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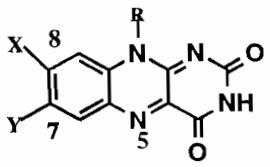
Electronic Effects of 7 and 8 Ring Substituents as Predictors of Flavin Oxidation-Reduction Potentials

D.E. Edmondson
Department of Biochemistry, Emory University, Atlanta, GA. USA
S. Ghisla
Faculty of Biology, University of Konstanz, Germany

Introduction

Flavin coenzyme analogues have been used extensively as probes of flavin binding sites in a wide variety of flavoenzymes [1]. In addition to their uses as structural probes, they also have been used profitably as mechanistic probes of flavoenzyme function. Flavin analogues with substituents in the 7 and 8 positions (Scheme 1) are especially valuable for systematically altering the oxidation-reduction potential of the coenzyme. Since the redox active center of the isoalloxazine ring is at the N(5) and C(4a) positions, the 7 and 8 positions of the benzenoid ring can be considered meta- and para- positions, respectively.



A previous attempt to provide a quantitative correlation of Hammett σ parameters with E_m for a series of lumiflavin analogues at two pH values treated the contribution of the 7 substituent equal with that of the 8 substituent [2]. In this paper, we have assembled from the literature the $E_{m,7}$ values of 20 flavin analogues with varying substituents in the 7 and 8 positions and performed multivariate regression analysis to quantify their individual contributions. The results of this analysis demonstrates the feasibility of such a correlation and provides a method for prediction of redox potentials for 7,8-substituted flavins.

Results and Discussion

A search of the literature yielded the assembled potentials of the flavin analogues at their riboflavin, FMN or FAD levels which are listed in Table 1. Although the FAD level alters the redox potential of the flavin ring relative to riboflavin or FMN by 10-15 mV [3], this small change is considered insignificant relative to the shift of potentials induced by the 7 and 8 substituents and, therefore, the analysis is performed without regard to the coenzyme level of the flavin analogue.

The correlation of measured $E_{m,7}$ values for the flavin analogues listed in Table 1 with Hammett σ values is expressed by the following equation:

$$E_{m,7}(X_p,Y_m) = \rho_p \sigma_p + \rho_m \sigma_m + E_{m,7}(H,H) \quad \text{(eq.1)}$$

where σ_p and σ_m are the respective σ values for substituents in either the para (8) or meta (7) positions of the benzenoid portion of the flavin ring. $E_{m,7}$ (H,H) is the potential for the flavin with only H substituents at positions 7 and 8 and

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Table 1. List of Substituent Sigma Values and Experimental and Calculated Oxidation-Reduction Potentials of 7,8-Substituted-Flavin Analogues.

Flavin Substituent		[1] Sigma Values		[2] Potentials		Reference	Flavin
8	7	*para*	*meta*	(mV) (exptl)	[3] (mV) (calc)		No.
CH_3	CH_3	-0.170	-0.069	-208	-225	[3]	**1**
CF_3	CF_3	0.54	0.43	+20	-12	[4]	**2**
F	CH_3	0.062	-0.069	-167	-178	[5]	**3**
Br	CH_3	0.232	-0.069	-148	-144	[5]	**4**
Cl	Cl	0.227	0.373	-126	-83	[6]	**5**
H	Cl	0	0.373	-128	-129	[6]	**6**
Cl	CH_3	0.227	-0.069	-152	-145	[7]	**7**
CH_3	Br	-0.17	0.391	-154	-161	[7]	**8**
S^-	CH_3	-1.21	-0.069	-290	--	[8]	**9**
CH_3S	CH_3	0	-0.069	-204	-191	[5,8]	**10**
CH_3SO_2	CH_3	0.720	-0.069	-50	-45	[5]	**11**
CH_3SO	CH_3	0.49	-0.069	-161	--	[5]	**12**
O^-	CH_3	-0.81	-0.069	-340	-354	[9]	**13**
NH_2	CH_3	-0.660	-0.069	-330	-324	[5]	**14**
Me_2N	CH_3	-0.32	-0.069	-254	-255	[7]	**15**
MeO	CH_3	-0.268	-0.069	-260	-245	[5]	**16**
EtO	CH_3	-0.240	-0.069	-246	-239	[5]	**17**
H	CH_3	0	-0.069	-180	-191	[5]	**18**
CN	CH_3	0.660	-0.069	-50	-57	[10]	**19**
CHO	CH_3	0.47	-0.069	-90[4]	-96	[11]	**20**
COO^-	CH_3	0	-0.069	-165	-191	[12]	**21**
CH_2OH	CH_3	0	-0.068	-170	-191	[12]	**22**

