Water Soluble Complexes of the Antiviral Drugs, 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine and Acyclovir: The Role of Hydrophobicity in Complex Formation

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Abstract □ We investigated water-soluble complexes of various ligands with the antiviral drugs, 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG). For comparison, we also examined the "parent" compounds, guanine and guanosine, as substrates for complex formation. Using the phase-solubility technique, we measured formation constant (K_1) values at 23 °C in pH 7 buffer. For a single substrate, formation constants with different ligands varied in the order: caffeine > pyridoxine ~~ cytidine > nicotinamide > sucrose. With caffeine as the ligand, formation constants with different substrates varied in the order: guanine > guanosine ~~ acyclovir > DHPG. The largest formation constant observed was 58 M^{-1} (for guanine–caffeine), and the smallest formation constant was 0.29 M^{-1} (for DHPG–sucrose). Examining the literature for formation constant data on compounds related to DHPG, and comparing literature data with our own, reveals a significant correlation between formation constants and ligand hydrophobicity. For 41 substrate–ligand pairs, least squares linear regression analysis of log K_1 values versus various parameters reflecting donor–acceptor abilities (e.g., substrate and ligand HOMO and LUMO values, or substrate oxidation potentials) failed to significantly correlate. We conclude that ligand hydrophobicity is a general determinant of water soluble complex formation, but not necessarily the exclusive or dominant controlling factor for all complexes. Charge–transfer interactions are not important determinants of complex formation for the substrate–ligand combinations that we have considered.

Purine and pyrimidine nucleosides play critical roles in many biochemical processes, and commensurate with this importance is the intensity of study directed toward elucidating the molecular factors that control formation of complexes between the nucleosides and various organic molecules. Such studies offer to advance our understanding of polynucleotides with respect to their structure and their interactions with therapeutic drugs, mutagens, and other xenobiotics.

Our interest in nucleoside complexes originates in developing parenteral formulations for two antiviral drugs that are structurally related to 2′-deoxyguanosine: 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir);[1] and 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG).[2-6] For both DHPG and acyclovir, it is difficult to achieve therapeutic intravenous doses with drug concentrations at or below the intrinsic solubility limits of 3 and 1 mg/mL, respectively. Furthermore, the drug acid–base dissociation constant values (pK_{a1} = 2.5 and pK_{a2} = 9) prevent solubility enhancement through pH selection if the formulation pH is to remain near physiological values.

As an alternative to formulation pH modification, we elected to attempt DHPG and acyclovir solubility enhancement at neutral pH via formation of water-soluble complexes between the drugs and various organic molecules. Recommending this approach were literature reports[7-14] that intramolecular complexes can increase the aqueous solubilities of various drugs, including some nucleoside analogues.[15,16]

Accordingly, we have used the phase-solubility[14] technique to determine formation constants for complexes between DHPG or acyclovir and: sucrose, nicotinamide, thiamine, pyridoxine, cytidine, and caffeine. To identify the molecular interactions that influence complex formation, we extended our investigation to include determinations using guanine and guanosine as solubility-limited substrates, and the *N*-ethyl derivative of nicotinamide as a complex-forming ligand.

Our studies reveal that ligands such as nicotinamide and caffeine afford significant DHPG and acyclovir solubility increases at moderate (but not necessarily nontoxic) ligand concentrations. Furthermore, correlation analysis clearly demonstrates that hydrophobic interactions strongly contribute to DHPG complex formation, whereas charge–transfer interactions are unimportant. Comparing our own data with relevant literature data suggests that ligand hydrophobicity may generally be a dominant factor controlling complex formation in aqueous solutions.

