2′-Oxoethyl Flavin Revisited

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The present work investigates the possibility of using 2′-oxoethyl flavin (2) as a starting material for the construction of more complicated flavin-based molecules. 2′-Oxoethyl flavin (2), prepared by oxidative degradation of commercially available riboflavin 1, is however a rather untypical aldehyde. It prefers the hydrated gem-diol form 4 in aqueous solution. Ab initio electronic structure calculations, carried out at the level of Møller-Plesset perturbation theory of second order (MP2), predict the existence of an intramolecular hydrogen bond between one of the hydroxy groups of the diol and the N1 atom of the flavin skeleton. This result is supported by 1H NMR measurements which indicate an interaction between the hydroxy groups and the conjugated ring system. We postulate that this rather strong intramolecular hydrogen bond is the origin of the enhanced stability of the gem-diol over the aldehyde form 2.

Synthetic applicability of 2′-oxoethyl flavin 2 is limited by low solubility in most organic solvents and sensitivity to basic conditions. The aldehyde functional group is surprisingly reluctant to nucleophilic attack, and several reactions quite typical for aldehydes failed. Nevertheless, reductive amination led to the expected secondary amine 7. Solubility of the molecule thus increased, and a new amino group was introduced.

Key words: Flavin, Aldehyde, Hydration, Nucleophilic Attack, Reductive Amination, Local MP2

Introduction

The flavin (7,8-dimethylbenzo[g]pteridine-2,4-dione) unit is the functional component of many redox enzymes [1, 2], often in the form of flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) co-factor (Scheme 1), and is responsible for a variety of biochemical redox transformations. For humans, it is an essential compound, and must be acquired from the nutrition in the form of riboflavin, vitamin B2. Redox activity of the flavin unit, even enhanced upon irradiation, was studied in flavoenzyme models and applied for the construction of de novo functional molecules [3 – 26]. In organic synthesis, flavins are usually accessed via the Kuhn synthesis [5 – 8, 11, 13, 14, 18, 19, 21 – 23, 27 – 29] which is a multi-step procedure to be repeated every time a change in the design is required, and there is a logical interest to prepare flavin-based molecules in a quicker and modular fashion from an easily available common intermediate. Therefore, our objective was to investigate the reactivity of 2′-oxoethyl flavin (2), known product of riboflavin (1) oxidative degradation (Scheme 2) [30].
the modification of amino-group containing polymers [32, 33] and surface-bound monolayers [34, 35], 2′-oxoethyl flavin (2) has been mostly considered a product of riboflavin (1) photodegradation [36, 37]. Reduction of 2′ to the corresponding alcohol 3 has been described (Scheme 3) [38], but to the best of our knowledge, no synthetic application thereof – apart from simple acetylation [38] – has been reported. We wondered whether 2′-oxoethyl flavin (2), containing the versatile aldehyde functional group, can be used to establish a route to more complicated flavin-based molecules using nucleophilic addition to the aldehyde. We have therefore accomplished a synthetic study on 2′-oxoethyl flavin (2), extended its original characterisation by modern spectroscopic methods, and prepared a new derivative.