[1] Sigma values were taken from tables in Hansch and Leo [13].

[2] All potentials listed are for the 2-electron couple of the unbound flavin analogue *versus* the normal Hydrogen electrode

[3] The calculated $E_{m,7}$ values for each flavin analogue are from Equation 3.

[4] The listed potential is for the uncomplexed 8-formyl group. Intra-molecular hemiacetal formation with a side chain hydroxyl group lowers the potential to -159 mV [11].

$E_{m,7}$ (X_p,Y_m) the potential for a flavin analogue with substituents other than H. This treatment allows the relative contributions of the electronic effects of the 7 and 8 positions to be evaluated separately since, for a complex heterocyclic ring system such as a flavin, there is no reason to believe that they would follow an additive

behavior. There[illegible], the analytical expression shown in eq.1 is preferable to one where the σ values for the para and meta positions are combined as shown in eq. 2.

$$E_{m,7}\,(X_p,Y_m) = \rho\,(\sigma_p+\sigma_m) + E_{m,7}\,(H,H) \quad \text{(eq.2)}$$

With the availability of statistical software that can perform multi-parameter linear regression analysis, the experimental oxidation-reduction potential data in Table 1 can readily be correlated with the published σ values for the various 7 and 8 substituents (17). When this analysis is performed, the constants in eq.1 are calculated for the flavin ring system as follows:

$$\rho\, p = 202\ (\pm\ 11)\ \text{mV}/\ \sigma p\ ,\ \rho\, m = 140\ (\pm\ 22)\ \text{mV}\ /\ \sigma m\ , \text{and}$$

$$E_{m,7}(H,H) = -181\ (\pm\ 4.5)\ \text{mV}$$

The statistical values for this correlation are: $F_{2,18} = 234$ ($P<.001$) and $r^2 = 0.97$ which allows eq. 1 to be rewritten:

$$E_{m,7}\,(X_p,Y_m) = 202\ (\pm 11)\ \sigma_p + 140\ (\pm 22)\ \sigma_m - 181\ (\pm 4)\ \text{mV} \quad \text{(eq.3)}$$

The respective ρ values show the 8 substituent has a stronger electronic influence on the flavin $E_{m,7}$ value than that at position 7. Therefore, expressions which simply add them together (such as eq. 2) do not provide as rigorous a treatment as the one in eq. 1. Equation 3 allows the prediction of the oxidation-reduction potential for any 7,8-substituted flavin analogue in which the σ values for the substituents are known.

Of the twenty-two flavin analogues listed in Table 1, only two analogues exhibit anomolous behavior. The measured oxidation-reduction potential for the 8-nor-8-thioflavin correlates with a σ value intermediate between the thiolate anion (pK = 3.8) and the thioquinoid tautomer which demonstrates both forms to exist in aqueous solution. The only other flavin analogue exhibiting anomolous behavior is 8-nor-8-methylsulfinylflavin(Table 1). This is probably due to the reduction of the sulfinyl group during the reductive phase of the redox titration to form the 8-thioether and thus lead to an observed potential between that of a two forms.

To test the value of eq. 3 for its ability to predict the redox potentials of a variety of 7- and 8-substituted flavin analogues, the respective σ values shown in Table 1 for the appropriate substituents were used in equation 3 to calculate the oxidation-reduction potentials of the analogues listed (Table 1). These calculated potentials are listed in Table 1 and their correlation with experimental values are shown in Fig.1.

