Spectral and redox properties of benzodipteridine. A pulse radiolysis, laser flash photolysis and semi-empirical molecular orbital study

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The properties of the flavin enzyme mimic benzodipteridine (BDP) have been studied by pulse radiolysis, laser flash photolysis and semi-empirical molecular orbital (AM1) methods. The redox, protolytic and tautomeric equilibria in the ground state and excited triplet state were compared to the parent flavin molecule (FL). Pulse radiolysis was used to detect the one-electron reduced form of BDP which was found to be less basic than flavins, in agreement with the prediction of semi-empirical calculations. BDP is more electrophilic than FL in the ground state, but not in the excited triplet state, due to the lower triplet energy of the former as predicted by semi-empirical calculations. In contrast to FL, the triplet state of BDP does not appear to be more basic than the ground state, in agreement with the calculated shift in electron density upon excitation.

Introduction

Flavins are well known as redox active cofactors in many enzymic and photoenzymic processes. Many studies[1] of free flavins have been carried out as model systems for understanding the basic features of the enzymic reactions. However, in general, free flavins fail to emulate flavoenzymes in terms of their ability to oxidise a range of organic substrates. Recently, a benzodipteridine (BDP) flavin derivative was reported that
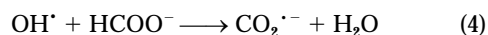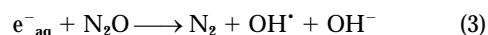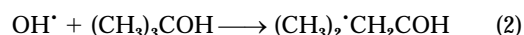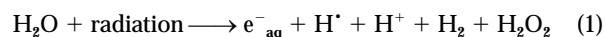

BDP   FL
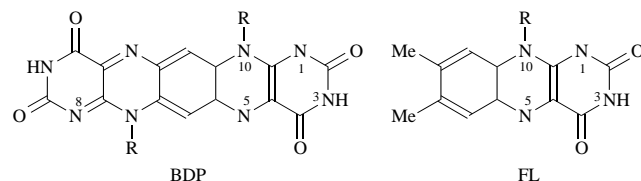
more closely mimics flavoenzymes.[2] For example, thiols and phenylhydrazine were oxidised 10^7-fold faster by BDP than with ordinary flavins. In contrast to the wealth of information on the chemical properties of natural flavins, little is known about the behaviour of benzodipteridines in their ground or excited states.

In this work we report the results of a laser flash photolysis study of the reactivity of triplet state benzodipteridine and a pulse radiolysis study of the one-electron reduced form of benzodipteridine. Semi-empirical (AM1) calculations[3] were performed to explain the differences in redox, protolytic and spectral properties of BDP compared with the parent flavins.

Experimental

Benzodipteridine (BDP) was synthesised as previously described.[2] All other chemicals were of AnalaR grade and were supplied by BDH. The solutions were typically $1–4 \times 10^{-5}$ mol dm^{-3} in BDP. The solutions for pulse radiolysis were made up in triply distilled water and the acidity controlled by buffering (0.01 mol dm^{-3}) with Na$_2$HPO$_4$–KH$_2$PO$_4$ (pH 4–8) or H$_3$BO$_3$–NaOH (pH 8–10).

The reducing pulse radiolysis conditions were argon-saturated solutions containing 0.1 mol dm^{-3} 2-methylpropan-2-ol to scavenge the OH$^{\bullet}$ radical [reactions (1) and (2)] or nitrous oxide saturated solutions of 0.1 mol dm^{-3} sodium formate [reactions (1), (3) and (4)].