Results and Discussion

2′-Oxoethyl flavin (2) was prepared according to the known procedure [30]. Starting from commercially available riboflavin (1), oxidative degradation by periodic acid [39] yielded up to 5 g of 2 in one batch. Additionally, we have clarified the structure of the hydrate, mentioned first by Petering [40], using 1H NMR spectroscopy. We observed that the raw product spectrum contains two sets of resonance signals: the first belongs to the desired aldehyde product 2, while the second, more intensive set of signals does not include the typical aldehyde proton resonance and its side chain resonance signals exhibit higher multiplicity than expected. The second set of signals was assigned to the geminal diol 4, formed by an equilibrium hydration reaction of the aldehyde 2 (Scheme 4). Chemical shifts of these signals were in accordance with the generally observed pattern [42]: the resonance of the protons in position 2′ (see Scheme 2 for numbering) is observed at 5.31 ppm in the gem-diol 4, shifted by 4.43 ppm upfield from the aldehyde resonance at 9.74 ppm (generally −4.6 to −5.0 ppm), and the resonance of α-protons is observed at 6.27 ppm in the gem-diol form 4, shifted by 0.63 ppm downfield from the resonance of α-protons at 5.64 ppm in the aldehyde form (generally +0.7 to 0.9 ppm). Interestingly, even the resonance of aromatic protons 6-H and 9-H is influenced by the hydration reaction, although rather little. On hydration, the resonance of 9-H, observed at 7.71 ppm, shifts 0.16 ppm downfield to 7.87 ppm, while the resonance of 6-H, observed at 7.94 ppm, shifts 0.03 ppm upfield to 7.91 ppm. This observation suggests an interaction between the aldehyde gem-diol group and the conjugate flavin system. Azeotropic drying of the crude product with toluene (see Experimental Section) forces the equilibrium to the aldehyde 2, and only the corresponding resonance signal set can be observed in the 1H NMR spectrum after this treatment.

To probe this unexpected tendency of preferring the gem-diol form 4 rather than the aldehyde form 2 the equilibrium constant of hydration was determined. A sample of the azeotropically dried aldehyde 2 in a mixture of [D6]-dimethylsulphoxide and deuterium oxide (1:1 v/v) was prepared, and the changes of the gem-diol:aldehyde 2 ratio were monitored by 1H NMR spectroscopy (Fig. 1). The intensity of the aldehyde 2 set of resonance signals steadily decreased, accompanied by an increase in the gem-diol 4 signal intensity [41]. After 16 days of standing at ambient temperature, the concentration of aldehyde fell under the detection level, and the ratio gem-diol 4:aldehyde 2 must have therefore reached at least 100 [42]. This is highly unusual: typical examples of aldehydes or ketones which form hydrates of such stability are
those which contain electronegative substituents in the α-position increasing the polarisation of the aldehyde group, such as trichloroacetaldehyde (equilibrium constant of hydration 2000), and those where hydration reduces deviations from “ideal” geometry, such as cyclopropanone [43]. However, in the case of 2′-oxoethyl flavin (2), there are no electronegative substituents in the α-position nor geometric strain to be released.

For comparison, a similar experiment was carried out with structurally related 2-phenylpropionic aldehyde 5 (Scheme 5). Again, an intensity increase of the gem-diol 6 signals at the expense of the signals for aldehyde 5 was observed, but in this case, the mixture equilibrated at 45:55 (gem-diol 6: aldehyde 5) ratio which corresponds to an equilibrium constant of 0.8, as expected for aldehydes in general.

In order to understand the preference of the gem-diol over the aldehyde form, ab initio electronic structure calculations at the level of second-order Möller-Plesset perturbation theory (MP2) were performed. Basis set superposition error (BSSE) contaminations of the interaction energy (which in the context of an intramolecular hydrogen bond cannot be corrected for with the counterpoise procedure of Boys and Bernardi [44]) were to a large extent avoided by performing local MP2 (LMP2) calculations. Correlation energies obtained from local correlation methods like LMP2 are much less affected by BSSE effects than the energies of the corresponding canonical methods, as was demonstrated before [45]. Geometry optimisations of 2′-oxoethyl flavin and its gem-diol form were carried out with the efficient analytic LMP2 energy gradient method described previously [46] using the aug-cc-PVDZ AO basis set of Dunning [47]. Single point energy calculations at these geometries were performed employing the more extended aug-cc-PVTZ and aug-cc-PVQZ sets, respectively, which were used to extrapolate the correlation energy at the basis set limit (two-point extrapo-
lation formula, ref. [48]). Analogous calculations were also performed for 2-phenylpropionic aldehyde and its gem-diol form to have as a reference system an aldehyde with “normal” chemical behaviour. The parameters specifying the calculations in detail are given in the Appendix.