A linear plot is observed with an $r^2 = 0.99$ which demonstrates the validity of eq. 3 for the flavins tested and, therefore, can be used with confidence as a predictor of potentials for other 7,8-substituted flavin analogues. To test this correlation further, several flavin analogues not listed in Table 1 were assembled from the literature, their potentials calculated using equation 3, and compared with published experimental values (Table 2). There is good agreement between experimental and calculated potentials in most examples which provides further confidence in the

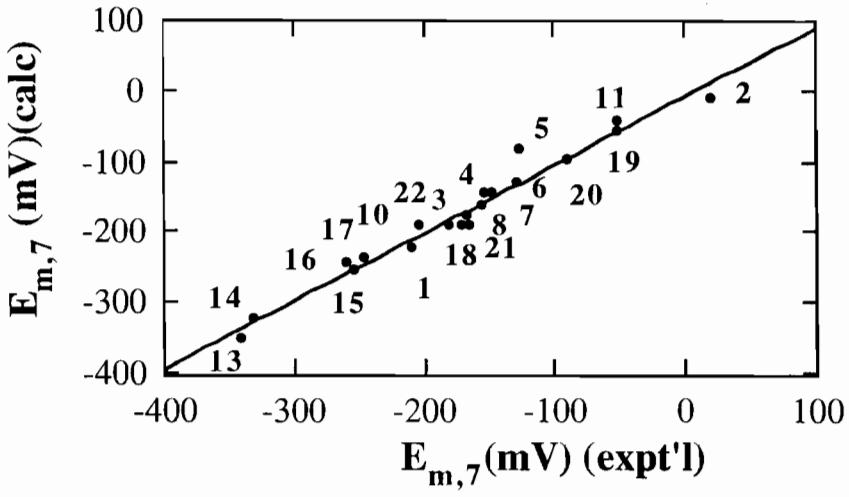

Figure 1. Plot of experimental versus calculated potentials of 7,8-substituted flavin analogues. The values for the potentials and the numbers (denoting each flavin analogue) are taken from Table 1.

validity of equation 3. For the two analogues in Table 2 where the two values differ by >40 mV, the experimental values for the potentials should be re-determined.

We have not extended this analysis to those flavins with substituents in the 6 position. Complications in such analysis can arise from steric effects of the ortho substituent in addition to the electronic effect. It should be noted that the full extent of substituent electronic effects on the properties of flavin analogues is expected to be more extensive than described here. In addition to oxidation-reduction potential modulation, it is anticipated that such effects probably also influence other important properties of flavins in flavoenzymes such as the pK_a values for neutral flavin semiquinones and possibly their respective hydroquinone forms. It is known that the ρ_p value for the pK_a of N,N-dimethylaniline radical cations is ~-7.4 whereas the value on the E^o of the N,N-dimethylaniline radicals is +540 mV/σ [15,16]. This latter ρ value is over 2-fold larger than that observed here for the 2 e^- flavin potentials. Even with a smaller electronic effect, it is probable that radical pK_a values of the analogues listed in Table 1 are probably altered considerably from the "normal" value of 8.3-8.4. This influence may have considerable consequences when these analogues are used in flavoenzyme systems as mechanistic probes.

Table 2. Calculated and Experimental Redox Potentials for 7,8-Substituted Flavin Analogues not used in the Formulation of Equation 3.

Flavin Substituent 8	7	[1]Sigma Values *para*	*meta*	Potentials (mV) (expl)	[2] (mV) (calc)	Reference
PheS-	CH_3	0.07	-0.069	-166	-177	[8]
$PheSO_2$-	CH_3	0.70	-0.069	-30	-50	[8]
SO_3-	CH_3	0.35	-0.069	-122	-120	[6]
Br	Br	0.232	0.391	-100	-79	[14]
MeO	MeO	- 0.268	0.12	-220	-216	[14]
H	EtO	0	0.13	-220	-161	[14]
H	MeO	0	0.12	-210	-168	[14]
H	NH_2	0	-0.16	-200	-199	[14]
Cl	H	0.227	0	-144	-135	[7]

[1] Taken from [13].
[2] Calculated using equation 3.

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