Experimental Section

Materials—9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG) and acyclovir were prepared by the Syntex Institute of Bioorganic Chemistry according to established procedures.[1,2] The *N*-ethyl derivative of nicotinamide was prepared by treating nicotinamide with ethyl iodide in refluxing isopropyl alcohol for 4 h, followed by crystallization from isopropyl alcohol. The structures and purities were established by the usual spectroscopic, chromatographic, and elemental analyses. The following compounds were purchased from Aldrich Chemical Co., were of reagent grade, and were used without further purification: guanine, guanosine, nicotinamide, pyridoxine hydrochloride, and ethyl idodide. Cytidine was reagent grade from Sigma Chemical Co., and caffeine was USP reference standard material.

Methods—Phase-solubility experiments were conducted by adding aqueous solutions (3 mL of 100 mM sodium phosphate buffer, pH = 7) of ligand at various concentrations to solid substrate and agitating for 24 h at 23±3 °C. In all cases, the quantity of added solid exceeded the substrate solubility limit in the aqueous phase. Substrate concentrations in filtered aliquots of the aqueous phase were measured by reversed-phase HPLC or by differential-pulse anodic voltammetry using previously described methods.[17,18]

Calculations—Formation constant (K_1) values for 1:1 complexes were defined according to eq. 1 and calculated according to eq. 2:[14]

$$K_1 = [SL]/[S][L] \quad (1)$$

$$K_1 = \text{Slope}/[S_0](1 - \text{Slope}) \quad (2)$$

where $[S]$, $[L]$, and $[SL]$ are concentrations of substrate, ligand, and water-soluble complex, respectively, and $[S_0]$ is the solubility of substrate for $[L] = 0$. In eq. 2, "slope" is the least squares regression slope for data plotted according to:

$$[S] = [S_0] + \text{Slope}^* [L] \quad (3)$$

The majority of ligand and substrate octanol–water partition coeffi-

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cient (log P) values were from Leo et al.[19] For some cases (e.g., the halo substituted uracils and 8-methoxy caffeine), log P values were calculated from tabulated values for the unsubstituted compound and values for the appropriate substituted and unsubstituted benzenes, i.e., according to:

$$\log P_{LX} = \log P_{L} + \log P_{PhH} - \log P_{PhX} \quad (4)$$

where LX and L refer to substituted and unsubstituted ligand or substrate, respectively, and PhH and PhX refer to the unsubstituted and substituted benzenes, respectively.

Least squares linear regression analyses were performed using the SAS (SAS Institute, Inc., Cary, NC) procedures, GLM and STEPWISE, on an IBM 3081 mainframe computer.

Results and Discussion

9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine (DHPG) and Acyclovir Complexes—We examined the effects of various ligands and solvent systems on the solubilities of DHPG and acyclovir. For comparison, we also included the "parent" compounds, guanine and guanosine, as substrates. Typically, data plots according to eq. 3 were linear, with no evidence of higher-order (SL_2, SL_3, etc.) complexes over the ligand concentration ranges employed. Figure 1 shows representative data for DHPG complexes with sucrose, caffeine, and nicotinamide as ligands in pH 7 aqueous buffer. Table I summarizes linear regression statistics and K_1 calculations for each substrate–ligand–solvent system investigated.

The first three entries in Table I demonstrate that the DHPG–nicotinamide K_1 values were essentially invariant over the pH range of 5 to 8. This pH independence is expected because neither the substrate nor the ligand change ionization state over the pH range investigated. Similarly, alcoholic solvents or cosolvents have small effects on the DHPG–nicotinamide K_1 values. Other investigators[11,20] also report small cosolvent effects on complex formation constants, and attribute the effects, at least in part, to reduction in solvent mixture surface tension relative to pure aqueous solution.

Having demonstrated the relative insensitivity of complex formation constants to medium effects, we explored the effects of various ligands on DHPG and acyclovir solubilities in a single solvent system (100 mM phosphate buffer at pH 7). For DHPG complexes, K_1 values depended strongly on the ligand employed (a 30-fold range from a low of 0.29 for sucrose to a high of 9.30 for caffeine). For acyclovir, guanine, and guanosine as substrates, formation constants also depended strongly on ligand type and the K_1 values varied in the same order, i.e., $K_{1(\text{caffeine})} > K_{1(\text{nicotinamide})}$, for all substrates investigated. For a single ligand (either caffeine or nicotinamide), formation constants varied in the order: $K_{1(\text{guanine})} > K_{1(\text{guanosine})} \sim\sim K_{1(\text{acyclovir})} > K_{1(\text{DHPG})}$.

