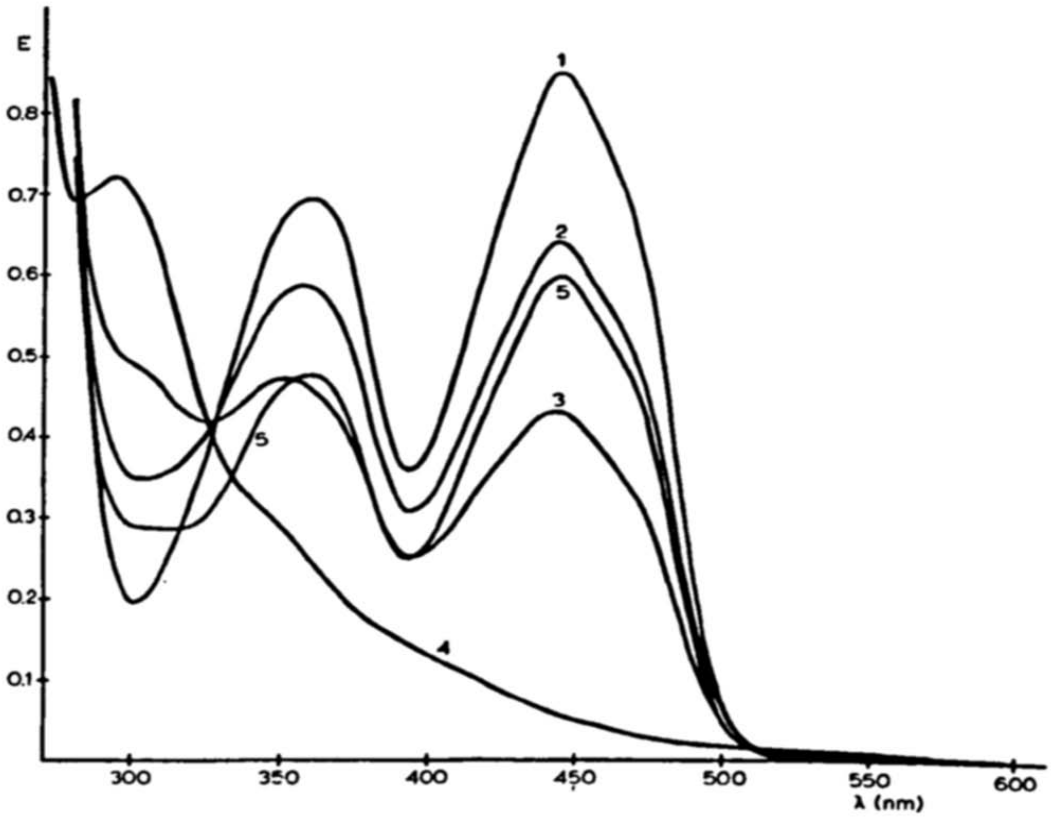


Fig. 1. Anaerobic photoreduction of 6.25×10^{-5} M 3-methyllumiflavin in 0.02 M aqueous Na-pyruvate by a 200 Watt tungsten lamp at 15 cm distance. Spectrum 1 is taken before irradiation, spectra 2–4 are taken at 20, 60, 600 seconds. Spectrum 5 shows partial re-autoxidation (2 min aeration). The difference between 1 and 5 indicates the amount of irreversible photoreduction, i.e. the amount of II formed. The cycle 1–5 may be repeated until irreversible reduction is complete. Full restoration of spectrum 1 is then found on addition of $NaNO_2$ and acetic acid (residual HONO removed in vacuo).