For the gem-diol form of the 2'-oxoethyl flavin molecule the calculations predict the formation of an intramolecular hydrogen bond between one of the hydroxy groups of the aldehyde and the nitrogen atom in position 1 of the flavin skeleton. The hydrogen bond is comparatively short, i.e., 1.96 Å vs. 2.05 and 2.07 Å for the water dimer and the water ammonia complex, respectively, calculated at the same level of theory. In order to assess the strength of this hydrogen bond additional constrained geometry optimisations were performed for a sequence of different C-1'-C-2'-OH dihedral angles. A barrier height of 8.34 kcal/mol at the basis set limit was so obtained for the rotation about this dihedral angle breaking the hydrogen bond. Due to the absence of sterical hindrance this barrier height appears to be a reasonable estimate for the strength of the intramolecular hydrogen bond, which is substantially stronger than the hydrogen bond of the water dimer (4.94 kcal/mol) or even of the water-ammonia dimer (6.48 kcal/mol).

For the electronic contribution to the hydration reaction energy of 2'-oxoethyl flavin (2) a value of −47.2 kcal/mol (extrapolated to the basis set limit, −46.8 kcal/mol for the aug-cc-pVQZ basis alone) was obtained. This is −5.5 kcal/mol more than for the reference system, again reflecting the enhanced stability of the former due to intramolecular hydrogen bond formation. Based on these electronic reaction energies, the related free energy differences at room temperature were assessed using harmonic vibrational frequencies calculated at the level of density functional theory (B3-LYP hybrid functional, TZVP basis set [49]). The free energy differences for the hydration reactions so obtained amount to −38.2 kcal/mol and −26.9 kcal/mol for 2'-oxoethyl flavin (2) and 2-phenylpropionic aldehyde (5), respectively. Due to the underlying approximation of an ideal solution these two values certainly have to be taken with care. However, the error imposed by this model is likely to cancel to a large extent in the difference between these two free energy differences, which amounts to −11.3 kcal/mol. Thus we can infer from these free energy calculations that (i) the free reaction energies are smaller (absolute value) than the corresponding pure electronic reaction energies, and (ii) zero-point energy corrections and finite temperature entropic effects disfavour the gem-diol form to lesser extent for 2'-oxoethyl flavin (2) than for the reference system, i.e. 2-phenylpropionic aldehyde (5).

To summarise, we conclude from our calculations and experimental findings, that the intramolecular hydrogen bond occurring in the gem-diol form of 2'-oxoethyl flavin (2) leads to a stabilisation of the diol over the aldehyde to such an extent that the aldehyde form 2 can barely be observed by the spectrometric methods applied in this work.

To explore the use of 2 in synthetic flavin chemistry, a range of reactions were attempted. Unfortunately, the poor solubility (less than 2 g/L in dimethylsulphoxide, < 305 mg/L in dichloromethane, < 200 mg/L in ethanol, and < 30 mg/L in tetrahydrofuran) and liability to base [50, 51] severely limit the synthetic use of aldehyde 2.

Reductive alkylation, for example by 2-methoxy-ethanol, would be among the synthetically interesting transformations, because it directly furnishes 10-(3',6'-dioxahept-1'-yl) flavin, a derivative of good solubility both in organic solvents and water [7, 8]. Unfortunately, neither the conditions used by Doyle et al. [52] nor by Bethmont et al. [53] led to the desired product. Knoevenagel or Wittig reactions which would introduce a synthetically versatile carboxylic acid function [54, 55] failed, too. The former, carried out in the presence of pyridine and piperidine under reflux [56], yielded a complex reaction mixture, while the latter, using an ylide pre-formed from triethyl phosphoacetate and sodium hydride, did not yield any products. Surprisingly, even the formation of acyclic and cyclic acetals failed in our hands, and the starting material remained intact under the reaction conditions. On the other hand, the reaction with highly basic butyl lithium yielded a complex reaction mixture, indicating decomposition of the flavin skeleton.