$$H_2O + \text{radiation} \longrightarrow e^-_{aq} + H^{\bullet} + H^+ + H_2 + H_2O_2 \quad (1)$$

$$OH^{\bullet} + (CH_3)_3COH \longrightarrow (CH_3)_2{}^{\bullet}CH_2COH \quad (2)$$

$$e^-_{aq} + N_2O \longrightarrow N_2 + OH^{\bullet} + OH^- \quad (3)$$

$$OH^{\bullet} + HCOO^- \longrightarrow CO_2{}^{\bullet-} + H_2O \quad (4)$$

The pulse radiolysis experiments were carried out with an 8–14 MeV Vickers electron linear accelerator (pulse lengths 5–50 ns) as previously described.[4] Typical experiments employed radiation doses of 1–5 Gy per pulse. Optical cells of 1.0 cm path length were employed. Variations in the dose from pulse-to-pulse were measured by charge collection in a secondary emission chamber. Ten wavelengths were detected simultaneously using a diode array system with a diode spacing of 16 nm.

Radiation doses were measured using the molar absorption coefficient of $(SCN)_2{}^{\bullet-}$ formed by pulsing nitrous oxide saturated solutions of 10^{-2} mol dm^{-3} KSCN and taking $G\varepsilon$ $[(SCN)_2{}^{\bullet-}]$ as 46 400 dm^3 mol^{-1} cm^{-1} at 480 nm. The following G values (10^{-7} mol J^{-1}) were assumed for e^-_{aq} (0.28) and Br$_2{}^{\bullet-}$ (0.61). Solutions for laser flash photolysis were either aqueous and buffered (0.01 mol dm^{-3}) with Na$_2$HPO$_4$–KH$_2$PO$_4$ (pH 4–8) or H$_3$BO$_3$–NaOH (pH 8–10) or in 1,2-dichloroethane.

The laser flash photolysis system was based on a JK Lasers System 2000 Neodymium:YAG Laser emitting pulses at 532 nm with energies in the range 50–100 mJ and a pulse duration of 20 ns.

A pulsed Xe lamp which produced light of constant intensity for 50 ms after the laser pulse was used as the analysing source. Measurements of transient changes in absorbance were recorded and analysed on a Philips PM3311 digital oscilloscope/Hewlett Packard 9153 computer combination. Pulse-to-pulse variations in the laser intensity were corrected for by monitoring the integrated laser intensity of each pulse. Samples were contained in quartz cells of 1 cm optical path length. Appropriate filters were placed in the analysis light path to reduce photolysis. Oxygen was removed by bubbling nitrogen through the solution for 45 min.

Calculations

The calculations were performed with the AM1 Hamiltonian using AMPAC 5.0 (Semichem) unless otherwise indicated. For calculations on the ground state, all geometric parameters were

optimised unless otherwise stated. Excited triplet state calculations were carried out using the ground state geometry. The keywords PRECISE and MMOK were included to increase precision 100-fold and to increase the rotational barrier in the amide group, respectively. RHF calculations were performed on the neutral molecules and UHF calculations on the anion radicals using CHARGE = −1. Hydration effects were included using the AMSOL method. AMSOL models the effect of solvent by using a theoretical model of solvation that assumes that the solute occupies a spherical cavity, the free energy of solvation being mainly due to electric polarisation of the solvent by the solute molecule and the free energy of cavity formation. The keyword DERINU was used, *i.e.* the gas phase geometry was further optimised to take into account the more polar environment.

Spectral calculations were performed using the ZINDO method within the Hyperchem software package using the AM1 calculated geometry. All ZINDO calculations are gas phase.
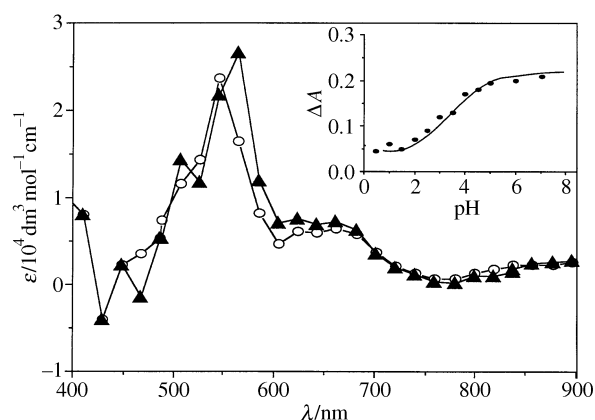
Results and discussion