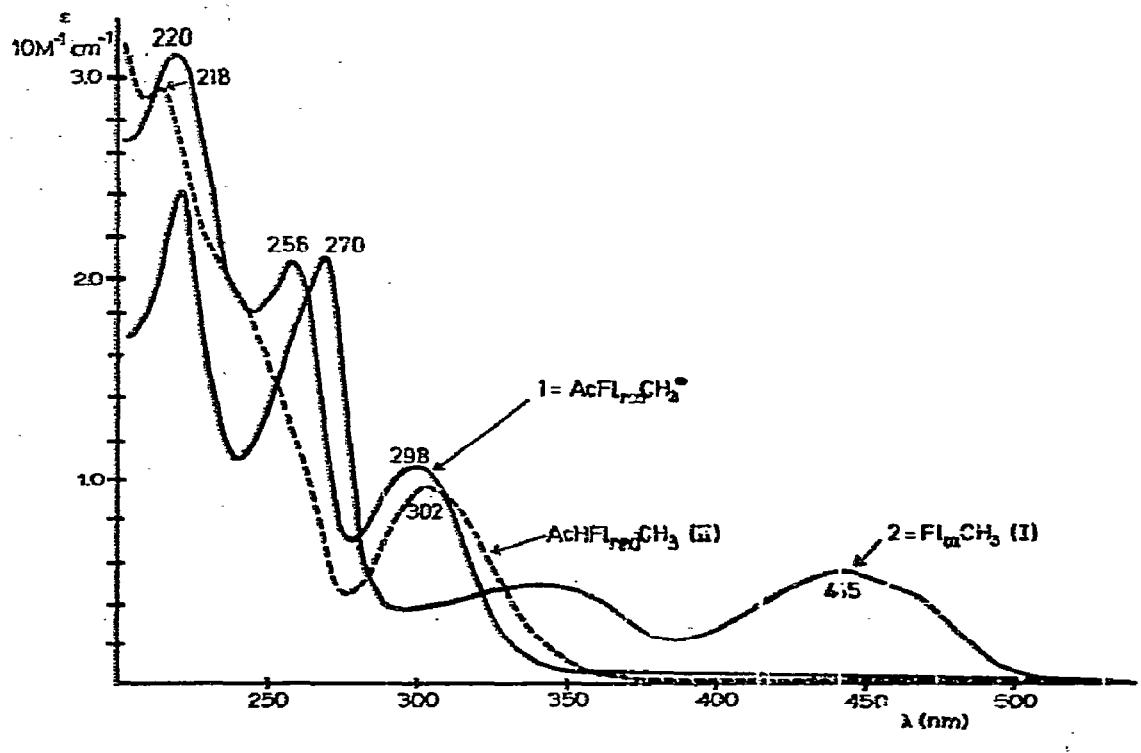
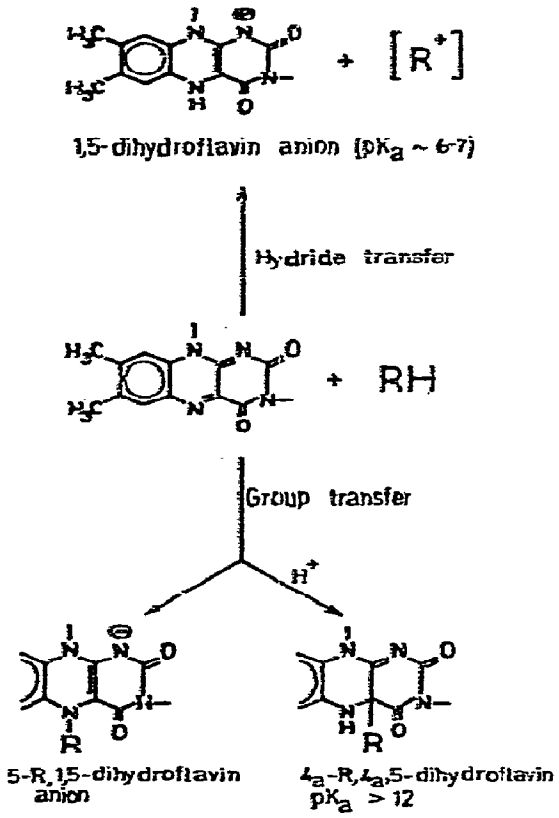


Fig. 2. Spectra of the pure "photoflavin", identical with those of authentic II. Broken line: II in CH_3OH. Curve 1: II in 0.1 M phosphate buffer pH 7.0 (anion). Curve 2 was obtained from curve 1 upon 60 minutes irradiation with a 250 mμ mercury lamp.



Scheme 2.

5-Acetylation renders the leucoflavin nucleus stable towards O_2, presumably by favoring its bent configuration through withdrawal of π-electrons at N(5) from tricyclic delocalisation. Removal of an electron by suitable acceptors as NO^+ leads to deacetylation even under strictly non-protolytic conditions (glacial acetic acid). O_2 will act as acceptor if the system is photoexcited (250 nm). This may be due to higher planarity of the photoexcited state. Therefore, one has to envisage the formation of 'active acetate' according to scheme 1. It remains to be established, whether such a mechanism could have biological importance. The question may be generalised in the following way:

Hitherto the first step of flavin dependent dehydrogenation is taken to be a 'hydride' transfer ('hydride' being written for 'proton + 2 electrons in one step'). It has to be envisaged, however, that this step may rather imply a group transfer (scheme 2) where the 'active' residue R is fixed to either of positions 4a or 5, as established for model reactions [8].

References

[1] A. De Kok and C. Veeger, Biochim. Biophys. Acta 131 (1967) 589.
[2] A. Ehrenberg, F. Mueller and P. Hemmerich, European J. Biochem. 2 (1967) 286.
[3] P. Hemmerich, Helv. Chim. Acta 47 (1964) 464.
[4] P. Hemmerich, C. Veeger and H.C.S. Wood, Angew. Chem. Internat. Edit. 4 (1965) 671.
[5] P. Hemmerich, B. Prijs and H. Erlenmeyer, Helv. Chim. Acta 43 (1960) 372.
[6] K.H. Dudley, A. Ehrenberg, P. Hemmerich and F. Mueller, Helv. Chim. Acta 47 (1964) 1354.
[7] K.H. Dudley and P. Hemmerich, Helv. Chim. Acta 50 (1967) 355.
[8] W. H. Walker, P. Hemmerich and V. Massey, Helv. Chim. Acta 50 (1967) 2269.