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SYNTHESIS OF THE FLAVOCOENZYME OF MONOAMINE OXIDASE

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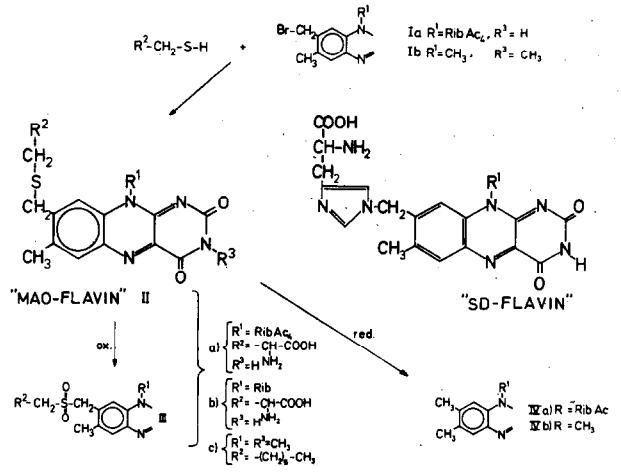
1. Introduction

Previous studies of Igaue, Gomes and Yasunobu [1] and Erwin and Hellerman [2] suggested that the liver and kidney monoamine-oxidase (MAO) coenzyme, an FAD derivative, is covalently linked to the protein. Upon hydrolysis it is degraded in consecutive steps to the mononucleotide and the riboflavin-oligopeptide ("MAO-flavin") level. ESR and absorption spectra of MAO-flavin [3] indicated that the peptide chain is coupled to the flavin nucleus at position 8α as in the case of flavin in succinate dehydrogenase ("SD flavin") [4–7]. In contrast to SD-flavin (cf. the scheme 1) the fluorescence of MAO-flavin as compared to that of riboflavin is quenched over the whole pH range. Recently the peptide sequence of MAO-flavin was determined and the amino acid linked to the 8α-carbon was shown to be cysteine in thioether linkage [6]. This structure is entirely in accord with our own conclusion that the 8α-substituent in the case of MAO-flavin must be a functional group devoid of acid-base properties, though capable of donating electrons to and accepting excitons from the flavin nucleus. Hence, among the functional groups contained in proteins only the aromatic systems of tryptophan and tyrosine, and the cysteine sulphur could be considered as links between peptide and the flavin nucleus in MAO-flavin. As we found, the model compound 8α-(*S*-*n*-octylmercapto)-3-methyl-lumiflavin (IIc) simulated the spectral and chemical properties of the MAO-flavin coenzyme. We synthetized therefore 8α-(*S*-cysteinyl)-riboflavin (IIb) which in the present communication is shown to be identical with the flavoenzyme isolated by Walker, Kearney and Singer [6] from liver monoamine-oxidase.



Reaction scheme

2. Synthesis

The 8α-bromo-tetraacetyl-riboflavin (Ia) [7] was allowed to react with cysteine hydrochloride in absolute dimethylformamide for 48 hr at room temperature. After evaporating the solvent in vacuo, phosphate buffer (pH 7) was added and the mixture extracted with chloroform in order to remove all unreacted flavin. To remove unreacted cysteine, the aqueous phase, containing the 8α-(*S*-cysteinyl)-tetraacetyl-riboflavin (IIa) was adjusted to pH 4 with sulphuric acid, saturated with ammonium sulphate and the flavin extracted with *n*-butanol. Protection of the cysteine amino function was found to be unnecessary in this reaction because of the nucleophilicity of the sulphydryl residue, which ensures selective substitution of the bromine by sulphur. Mild acid hydrolysis of the

acetyl groups gave the desired 8α-(*S*-cysteinyl)-riboflavin (IIb).

Similarly 8α-bromo-3-methyl-lumiflavin (Ib) [8] was reacted with *n*-octyl-mercaptan in absolute dimethylformamide and diisopropyl-ethyl-amine at 40° for 1 hr to yield 8α-(*S*-*n*-octyl-mercapto)-3-methyl-lumiflavin (IIc). The reaction mixture was dissolved in chloroform, the base extracted with dilute acid and the flavin separated from unreacted octyl-mercaptan by precipitation with hexane. The crude product, dissolved in toluene, was then separated from unsubstituted flavin by extraction with a large amount of water.

Reductive cleavage of the thioether linkage of compounds II was performed by refluxing in glacial acetic acid with zinc dust over 30 min. Addition of water and extraction with chloroform gave quantitative yields of the corresponding 8α-unsubstituted flavin derivatives IV as shown by chromatography and absorption spectra.