The ground state

One-electron reduction by pulse radiolysis. Immediately after the pulse, the well-known absorption ($\lambda_{max} = 700$ nm) of the hydrated electron (e^-_{aq}) was observed. However, in the presence of BDP at pH 6, e^-_{aq} decayed rapidly ($\tau < 1$ μs) and a concomitant growth of a transient with a sharp maxima at 560 nm and a broad absorption from 600–720 nm was observed. The spectrum of this species is shown in Fig. 1 after correcting for ground state depletion. This new species was not observed upon purging with N_2O to remove e^-_{aq}. The rate constant of the reaction of BDP with e^-_{aq} was determined as 1.6×10^{10} dm^3 mol^{-1} s^{-1} by measuring the first-order rate of decay of e^-_{aq} as a function of BDP concentration.

The above experiments were repeated in nitrous oxide saturated solutions containing formate, to convert all the primary radicals into the reducing radical $CO_2^{\cdot-}$. The reaction of $CO_2^{\cdot-}$ with BDP was observed to occur over 100 μs to yield at pH 6 a species essentially identical to that observed with e^-_{aq} and BDP. The experiments with $CO_2^{\cdot-}$ were repeated at pH values in the range 0.1–7.0 and at pH < 2.8 a different transient species ($\lambda_{max} = 565$ and 663 nm) to that observed at pH 6 was observed [Fig. 1 (inset)]. Variation of the pH in the range 0.5–8 yielded an associated pK_a value of 3.4 ± 0.4. It is proposed that this pK_a value represents the protonation of the radical anion of BDP. Hence BDP$^{\cdot-}$ is substantially less basic than FL$^{\cdot-}$ by 4.9 pK_a units (*cf.* pK_a of FL$^{\cdot-}$ = 8.3).[5]

Using semi-empirical molecular orbital calculations, the difference in basicity (assuming protonation at N5, see later) of

BDP$^{\cdot-}$ and FL$^{\cdot-}$ can be predicted to be 3.1 pK units (FL$^{\cdot-}$ being the more basic) using eqn. (5),

$$\Delta pK_a = [\Delta G_{prot}(\text{BDP}^{\cdot-}) - \Delta G_{prot}(\text{FL}^{\cdot-})]/2.303RT \quad (5)$$

where $\Delta pK_a = pK_a(\text{BDP}^{\cdot-}) - pK_a(\text{FL}^{\cdot-})$ and ΔG_{prot} equals the free energy of protonation of the hydrated BDP$^{\cdot-}$ or FL$^{\cdot-}$ radical anions using the AM1/AMSOL method. In fact the difference in gas phase basicities was predicted to be much larger (12 pK_a units). However, the loss of solvation stabilisation of FL$^{\cdot-}$ upon protonation is much greater than the corresponding loss for BDP$^{\cdot-}$.

EPR studies[6] indicate that the FL$^{\cdot-}$ radical anion protonates at N5. For FL, the AM1 method predicts that the N5 protonated (and hydrated) radical is 10 kJ mol^{-1} more stable than its N1 protonated tautomer. In contrast, for BDP, both N1 and N5 protonated species are of approximately equal stability.

Redox properties of BDP. Free energies of one-electron reduction (ΔG_{red}) of BDP were calculated by combining calculated heats of formation (H_f), entropies (ΔS) and free energies of hydration (G_{hydr}) for both the neutral oxidised and one-electron reduced radicals [eqn. (6)] where $\Delta G_{red} = G_f(\text{BDP}^{\cdot-}) - G_f(\text{BDP})$.
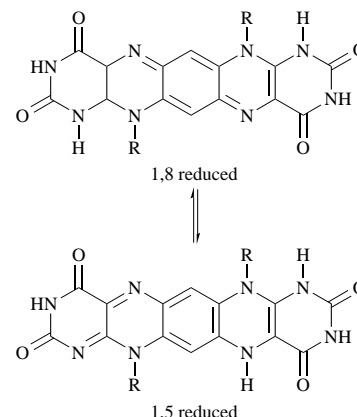
$$\Delta G_{red} = [H_f - T\Delta S + G_{hydr}](\text{BDP}^{\cdot-}) - $$
$$[H_f - T\Delta S + G_{hydr}](\text{BDP}) \quad (6)$$

