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Model systems for flavoenzyme activity: an investigation of the role functionality attached to the C(7) position of the flavin unit has on redox and molecular recognition properties†‡

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We describe the role functionality attached to the C(7) position of a family of flavin derivatives has in tuning their redox and recognition properties and the subsequent exploitation of two of these derivatives as a three-component electrochemically controllable molecular switch.

Flavoenzymes are a class of proteins that catalyse a range of redox transformations and mediate one- and two-electron transfer processes in biological systems.1 Supramolecular interactions between the flavin cofactor and the apoenzyme modulate the redox potential of the flavin moiety by over 500 mV, tuning the redox properties of the flavin units to match their biological function.2 X-Ray crystallography of flavoenzymes has provided the redox potential of the flavin moiety by over 500 mV, tuning the redox properties of the flavin units to match their biological function.2 X-Ray crystallography of flavoenzymes has provided important information regarding the nature and relative position of functionality in the flavoenzyme active site; however, little information is provided relating to the role individual interactions play in modulating flavin properties.3 In other studies, the apoenzymes of flavoenzymes have been reconstituted with artificial flavins to probe the role non-covalent interactions around the flavin binding site play in modulating the reactivity and properties of the cofactor.4

Small molecule model system studies of flavoenzymes provide insight into biological processes while simultaneously providing access to new functional molecular systems. Synthetic model systems have been utilised to explore the role of non-covalent interactions in modulating redox properties of flavins.5 Furthermore, structurally modified flavin units in their C(7) and C(8)

positions have been used to quantify the role structural modification of this type has in controlling their physical properties in aqueous and non-aqueous media.6 However, in these investigations the role functionality attached to the non-biomimetic C(7) position has largely been overlooked. In this communication, we report the role functionality attached to the C(7) position of the flavin unit has in modulating both the redox and recognition properties. We then use this modulation to create a three-component electrochemically controllable molecular switch from flavin derivatives 1 and 6 and complementary diaminopyridine (DAP) derivative 7.

A family of synthetic flavin derivatives 1–6 have been synthesised with a range of electron donating and withdrawing functionality attached to the C(7) position (see ESI†). Slow evaporation of solvent from a solution of 1 dissolved in toluene resulted in the formation of X-ray quality crystals.§ The X-ray structure of compound 1 is shown in Fig. 1, and unequivocally confirms the presence of a –NMe 2 group attached to the C(7) position of the flavin. In the solid state, the flavin units self-assemble via two point hydrogen bonding between the imide moieties of adjacent flavin units, resulting in a dimeric structure.

Fig. 1 X-Ray crystal structure of flavin 1. Hydrogen bonding interactions are highlighted in light blue. The molecular structure was visualised using Mercury 1.4.2.

We have investigated the complexation of flavin derivatives 1–6 with complementary derivative 7 using NMR titrations. It has previously been shown that DAP derivative 7 serves as an effective host for flavin derivatives and mimics the three point hydrogen bonding interactions with the complementary residues of the apoenzyme.7 The titration resulted in a smooth downfield shift in the hydrogen attached to N(3) of the flavin upon addition of aliquots of 7. The titration data were successfully fit to a 1 : 1 binding isotherm using non-linear curve fitting methods to afford the association constants (K a) provided in Table 1. It is clear from these data that the electronic characteristics of the functionality attached to

† Electronic supplementary information (ESI) available: Synthesis of derivatives 1–6. CCDC 715445. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b900269n

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of the chemical–chemical–electrochemical (e–c–e) process where some whereas the second reoxidation waves are due to an electro-

free flavin derivative), indicating that significant stabilisation mV shift in the reduction of the flavin unit (compared to the

Indeed, upon exchanging the electron donating –NMe 2 group role in controlling the redox properties of the flavin unit.

CVs of compound 1 (blue line) and compound 6 (red line) recorded in CH2Cl2 (~1 mmol). Scan rate = 100 mV s −1.