The thioether groups of II were smoothly oxidized with a small excess of peracetic acid or with permanganate in acetic acid at 10° to yield the sulphones III. These results are in full agreement with the known reactivity of thioethers [9].

3. Physical properties and comparison of synthetic and natural MAO-flavin

Comparison of the fluorescence spectra of synthetic and natural MAO-flavin IIb is made in fig. 1. The thioether residue at position 8α of compound II indeed causes a strong quenching of the fluorescence over the whole pH range. Oxidation of compounds II leads to the sulphones IV, which exhibit IR stretching vibrations at 1315 and 1225 cm^{-1}, characteristic of sulphones [10, 11] (fig. 2). Partial oxidation to the corresponding sulphoxyl derivatives can be excluded due to the absence of the characteristic absorption at 1050 cm^{-1}. As compared to the flavin-8α-thioethers II, the sulphones III show a strong increase in fluorescence (fig. 1), which reflects oxidation of the non-bonding electron pairs of sulphur. An analogous reduction of quenching has been observed upon protonation of the imidazole moiety of SD-flavin [7]. A similar effect, due to the electron donating properties of bivalent sulphur [12], is the cause of a slow internal redox reaction leading to reduction of the flavin nucleus and oxidation of the homoconjugated sulphur atom. This explains the instability of compounds II [6].

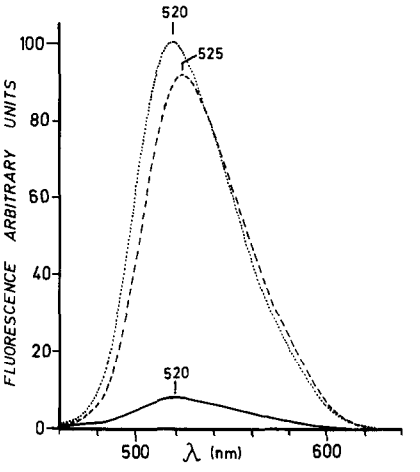


Fig. 1. Relative fluorescence intensitites of synthetic MAO-flavin (IIb) (——) as compared with riboflavin (IVa) (....). Oxidation of MAO-flavin to the sulphone IIIb (- - - -) increases the fluorescence up to 90%. The values for the natural compounds are 10% and 70–80% for IIb and IIIb, respectively [6]. Solutions 4 × 10^{-5} M in phosphate buffer, pH 7, measured with a Beckman fluorometer model SF 1078, excitation 445 nm, λ_{max} values uncorrected.

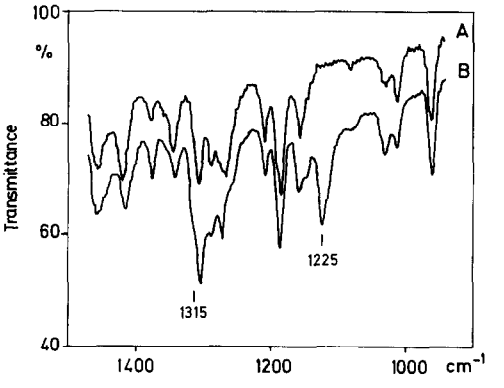


Fig. 2. Comparison of IR spectra of the 8α-thioether-flavin IIc, (A) and of the corresponding sulphone IIIc (B). KBr pellets measured with an Perkin Elmer model 621 spectrometer.

The influence of 8α-substitution on the absorption spectra of the neutral molecules is shown in fig. 3. The increasing –I effect of 8α-substituents in the

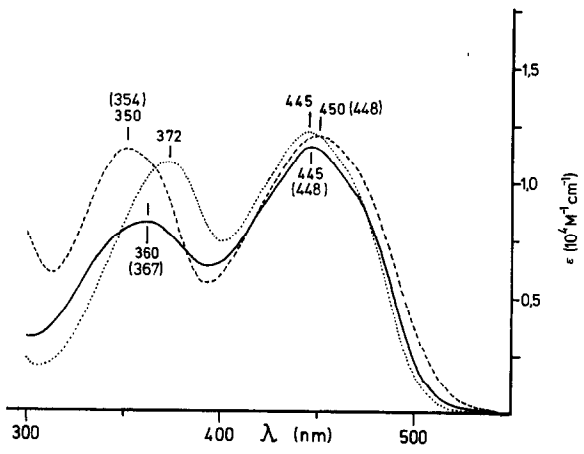


Fig. 3. Neutral spectral of riboflavin (IVb) (. . . .), synthetic MAO-flavin (IIb) (——) and the sulphone IIb (- - - -) in phosphate buffer at pH 7. The λ_{max} values of the natural products [6] are given in brackets.