Previously, this approach has been used successfully to predict trends in two electron redox potentials for a structurally related series of compounds.[7] Here the same method is used, but applied to one-electron redox potentials. As a reference compound, lumiflavin (FL) was used. In this case the extra pyrazine and pyrimidine rings of BDP are being treated as a substituent. The difference in one-electron redox potential between BDP and FL was calculated using eqn. (7).

$$\Delta[E^o(\text{BDP}) - E^o(\text{FL})] = [\Delta G(\text{BDP}) - \Delta G(\text{FL})]/F \quad (7)$$

The results predict that the one-electron reduction potential of BDP should be 0.24 V more positive than FL ($E = -0.27$ V for FL[8]), *i.e.* reduction of BDP is more favourable than FL. This is in reasonable agreement with experimental data using cyclic voltametry[2] which shows that $E^o(\text{BDP/BDP}^{\cdot-})$ is 0.319 V higher than $E^o(\text{FL/FL}^{\cdot-})$ in aqueous solution. There are two main contributions to the difference in redox potential between BDP and FL. The first is the enthalpy of reduction, which is much more favourable for BDP due to the greater delocalisation of the extra electron added to the LUMO. Partly counteracting this latter effect is the greater free energy of solvation of the lumiflavin anion compared with the BDP anion, due to the greater electric polarisation of the solvent by the higher charge density on the FL$^{\cdot-}$ radical anion.

Tautomeric equlibria in the fully reduced state. The fully (two electron) reduced form of BDPH$_2$ can exist in two tautomeric forms, either 1,5- or 1,8-H$_2$.

Fig. 1 The transient absorption spectrum observed (after correction for loss of the ground state) following the reaction of e_{aq}^- with BDP 10 μs after the pulse at pH 6 (▲) and pH 1 (○). Inset: variation of ΔA with pH measured at 560 nm.

Table 1 Calculated heats of formation (AM1) in the gas phase and in simulated aqueous environments (AMSOL method) and calculated electronic spectra (ZINDO) of fully reduced BDP in its various tautomeric forms

	$\Delta_f H$/kJ mol^{-1}		
	Gas	Water	Computed λ_{max}/nm
BDP$_{ox}$			502
1,8-H$_2$	70.1	−52.3	555
1,8-H monoanion	−185.4	−469	631
1,5-H$_2$	48.6	−74.1	476
1,5-H monoanion	−149.4	−481	763
1,8- or 1,5-dianion	−195.1	−885	643

To try and resolve the question of which is the predominant tautomeric form, calculations were carried out on the stabilities (heats of formation) and spectroscopic properties of the various possible tautomeric forms.

The heats of formation of the various tautomeric–protolytic forms were calculated in the gas phase and in an aqueous environment (using the AMSOL method) and are shown in Table 1.

In principle, the species with the lowest heat of formation will be the one expected to be the predominant form. However in practice the small differences in the heats of formation of the two tautomers are not enough to predict the predominant form with certainty. In fact, higher level *ab initio* methods are needed to obtain reliable thermodynamic data.[9]

Calculations of the UV–VIS spectra of BDP, BDPH$_2$ and BDPH were made by ZINDO/s, a semi-empirical molecular orbital method specifically parametrised to reproduce UV–VIS spectra of organic molecules. The starting molecular geometry was that calculated earlier by the AM1 method. The precise value of the computed λ_{max} is dependent upon the parametrisation chosen since ZINDO contains adjustable parameters controlling factors such as π–π overlap. Hence it is the spectral differences between the redox forms that are important here, rather than the absolute λ_{max}. The computed λ_{max} for the various redox and protolytic forms are given in Table 1.