C(7) position plays little role in modulating the electron density of the carbonyl oxygen atoms O(2) and O(4) of the flavin moiety, and thus the binding efficiency with com-

We next investigated the electrochemical properties of derivat-

Table 1 Calculated and LFER predicted binding and electrochemical properties of flavins 1–6

<table>
<thead>
<tr>
<th>K D /M −1a</th>
<th>E, SWV/V</th>
<th>ΔGbinding exp b / kcal mol −1</th>
<th>ΔGbinding LFER c / kcal mol −1</th>
<th>ΔGredox exp d / kcal mol −1</th>
<th>ΔGredox, LFER e / kcal mol −1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>446 ± 22</td>
<td>−1.40</td>
<td>−3.61</td>
<td>−2.53</td>
<td>32.29</td>
</tr>
<tr>
<td>2</td>
<td>455 ± 23</td>
<td>−1.36</td>
<td>−3.61</td>
<td>−3.15</td>
<td>31.36</td>
</tr>
<tr>
<td>3</td>
<td>460 ± 23</td>
<td>−1.33</td>
<td>−3.63</td>
<td>−3.63</td>
<td>30.67</td>
</tr>
<tr>
<td>4</td>
<td>482 ± 24</td>
<td>−1.21</td>
<td>−3.66</td>
<td>−6.60</td>
<td>27.90</td>
</tr>
<tr>
<td>5</td>
<td>434 ± 22</td>
<td>−1.15</td>
<td>−3.60</td>
<td>−7.50</td>
<td>26.52</td>
</tr>
<tr>
<td>6</td>
<td>436 ± 22</td>
<td>−1.10</td>
<td>−3.60</td>
<td>−9.60</td>
<td>25.37</td>
</tr>
</tbody>
</table>

a Determined in CDCl3 at 298 K. b Calculated using: ΔG = −RTlnK D . c Calculated using: ΔG(X,Y) = ρmσm(X) + ρpσp(Y) + ΔG(H,H), where ρm = −6.9 kcal mol −1 σ −1 and ρp = −4.6 kcal mol −1 σ −1. d Calculated using: ΔG = −nFE.

are subsequently reoxidised at a less negative potential than 1rad and 2rad . However, it must be stressed that the intensity of the second reoxidation wave is suppressed compared to the electro-

We have used linear free energy (LFER) relationships to

Fig. 2 Graphical representation of the good correlation between experimentally determined and LFER predicted ΔG values for redox properties of 1–6. Inset shows the near-zero slope of the experi-

Fig. 3 Graphical representation of the good correlation between the experi-

Graphical representation of the good correlation between experimen-

This e–c–e process is largely suppressed with derivatives 5 and 6, suggesting that the electron withdrawing nature of the –CN and –NO 2 groups significantly lowers the pKa of their radical anions to below that of the imide of the oxidised forms of these derivatives.

We have used linear free energy (LFER) relationships to further corroborate our experimental work and gain further insight into the role functionality attached to the C(7) position of the heterocycle has upon the redox and recognition properties of the flavin. The experimental and LFER predicted values for host–guest binding and redox properties were determined using our previously described methodology and are summarised in Table 1 and represented graphically in Fig. 3, also see ESI. Good correlation exists between the experimental and LFER predicted ΔG values for the electrochemical properties of the flavin derivatives, further supporting the importance electronic effects of functionality attached to C(7) have in modulating redox properties. In contrast, a slope of essentially zero was observed for the plot of experimental versus LFER predicted ΔG values for host–guest binding, indicating that the electronic characteristics of functionality attached at the C(7) position do not significantly influence recognition of DAP. However, the CV studies suggest that the electronic effects may weakly influence the acidity of the N(5) moiety of the radical anions derived from derivatives 1 and 2. We attribute the differing role functionality attached to the C(7) position has in modulating recognition and redox properties as follows. Functional groups attached to the C(7) position of flavin are inductively connected to the O(2) and O(4) carbonyls.
that dictate binding affinity via the “meta” position of the phenyl unit. In contrast, the C(7) position is in direct resonance communication with the central redox core of the flavin unit, thereby significantly influencing its redox properties.