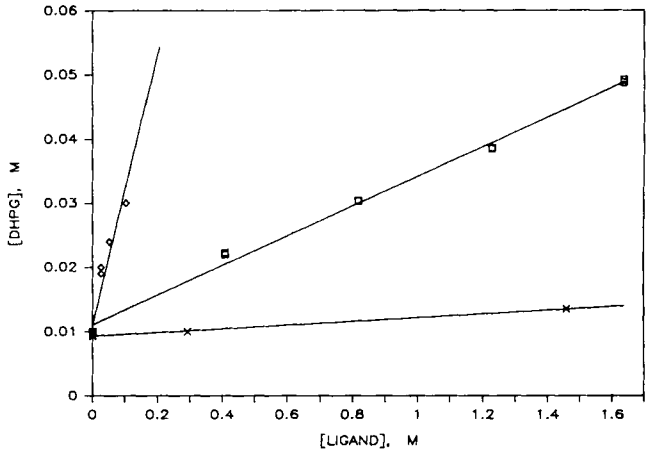

Figure 1—*Dependence of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG) solubility on ligand concentration for phase-solubility determinations with nicotinamide (□), caffeine (◇), and sucrose (×) as ligands. All determinations at 23 °C in pH 7 phosphate buffer.*

A primary objective of our investigation was to identify nontoxic ligands that elevate DHPG and acyclovir concentrations to levels consistent with therapeutic dosing regimens. For a 500-mg daily intravenous dose of either acyclovir or DHPG, a 5-mg/mL drug concentration would be necessary to maintain infusion volumes of <100 mL. Table II shows the calculated ligand molar concentrations needed to elevate DHPG and acyclovir concentrations to 5 mg/mL, and also summarizes calculated ligand doses (in grams) that would accompany a 500-mg drug dose.

From the information in Table II it is evident that rather low (33 to 227 mM) concentrations of caffeine are needed to provide 5 mg/mL of DHPG and acyclovir, respectively. None of the other ligands investigated equaled caffeine with respect to providing significant DHPG and acyclovir solubility

Table I—Formation Constant (K_1) Data for 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine (DHPG), Acyclovir, Guanine, and Guanosine.

Substrate	Ligand	Medium[a]	Slope[b] × 100	Intercept,[b] mM	r[b]	K_1,[c] M^{-1}
DHPG	Nicotinamide	pH = 7	2.31	11.1	0.997	2.08
DHPG	Nicotinamide	pH = 5	2.28	10.2	0.995	2.23
DHPG	Nicotinamide	pH = 8	2.64	11.3	0.998	2.89
DHPG	Nicotinamide	100% MeOH	0.296	0.93	0.987	3.17
DHPG	Nicotinamide	10% EtOH	2.13	13.8	0.993	1.54
DHPG	Nicotinamide	10% Propylene Glycol	2.12	13.5	0.996	1.57
DHPG	Nicotinamide	10% Tween 20	2.36	10.9	0.999	2.16
DHPG	Sucrose	pH = 7	0.284	9.33	0.998	0.29
DHPG	Cytidine	pH = 7	2.83	9.04	0.996	3.13
DHPG	Caffeine	pH = 7	14	15	0.992	9.30
DHPG	Thiamine	pH = 7	3	15	0.998	2.11
DHPG	Pyridoxine	pH = 7	5	15	0.998	3.57
DHPG	*N*-Ethyl derivative of nicotinamide	pH = 7	1.87	9.5	0.983	1.97
Acyclovir	Nicotinamide	pH = 7	2.11	6.31	0.997	3.34
Acyclovir	Caffeine	pH = 7	7.33	5.93	0.961	12.4
Guanine	Nicotinamide	pH = 7	0.00758	0.0165	0.989	4.58
Guanine	Caffeine	pH = 7	0.003359	0.000575	0.996	58.2
Guanosine	Nicotinamide	pH = 7	0.879	1.82	0.987	4.82
Guanosine	Caffeine	pH = 7	2.23	0.202	0.984	11.1

[a] In 100 mM phosphate buffer. [b] By least squares linear regression according to eq. 3. [c] Calculated according to eq. 2.