As can be seen, it is predicted that upon reduction, a red or blue shift would result depending upon whether the neutral 1,8- or 1,5-tautomer was formed, respectively. In fact a red shift[2] is observed upon reduction to the neutral form (pH < 3.6, 548–614 nm). This agrees with the earlier suggestion that the observed red shift was due to the more extensive conjugated character of the 1,8-tautomer.[2] Upon deprotonation to give the monoanion, a small red shift is observed[2] (614–620 nm). This compares with the 76 nm red shift predicted for formation of the 1,8-monoanion and the very large red shift (+287 nm) for formation of the 1,5-monoanion from the 1,5-neutral form. Although the agreement with the observed values is not close, the results still suggest that the 1,8-dihydro form is predominant in the monoanionic species. For dianion formation, a small red shift is predicted, although a 46 nm blue shift is actually observed. However, in the case of the dianion, the 1,5 and 1,8 forms are equivalent as they represent resonance hybrids.

The excited triplet state

Laser flash photolysis of BDP in 1,2-dichloroethane under anaerobic conditions revealed a transient species (Fig. 2, λ_{max} = 520 and 780 nm). This species decayed by first-order kinetics with τ = 4 μs and little residual absorption was observed. Oxygen quenched this species with a rate constant (k_q) of 1.2×10^9 dm^3 mol^{-1} s^{-1}. The addition of 10^{-1} mol dm^{-3} of 1,2-dimethoxybenzene quenched the initially formed species with a rate constant (k_q) of 1.6×10^7 dm^3 mol^{-1} s^{-1} to form a second species, which can be assigned on the basis of its spectrum (data not shown) as the one-electron reduced species BDP$^{\cdot-}$ (see
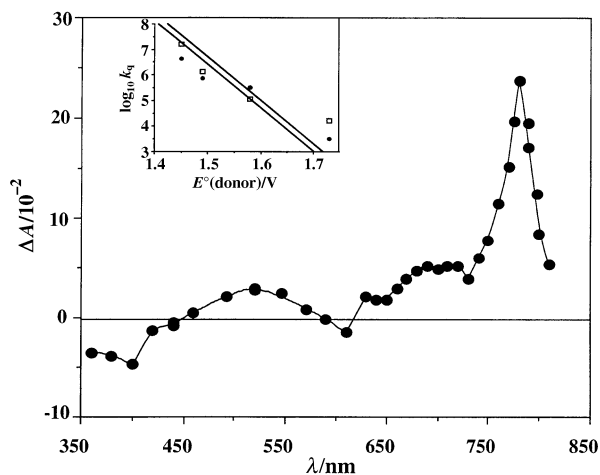
Fig. 2 Laser flash photolysis difference spectra observed for BDP alone, 50 ns after excitation. Inset: a plot of $\log_{10} k_q$ for quenching of ^{3}FL (●) and ^{3}BDP (□) *versus* the one-electron oxidation potential (E^0, D$^{\cdot+}$/D) of the donor. Donors were 1,2- and 1,3-dimethoxybenzene, 1,2,3,5-tetramethylbenzene and pentamethylbenzene.

pulse radiolysis section). Hence the initial species can be confidently assigned to the triplet state of BDP and its quenching by 1,2-dimethoxybenzene can be attributed to electron transfer.

The rate constants of electron transfer (k_{ET}) of ^{3}BDP with four substituted benzenes of known one-electron oxidation potential[10,11] were measured and are shown in Fig. 2 (inset) as a plot of $\log_{10} k_q$ *versus* the one-electron oxidation potential (E^0, D$^{\cdot+}$/D) of the donor. Each of the donors used has been shown previously to quench the riboflavin triplet state (^{3}FL) by electron transfer[12] and those results are also shown for comparison.