Reductive amination under catalytic hydrogenation conditions yielded the expected secondary amine 7 (Scheme 6). The amine 7 was the only organic product of the reaction, and the yield could be increased up to 80 % either by increasing the reaction temperature or using neat 2-methoxyethyl amine as solvent. It was not possible to reduce the intermediate imine by sodium cyanoborohydride [57], sodium triacetoxyborohydride [58], or sodium borohydride [59]. These reducing agents were too basic and similar to the aforementioned examples, the reaction yielded a palette of
flavin decomposition products. Secondary amine 7 is better soluble in organic solvents than the starting material, and the amino group may be a target of subsequent derivatisation for example by acylation.

In conclusion, we have extended the characterisation of 2'-oxoethyl flavin (2) and clarified the structure of its gem-diol form 4. In solutions containing water, the gem-diol form 4 is highly favoured and the equilibrium constant of aldehyde 2 and hydrated form 4 was found higher than 100, far from the range typical for aldehydes in general. We have shown by ab initio electronic structure calculations that the gem-diol 4 is stabilised by a hydrogen bond between one of the hydroxyl groups and the nitrogen atom in position 1 of the flavin skeleton. A variety of nucleophilic addition reactions were attempted to employ 2 as a building block for the construction of more complicated flavin-based molecules. However, the reactivity of the aldehyde group is influenced by the flavin skeleton, as the unusual stability of the gem-diol 4 indicates. Reductive amination under hydrogenation conditions was the only successful chemical transformation of the aldehyde 2 giving the secondary amine 7 in good yield.

Appendix – Computational Details

The ab initio calculations were performed with the local MP2 method as implemented in the MOLPRO [60] program package, employing the density fitting approximation for the electron repulsion integrals [61,46]. The augmented correlation consistent AO basis sets aug-cc-pVXZ of Dunning [47,62] were used (X = D for geometry optimizations, X = T, Q for single point energies), along with the related fitting basis sets optimized for DF-MP2 [63]. For the Hartree-Fock energy and the related component of the LMP2 gradient the JK-fitting basis sets of Weigend [64] related to the cc-pV(X+1)Z AO basis, respectively, were employed. Local orbitals were generated according to the Pipek-Mezey localisation scheme [65]. Pair domains were constructed with the Boughton-Pulay procedure [67] using a completeness criterion of 0.98. For the single point energy calculations, the BP domains then were extended by all next nearest neighbour centres. The occupied orbital pair list remained un-truncated in all calculations.

The density functional calculations were carried out by using the TURBOMOLE programme package [66].

Experimental Section

General

All chemicals were obtained from commercial sources, checked by 1H NMR, and used as received. Solvents were distilled before use and dried by usual methods if required by the experimental procedure. 1H NMR spectra were recorded at a Bruker spectrometer with working frequency of 300 or 600 MHz as indicated. The course of the reaction was monitored by thin-layer chromatography using silica gel 60 F-254 plates with UV indicator from Merck. Preparative thin-layer chromatography was carried out on home-made glass plates (20 × 20 cm) coated with silica gel 60 GF254 (20 g, Merck). Column chromatography was carried out on silica gel Geduran 60 (Merck) or silica gel 60 M (Macherey-Nagel) using a dry load of the mixture. The electrospray ionisation mass spectra (ES) were measured on a ThermoQuest Finnigan TSQ 7000 spectrometer, a high-resolution mass spectrum was measured on a ThermoQuest Finnigan MAT 95 spectrometer. Melting points were measured on a Büchi SMP-20 melting point apparatus using a glass capillary immersed in heated silicon oil, and are uncorrected.

Equilibration experiments

A solution of aldehyde 2 or 5 (2 × 10^{-3} M) and trifluoroacetic acid (2 × 10^{-4} M) in a mixture of deuterium oxide and [D_8]-dimethylsulphoxide (1 : 1 v/v) was prepared, left stand at ambient temperature, and regularly monitored by 1H NMR spectrometry on a Bruker spectrometer with working frequency 300 MHz, using 64 transitions. The contents of the aldehyde and gem-diol form were calculated from the integrals of protons 6 and 9 (aldehyde 2) or β-protons (aldehyde 5).