Table II—Ligand Effects on 9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine (DHPG) and Acyclovir Aqueous Solubility at pH = 7

Substrate	Ligand	Ligand, M, Needed to Provide Substrate = 5 mg/mL[a]	Ligand Dose, g, for 500 mg Substrate Dose[b]
DHPG	Nicotinamide	0.368	4.5
DHPG	Sucrose	3.62	123
DHPG	Cytidine	0.373	9.1
DHPG	Caffeine	0.033	0.64
DHPG	Thiamine	0.153	5.2
DHPG	Pyridoxine	0.092	1.9
DHPG	*N*-Ethyl derivative of nicotinamide	0.541	10.2
Acyclovir	Nicotinamide	0.773	9.4
Acyclovir	Caffeine	0.227	4.4

[a] Calculated according to eq. 3 and the slope and intercept values of Table I. [b] Ligand dose = 0.1 × (500-mg dose of drug)/(5 mg/mL) × [ligand] × ligand M_r.

enhancements at low ligand concentrations. Nicotinamide also elevated DHPG and acyclovir solubilities significantly, albeit at ligand concentrations higher than those required for caffeine.

Whether caffeine and nicotinamide actually have potential practical application as solubility enhancers depends on the physiological effects elicited by the ligands at the daily doses shown in Table II. For comparison, LD_{50} values for the ligands (normalized to a 70-kg body weight) are:[21] 14 g/70 kg for caffeine (subcutaneous in rats); and 119 g/70 kg for nicotinamide (orally in rats). Although evaluating tolerance to intravenous nicotinamide or caffeine injection strictly requires direct experimental determinations, we consider that the ligand daily doses shown in Table II are too close to lethal doses to confidently recommend either caffeine or nicotinamide as complexing agents for DHPG or, especially, acyclovir.

Factors Controlling Complex Formation—Because ligand and substrate properties strongly influence K_1 values, we searched the literature for formation constant data on analogous structures, with the objective of identifying molecular parameters that control complex formation. The literature[11,14,15,22] frequently subdivides the forces controlling aqueous complex formation into five categories:

1. Electrostatic forces.
2. Inductive effects.
3. Hydrogen bonding.
4. Charge–transfer (electron donor–acceptor) interactions.
5. Hydrophobic effects.

Electrostatic forces should be unimportant for uncharged molecules, and essentially all the compounds considered below are un-ionized under the conditions investigated. Inductive effects and hydrogen bonding are possibly important interactions, but these interactions are small in magnitude and difficult to quantify. By comparison, both donor–acceptor and hydrophobic interactions are frequently large in magnitude and relatively simple to express quantitatively. Moreover, hydrophobic and, especially, donor–acceptor forces find frequent reference in the literature[9–11,15–16,20,23] as controlling factors in water-soluble complex formation. Consequently, the following discussion focuses on donor–acceptor strengths and hydrophobicity as factors that could correlate with complex formation constant values.

Relative donor–acceptor strengths can be quantitated on the basis of either theoretical calculations or experimentally determined molecular properties. In the latter category are solution-phase oxidation potentials; for example, as measured by anodic differential-pulse voltammetry.[18,24] For a series of structurally related molecules, differential-pulse peak potentials should vary inversely with donor strength (i.e., good donors have low oxidation potentials).