In comparing the reactivity of ^{3}BDP *versus* ^{3}FL we can make use of the relationship between the overall free energy change for electron transfer (ΔG_{et}) and the rate constant of electron transfer as derived by Rehm and Weller.[13] According to such theoretical considerations, plots of $\log_{10} k_{ET}$ *versus* the one-electron oxidation potential of the donor (E^0, D$^{\cdot+}$/D) should be linear provided that $\Delta G_{et} > 0$.

As can be seen, in Fig. 2 (inset), the very small difference in intercept on the y axis represents only a small enhancement of the rate of electron uptake by ^{3}BDP compared with ^{3}FL. From Fig. 2 it can be predicted that the value of E^0(^{3}BDP/BDP$^{\cdot-}$) is only *ca.* 16 ± 15 mV more positive than the corresponding value for FL. This is despite the considerably more positive redox potential of the ground state of BDP compared with FL, computed earlier using the AM1 method (E^0 = −0.03 and −0.27 V, respectively). Unfortunately, no phosphorescence could be detected from BDP (ethanol glass at 77 K) and so the triplet energy level could not be determined. The triplet energy level of BDP can be estimated from the observed difference in triplet reactivity and the calculated difference in ground state reduction potential using eqn. (8).

$$\Delta E^0(^3\text{BDP} - {}^3\text{FL}) = \Delta E^0(\text{BDP} - \text{FL}) +$$
$$\Delta E_{o,o}(^3\text{BDP} - {}^3\text{FL}) \quad (8)$$

Using a value[13] of $E_{o,o}(^3$FL) of 210 kJ mol^{-1}, a triplet energy level of 180 kJ mol^{-1} for BDP can be estimated, *i.e.* a difference of 30 kJ mol^{-1}. An alternative approach was followeed by using semi-empirical methods to estimate the difference in triplet energy levels. Using the ground state geometry but calculating the energy of the lowest triplet state in the absence of solvent yielded a triplet energy difference betwee FL and BDP of 20 kJ mol^{-1} and 15 kJ mol^{-1} depending upon whether configurational interaction was excluded or included, respectively. Further, inclusion of hydration effects lowered the difference to 18 and 13 kJ mol^{-1}, respectively.

Laser flash photolysis of BDP was also carried out in aqueous solution in the pH range 1.5–7.0. The initial transient spectrum was very similar to that observed in 1,2-dichloroethane at all pH values. In contrast[12] to ^{3}FL ($pK_a = 5$) there appears to be no pH dependence in the spectrum of the BDP triplet state.

Earlier studies using laser flash photolysis[15] and phosphorescence[16] have shown that FL possesses a pK_a of *ca.* 5 which probably represents protonation at the N5 position, in contrast to protonation of the ground state which occurs at N1 ($pK_a \sim$ 0).[1] Molecular orbital calculations[16] (PPP and CNDO methods) explained the change in protonation site in FL by showing that a substantial shift in electron density towards N5 occurs in the triplet state relative to the ground state. The issue of excited state pK_a values has been reviewed recently.[17] The same approach was followed in this work using the AM1 method and similarly a substantial shift in charge from N1 to N5 was found for FL. For example, for a representative flavin such as lumiflavin, an increase in total electron charge at N5 of 0.11 is predicted, but a decrease at N1 of 0.09. In contrast, using the AM1 method for BDP, hardly any increase in the electronic charge at N5 is predicted in going from the ground state singlet to the triplet state. Hence it may be that BDP protonates at the same position, probably N1, in both the ground and excited triplet states. Thus the lack of any pH dependence of the BDP triplet state may be explained by the fact that the triplet state pK_a of BDP is similar to the ground state, which is expected to be *ca.* 0.

Conclusions

This study has demonstrated that BDP possesses significantly different photochemical properties to those of the parent flavin molecule. However, where differences exist, they are readily explicable in terms of changes in the overall electron density in the ring or the electron density at specific atoms, namely N1 and N5, as computed by semi-empirical molecular orbital methods.

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