2'-Oxoethyl flavin (2)

Riboflavin 1 (5.60 g, 14.9 mmol, 1 eq.) was suspended in dilute sulphuric acid (4 mL acid in 140 mL of distilled water) and the suspension was cooled to 0–5°C using an ice bath. A solution of periodic acid (12.50 g, 54.8 mmol, 3.7 eq.) in water (90 mL) was added dropwise, keeping the

Scheme 6. Reductive amination of 2'-oxoethyl flavin (2); conditions: ethanol, hydrogen (10 bar), 10% palladium on charcoal, r. t., 28%, or (ii) ethanol, hydrogen (40 bar), 10% palladium on charcoal, 60°C, 80%.
temperature between 0 and 5 °C. Once the addition was complete, the cooling bath was removed, and the temperature was allowed to rise to ambient temperature. During ca. 60 min of stirring at ambient temperature, all solids dissolved. Active charcoal was added to the reaction mixture and the suspension was gently stirred for 30 min. The solid was filtered off and the pH of the filtrate was adjusted to 3.9 by the addition of concentrated sodium hydroxide solution, keeping the temperature between 20 and 25 °C. The mixture was cooled to +1.5 °C using an ice bath. The product precipitated and was separated by filtration using a Büchner funnel. The filtration cake was thoroughly washed with ice-cold water and dried on the vacuum pump. The product was filtered off and the pH of the filtrate was adjusted to 3.9 and the suspension was gently stirred for 30 min. The solid was suspended in toluene (200 mL), the suspension was heated to reflux and small portions of the distillate were removed using the Dean-Stark trap. Once the suspension was concentrated to ca. 50 mL (ca. 6 hrs.), it was evaporated to dryness in vacuo and dried on the pump. Yield 2.08 g (49%). – 1H NMR (300 MHz, [D6]-DMSO): δ = 2.40 (s, 3 H, 7-CH3), 2.46 (s, 3 H, 8-CH3), 5.64 (s, 2 H, CH2), 7.71 (s, 1 H, 9-H), 7.94 (s, 1 H, 6-H), 9.74 (s, 1 H, CHO), 11.39 (br, 1 H, 3-H). – 13C NMR (150 MHz, [D6]-DMSO): δ = 18.5 (7-CH3), 20.4 (8-CH3), 53.7 (CH2-1), 116.2 (C-9), 130.6 (C-6), 130.9 (C-6a), 149.9 (C-10a), 195.1 (CHO). The 13C NMR spectrum could not be measured directly because of insufficient solubility in organic solvents, but could be partially re-constructed from heteronuclear correlation experiments (HSQC, HMBC). The remaining carbon atoms could not be detected. – M. p. > 271 °C (methanol, decomposition). This value coincides with the one reported in the literature (23) [M + H2O]− + H+.

The mixture was therefore azeotropically dried with toluene: –1H NMR (300 MHz, methanol-D4): m/z (%) = 284.9 (6) [M + H]+, 303.0 (38) [M + H2O + H]+, 605.3 (1) [2 M + 2 H2O + H]+, 633.3 (2) [2 M + 2 CH2OH + H]+, 655.3 (6) [2 M + 2 CH2OH + Na]+, – MS (−)-ES: m/z (%) –282.7 (100) [M – H]+, 300.8 (23) [M + H2O – H]+, 314.7 (52) [M + CH2OH – H]+, – Rf = 0.57 (ethyl acetate : methanol = 5 : 2). – IR (KBr disc): v = 3436 (broad signal, O–H), 3148 (aromatic C–H), 3025 (aromatic C–H), 2819 (C–H aldehyde), 2361, 1705 (C4=O and N3 wagging, reported at 1703 [68], C4=O and C2=O, reported at 1712 [34]), 1652 (C2=O and N3 wagging, reported at 1646 [68], C2=O, N3–H bending and C4=O, reported at 1677 [34]), 1577 (C4a–N5, reported at 1578 [68], C4a–N5 and in-phase C10a–N1, reported at 1574 [34]), 1539 (C10a–N1, reported at 1546 [68], C4a–N5 and out-of-phase C10a–N1, reported at 1548 [34]), 1456 (aromatic C=C, C1398 (CH3), 1349, 1275 (CH3), 1244 cm−1, complex fingerprint area [69].