Concerning theoretical measures, Pullman and Pullman[25] elaborate extensively on the relationships between calculated orbital energies and relative donor and acceptor strengths. Specifically, the highest occupied molecular orbital (HOMO) energy correlates directly with donor strength, and the lowest unoccupied molecular orbital (LUMO) energy correlates inversely with acceptor strength. When orbital energies are expressed as:

$$E = \text{alpha} + k^*\text{beta} \qquad (5)$$

a low positive value of k (the resonance integral coefficient) corresponds to a high HOMO and high donor strength, and a low negative k value corresponds to high acceptor potential.

Hydrophobicities can be estimated on the basis of octanol–water partition coefficients, i.e., log P values. Extensive log P value compilations exist,[19] and additivity methods for log P value estimation are also known.[26] By convention, positive log P values are assigned to compounds that preferentially partition into octanol.

To identify the degree to which donor–acceptor and hydrophobic interactions control DHPG and acyclovir complex formation, we have compiled log P, oxidation potential, and orbital energy parameters for the substrates and ligands reported in this investigation. We have also extended the compilation to include literature data[15] for complexes of various ligands with the several purines (adenine, deoxyguanosine, guanosine, and hypoxanthine), and with a structurally unrelated molecule, the napthoquinone derivative, menadione.[10]

Table III summarizes K_1 values for 43 different substrate–ligand combinations in aqueous solution. The table also gives substrate and ligand log P values, HOMO and LUMO energies (expressed in multiples of the resonance integral, beta), anodic peak potentials, and substrate intrinsic solubilities. Using the data in Table III, we sought additional thermodynamic quantitative structure–activity relationships using linear regression analyses with log K_1 as the dependent variable. A preliminary stepwise multivariate regression analysis showed the ligand log P value to be the only significant dependent variable (at the 0.15 probability level). Following this lead, we then performed univariate regression analyses on subsets of the data in Table III to further define the quantitative relationships between K_1 and hydrophobicity or donor–acceptor strength. Table IV summarizes the univariate analysis results (and identifies the data subsets by reference to the substrate–ligand complex numbers listed in Table III).

Hydrophobic Interactions—Equation 6 in Table IV shows the correlation between log K_1 and ligand log P values for the 41 substrate–ligand pairs with available log P data. The squared correlation coefficient ($r^2 = 0.788$) indicates that the regression line accounts for a reasonably high fraction (78.8%) of all the data. Figure 2 graphically demonstrates the same correlation. The failure of eq. 6 to account for 100% of the data indicates that factors other than hydrophobic interactions partially control complex formation. Nevertheless, the degree to which hydrophobicity does account for complex formation is impressive, especially considering the structural dissimilarities between ligands and substrates, the wide range (7.2 log units) of log P values, and the wide range (factor of 6500) of K_1 values included in the correlation.

Equations 7–10 demonstrate correlations between log K_1 and ligand log P values for four individual substrates: DHPG, acyclovir, deoxyguanosine, and adenine. For DHPG