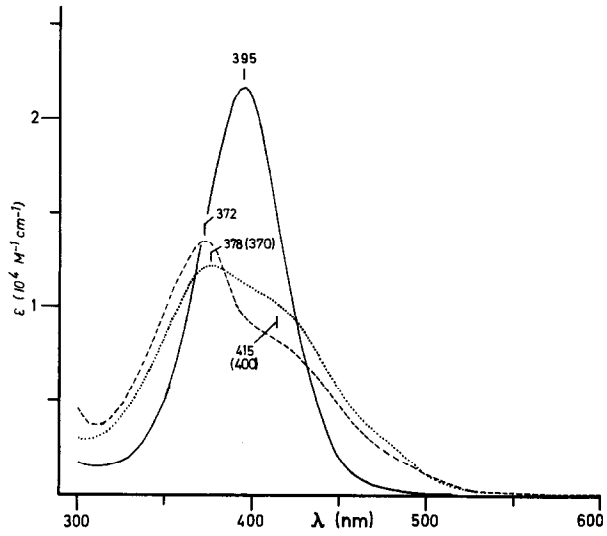


Fig. 4. Cationic spectra of riboflavin (IVa) (——), synthetic MAO-flavin (IIb) (. . . .) and its sulphone (IIIb) (- - - -) in 6 N HCl. The λ_{max} value of the natural product is given in brackets [6]. The stronger hypsochromic shift of the natural compound may be due to partial oxidation.

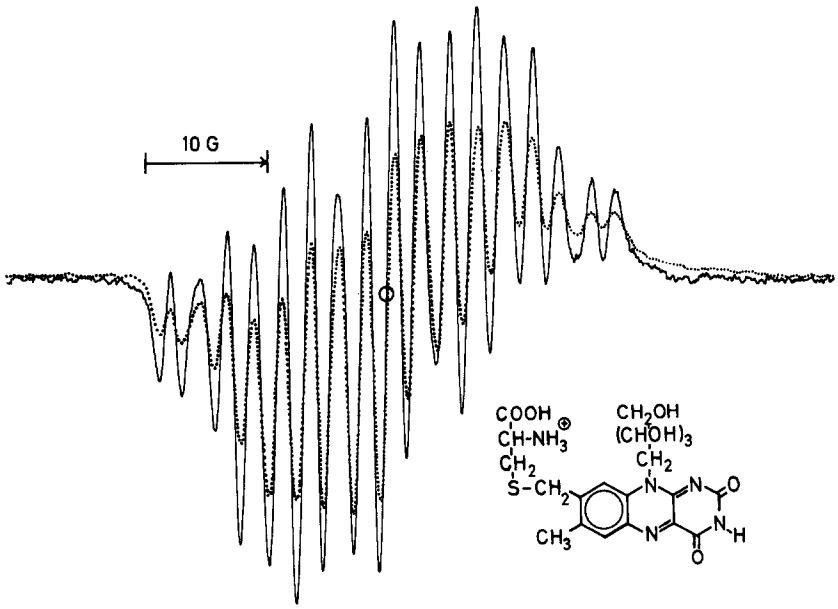


Fig. 5. Comparison of the ESR hyperfine structure of synthetical (——) and natural (- - - -) MAO-flavin (IIb) [3] radical cations in 6 N HCl, reduced with $TiCl_3$, measured with a Varian E 3 at room temperature, 8 mW power and with a modulation amplitude of 1.0 G.

series $-CH_3 \leqslant -CH_2 - S - R < CH_2 - SO_2 - R$ is reflected in a shift from 372 to 360 and 350 nm, respectively, in the second absorption band of the flavin chromophor. Significant spectral differences are likewise exhibited by the protonated molecules (fig. 4). These hypsochromic shifts can be attributed to restriction of resonance stabilization of the flavin excited state by electron withdrawing substituents at position 8α [13].

The ESR spectra of the natural [3] and the synthetic compound IIb in the radical cation state are compared in fig. 5. It is important to note, that the total signal width amounts to 46 as compared to 52 G for riboflavin (IVa). This reflects loss of ESR-active protons upon substitution at position 8α. Such a phenomenon has been attributed to hindered rotation of a large or strongly solvated functional group around the $-CH_2 - F1$ bond and is well documented in flavin radicals [14]. Similarly the spectrum of 8α-(*S*-*n*-octyl-mercapto)-3-methyl-lumiflavin (IIc) has a width of 45 as compared to 61 G for the corresponding lumiflavin (IVb) [15].

4. Acknowledgements

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