In recent years, an enormous amount of effort has been directed towards the development of molecular switches. Flavin–DAP systems are attractive moieties for developing switches as it is well established that the specific DAP-flavin recognition process affords a write state that can be read electrochemically due to the shift in flavin reduction potential that accompanies the hydrogen bonding mediated stabilisation of the flavin \( \text{rad}^{-} \) state. Systems 1–7 offer the exciting ability to create a novel three-component write–read molecular switch, whereby the binding between 7 and oxidised flavins can be sequentially electrochemically read by adjusting the redox window of the reduction process (Fig. 4). To test this hypothesis we have investigated the formation of a prototype three-component switch from derivatives 1, 6 and 7. The CV of an equinmol solution of 1 and 6 is shown in Fig. 5 and clearly shows that the disparate onset of their radical anion states allows the sequential formation of \( \text{I}_{\text{rad}}^{-} \) and \( \text{6}_{\text{rad}}^{-} \). Upon the addition of DAP and cycling the applied potential between \(-0.3 \) and \(-2.4 \) V, both of the redox waves are shifted to a more positive potential (100 mV), which corresponds to a new \( K_{\text{a}} \) of \( \approx 22000 \text{ M}^{-1} \) for \( \text{7}_{\text{rad}}^{-} \) and \( \text{7}_{\text{rad}}^{-} \). By adjusting the applied voltage to cycle between \(-0.2 \) and \(-1.6 \) V it is possible to only read the 7.6 binding event in the presence of derivative 1.

Thus, in this system electrochemistry not only provides a direct readout process for the DAP–flavin binding event through the change in voltage of the flavin reduction process but also provides a means of modulating the host–guest binding event.

In conclusion, we have shown that the electronic effects of functionality attached to the C(7) position of flavin derivatives play an important role in modulating the redox properties of the unit. In contrast, they do not significantly modulate the hydrogen bonding affinity for receptor 7. The similar binding efficiencies of the oxidised flavin derivatives 1 and 6 with compound 7 provide direct access to a prototype three-component redox controlled switch whereby the differing onset of the radical anion states of derivatives 1 and 6 can be sequentially read by the application of increasing negative potential. These results pave the way for the formation of new flavin-based molecular devices addressed by electrochemically controlled host–guest complexation. The results of our investigations in this area will be reported in due course.

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Notes and references

† This article is dedicated to Prof. Seiji Shinkai on the occasion of his 65th birthday.
§ Crystal data for 1: \( 4\text{C}_{10}\text{H}_{10}\text{N}_{2}\text{O}_{2}\text{C}_{10}\text{H}_{6} \), \( M_{r} = 1345.58, Z = 1, \) triclinic, \( \text{P}1, a = 10.3010(4), b = 11.8400(5), c = 16.1447(7) \text{ Å}, \) \( \alpha = 106.983(2), \beta = 108.228(2), \gamma = 106.322(2), \) \( F = 1631.48(7) \text{ Å}\(^3\), \( T = 100(2) \text{ K}, N_{\text{meas}} = 3946; N_{\text{th}} = 5795; R_{\text{calc}} = 0.041, R_{\text{obs}} \) (all data) = 0.096 (0.104).

11 The redox-based enhancement in recognition can be calculated using a thermodynamic cycle, which can be expressed mathematically using: \( K_{\text{a}}(\text{red})/K_{\text{a}}(\text{ox}) = e^{\Delta_{\text{f}}F/T}\), \( E_{1/2}(\text{bound}) - E_{1/2}(\text{unbound}) \), \( K_{\text{a}}(\text{red}) \) and \( K_{\text{a}}(\text{ox}) \) are the association constants in the reduced and oxidised forms, and \( E_{1/2}(\text{bound}) \) and \( E_{1/2}(\text{unbound}) \) are the half-wave redox potentials in the receptor bound and unbound states.