Table III—Chemical and Physical Data for Water-Soluble Complexes

Complex No.	Substrate	Ligand	K_1,[a] M^{-1}	Solubility, mM[b]	log P[c]	Substrate Potential,[d] volts	HOMO[e] beta	LUMO[e] beta	Log P[c]	Ligand HOMO[e] beta	LUMO[e] beta	Reference for K_1 Data
1	DHPG	Nicotinamide	2.07	11.7	−1.66[f]	0.97			−1.4	0.56[g]	−0.465[g]	f
2	DHPG	*N*-Ethyl derivative of nicotinamide	1.97	11.7	−1.66[f]	0.97			−2.02	1.03[h]	−0.356[h]	f
3	DHPG	Sucrose	0.2	11.7	−1.66[f]	0.97			−3.67			f
4	DHPG	Cytidine	3.13	11.7	−1.66[f]	0.97			−1.86			f
5	DHPG	Caffeine	9.3	11.7	−1.66[f]	0.97			−0.07	0.63[g]	−0.687[g]	f
6	DHPG	Thiamine	2.11	11.7	−1.66[f]	0.97				0.596	−0.807	f
7	DHPG	Pyridoxine	3.57	11.7	−1.66[f]	0.97						f
8	Adenine	Uracil	4.16	7.8[j]	−0.16	1.03	0.486	−0.865	−1.07	0.597	−0.96	15
9	Adenine	Uridine	4.03	7.8[j]	−0.16	1.03	0.486	−0.865	−1.62			15
10	Adenine	5-Fluorouracil	5.44	7.8[j]	−0.16	1.03	0.486	−0.865	−0.93[i]			15
11	Adenine	5-Chlorouracil	6.33	7.8[j]	−0.16	1.03	0.486	−0.865	−0.36[i]			15
12	Adenine	5-Bromouracil	7.04	7.8[j]	−0.16	1.03	0.486	−0.865	−0.21[i]			15
13	Adenine	5-Iodouridine	9.63	7.8[j]	−0.16	1.03	0.486	−0.865	−0.50[i]			15
14	Adenine	1,3-Dimethyluracil	7.14	7.8[j]	−0.16	1.03	0.486	−0.865	0.00[i]			15
15	Adenine	Thymine	7	7.8[j]	−0.16	1.03	0.486	−0.865	−0.44	0.51	−0.958	15
16	Adenine	Cytosine	4.89	7.8[j]	−0.16	1.03	0.486	−0.865	−1.46	0.595	−0.795	15
17	Adenine	Caffeine	45.1	7.8[j]	−0.16	1.03	0.486	−0.865	−0.07	0.63[g]	−0.687[g]	15
18	Adenine	8-Methoxycaffeine	80.2	7.8[j]	−0.16	1.03	0.486	−0.865	−0.09[i]			15
19	Adenine	Theophylline	31.1	7.8[j]	−0.16	1.03	0.486	−0.865	−0.02	0.656[g]	−0.690[g]	15
20	Adenine	Adenosine	21	7.8[j]	−0.16	1.03	0.486	−0.865	−1.1			15
21	Adenine	Inosine	8.25	7.8[j]	−0.16	1.03	0.486	−0.865	−2.08			15
22	Deoxyguanosine	1,3-Dimethyluracil	4.73	12.9[j]		1.03			0.00[i]			15
23	Deoxyguanosine	Cytosine	3.32	12.9[j]		1.03			−1.46	0.595	−0.795	15
24	Deoxyguanosine	Caffeine	26.5	12.9[j]		1.03			−0.07	0.63[g]	−0.687[g]	15
25	Deoxyguanosine	8-Methoxycaffeine	30.7	12.9[j]		1.03			−0.09[i]			15
26	Deoxyguanosine	Theophylline	14	12.9[j]		1.03			−0.02	0.656[g]	−0.690[g]	15
27	Deoxyguanosine	Adenosine	9.21	12.9[j]		1.03			−1.1			15
28	Deoxyguanosine	Inosine	8.25	12.9[j]		1.03			−2.08			15
29	Menadione	β-Hydroxy-naphthoic acid	1881		2.20		0.972[g]	−0.228[g]	3.63[i]	0.384[g]	−0.450[g]	10
30	Menadione	Salicylic acid	347		2.20		0.972[g]	−0.228[g]	2.26	0.438[g]	−0.555[g]	10
31	Menadione	Ethyl aminobenzoate	245		2.20		0.972[g]	−0.228[g]	2.57	0.363[g]	−0.386[g]	10
32	Menadione	Theophylline	56.3		2.20		0.972[g]	−0.228[g]	−0.02	0.656[g]	−0.690[g]	10
33	Menadione	Caffeine	41.25		2.20		0.972[g]	−0.228[g]	−0.07	0.63[g]	−0.687[g]	10
34	Menadione	Nicotinamide	5.08		2.20		0.972[g]	−0.228[g]	−1.4	0.56[g]	−0.465[g]	10
35	Guanine	Caffeine	58	0.017	−1.00	0.72	0.310	−1.05	−0.07	0.63[g]	−0.687[g]	f
36	Hypoxanthine	Caffeine	10.1	5.1[j]	−1.11	0.8	0.402	−0.882	−0.07	0.63[g]	−0.687[g]	15
37	Guanosine	Caffeine	11.3	1.83	−1.79	1.03			−0.07	0.63[g]	−0.687[g]	f
38	Guanosine	Caffeine	15.6	1.07[j]	−1.79	1.03			−0.07	0.63[g]	0.687[g]	15
39	Adenosine	Caffeine	39.4	19.2[j]	−1.10	1.3			−0.07	0.63[g]	−0.687[g]	15
40	Acyclovir	Caffeine	12.35	6.0		1.01			−0.07	0.63[g]	−0.687[g]	f
41	Acyclovir	Nicotinamide	3.34	6.0		1.01			−1.4	0.56[g]	−0.487[g]	f
42	Guanine	Nicotinamide	4.58	0.017	−1.00	0.72	0.310	−1.05	−1.4	0.56[g]	−0.487[g]	f
43	Guanosine	Nicotinamide	4.79	1.83	−1.79	1.03			−1.4	0.56[g]	−0.487[g]	f