**3'-Aza-6'-oxahept-1'-yl flavin (7)**

**Method I**

2'-Oxoethyl flavin (256 mg, 0.9 mmol, 1 eq.), 2-methoxy-ethyl amine (275 mg, 3.7 mmol, 4 eq.) and palladium on activated charcoal (10 %, 1 spatula tip) were suspended in ethanol (200 mL) in an autoclave. The autoclave was flushed five times with hydrogen and then filled with hydrogen up to the pressure of 10 bar. The reaction mixture was stirred for 17 hrs. at ambient temperature. The suspension was filtered over celite, and the filtrate was evaporated in vacuo.

The mixture of product and starting material was separated by column chromatography using silica gel as stationary and a mixture of ethyl acetate and methanol (5 : 2) as mobile phase. Yield 86 mg (28 %).

**Method II**

2'-Oxoethyl flavin (50 mg, 0.18 mmol, 1 eq.), 2-methoxy-ethyl amine (52 mg, 0.69 mmol, 3.8 eq) and palladium on activated charcoal (10 %, 55 mg) were suspended in ethanol (60 mL) in an autoclave. The autoclave was flushed five times with hydrogen and then filled with hydrogen up to the pressure of 10 bar and placed in a 60 °C oil bath. The reaction mixture was stirred for 19 hrs. After cooling, the reaction mixture was filtered over celite, and the filtrate was evaporated in vacuo. The product was purified by preparative thin-layer chromatography using a mixture of ethyl acetate and methanol (5 : 2) as mobile phase. The corresponding zone (Rf = 0.05) was extracted by methanol. The extract was filtered and the filtrate was evaporated in vacuo. Yield 48 mg (80 %). – 1H NMR (300 MHz, [D6]-DMSO): δ = 2.40 (s, 3 H, 7-CH3), 2.51 (br, > 3 H, DMSO + 8-CH3), 2.37 (t, J = 5.4 Hz, 2 H, CH2-3′), 2.90 (t, J = 6.6 Hz, 2 H, CH2-2′), 3.23 (s, 3 H, OMe), 3.35 (br, > 2 H, H2O + CH2-4′), 4.65 (t, J = 6.6 Hz, 2 H, CH2-1′), 7.89 (s, 3 H, 9-H), 7.91 (s, 3 H, 6-H), 11.32 (br, 1 H, 3-H). – 13C NMR (100 MHz, [D6]-DMSO): δ = 18.7 (7-CH3), 20.5 (8-CH3), 44.2 (C-1′), 45.8, 48.4, 58.0, 71.9 (C-2′,4′,5′,7′), 116.4 (C-9), 130.8 (C-9a), 131.2 (C-6), 133.8 (C-5a), 135.7 (C-7 or 8), 137.1 (C-4a), 146.4 (C-7 or 8), 150.2 (C-10a), 155.6, 159.9 (C-2,4). – M. p. > 232 °C (methanol, decomposition). – MS (−)-ES: m/z (%) = 344.1 (100) [M + H]+, 687.5 (4) [2 M + H]+. – HRMS (EI, 70 eV): m/z = 343.1640 (calcld. 343.1644 for C17H21N5O3, [M]+). – Rf = 0.05 (ethyl acetate : methanol = 5 : 2).

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[38] Upjohn Co., GB 760068, 1956.
[41] Fitting the experimental points by a linear (assuming zero-order kinetics) gave the reaction rate of $7 \times 10^{-14}$ mol/s.
[69] The two normal modes (obtained at the level of density functional theory, *vide supra*) involving the aldehyde C=O and the C4=O stretch coordinates strongly mix and cannot be clearly assigned to either one of these coordinates. Furthermore, the related harmonic frequencies are 1900 cm$^{-1}$ and 1902 cm$^{-1}$, respectively, i.e., they differ by merely 2 cm$^{-1}$. In such a situation Fermi resonance between these two modes is likely to occur, making the situation even more complicated.