[a] Calculated according to eq. 2. [b] $[S]_0$ values calculated according to eq. 2. Data of this work unless otherwise indicated. [c] Data of ref. 19, unless otherwise indicated [d] Differential-pulse anodic peak potential. Data of ref. 20. [e] Data of ref. 21, unless otherwise indicated. [f] Data of this work. [g] Data of ref. 10. [h] Data of ref. 21, using the values given for $NADH^+$. Calculated according to eq. 4.

Table IV—Univariate Linear Regression Analyses of log K_1 versus Several Independent Variables

Equation No.	Substrate(s)[a]	Ligand(s)[a]	Complexes Included[a]	Independent Variable[a]	Slope[b]	Intercept[b]	n	r^2
6	All	All	1–5,8–43	Ligand log *P*	0.479	1.35	41	0.788
7	DHPG	Various	1–5	Ligand log *P*	0.451	1.09	5	0.919
8	Menadione	Various	29–34	Ligand log *P*	0.446	1.53	6	0.948
9	Adenine	Various	8–21	Ligand log *P*	0.308	1.24	14	0.255
10	Deoxyguanosine	Various	22–28	Ligand log *P*	0.258	1.18	7	0.353
11	Various	Caffeine	17,33,35–40	Substrate log *P*	0.139	1.47	8	0.31
12	Various	Caffeine	5,17,24,35–40	Substrate Solub.	0.0111	1.23	9	0.048
13	Various	Caffeine	5,17,24,35–40	Substrate Potential	0.141	1.17	9	0.0056
14	Various	Caffeine	17,33,35,36	Substrate HOMO	0.188	1.41	4	0.0264
15	Various	Caffeine	17,33,35,36	Substrate LUMO	0.0742	1.56	4	0.0061
16	Menadione	Various	29–34	Ligand HOMO	−0.495	0.456	6	0.517

[a] See Table III. [b] For the expression: log K_1 = Intercept + Slope* (Independent Variable).

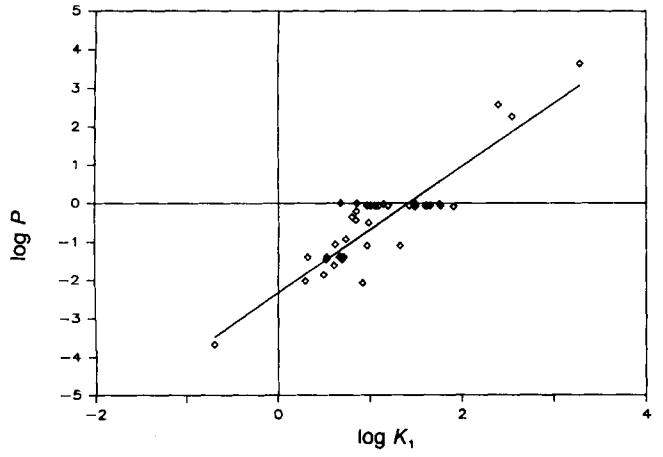

Figure 2—*Dependence of complex formation constant (log K_1) values on ligand octanol–water partition coefficient (log P) values for 41 ligand–substrate combinations. Data of Table III.*

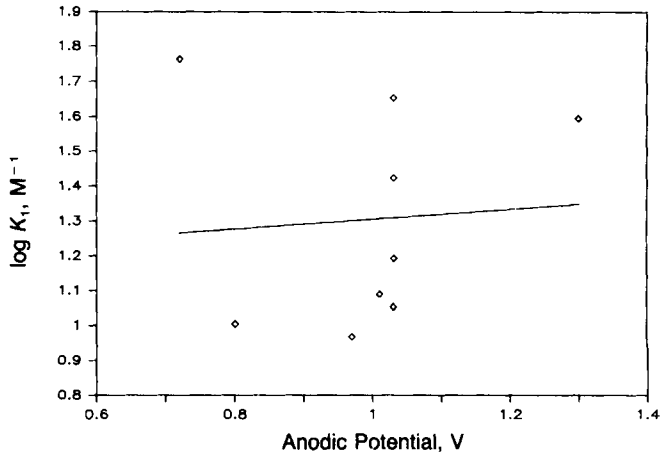

Figure 3—*Independence of complex formation constant (K_1) values and substrate oxidation (peak) potential values for caffeine complexes with various substrates.*

and menadione, log P accounts for >90% of the correlations. The log K_1 values for adenine and deoxyguanosine, however, correlated poorly with ligand log P values. Thus, hydrophobicity appears to be a strong, but not exclusive, determinant of complex formation.

Donor–Acceptor Interactions—Equations 12–16 in Table IV demonstrate that donor–acceptor properties for both ligands and substrates correlate very poorly with complex formation constant values. With caffeine as receptor, anodic peak potentials for nine different substrates failed to correlate significantly (r^2 = 0.0047) with log K_1 values. Figure 3 plots peak potentials versus log K_1 values and clearly shows the poor correlation. For a smaller subset of four substrates, eqs. 14 and 15 show that caffeine complex formation constants do not correlate with either substrate HOMO or substrate LUMO (r^2 = 0.0264 and 0.0061, respectively).

To probe for correlations of ligand orbital energies with formation constants we compared ligand LUMO values and log K_1 values for DHPG complexes with nicotinamide and the *N*-ethyl derivative of nicotinamide. Although the nicotinamide and the *N*-ethyl derivative of nicotinamide LUMO values differ substantially, K_1 values for DHPG are identical for both ligands within the limits of experimental uncertainty. Thus, no influence of ligand acceptor potential on complex formation is apparent.

Similarly, regressing ligand HOMO values versus log K_1 values demonstrated poor correlation (r^2 = 0.517) for menadione complexes with six different ligands. Interestingly, the same ligand-menadione subset gave an excellent correlation (eq. 8, r^2 = 0.948) between log K_1 values and ligand hydrophobicity.

Hata et al.[10] previously cited a "curvilinear" relationship between menadione formation constants and ligand HOMO for essentially the same data set as that used in eq. 8 and 16. Our results question the validity of the conclusions drawn by Hata and co-workers. Equations 8 and 16 require that hydrophobic forces, not charge–transfer interactions, predominantly control menadione complex formation.

Conclusions

The antiviral drugs DHPG and acyclovir form water soluble complexes with various ligands, including nicotinamide and caffeine. The complex formation constants vary over a modest range (30-fold range of K_1 values for DHPG as substrate) and depend strongly on ligand and substrate structure.

None of the DHPG or acyclovir complexes featured remarkably high formation constants; the acyclovir–caffeine complex K_1 value (12.4) was the highest value observed. Because of the low K_1 values, rather high ligand concentrations are necessary to elevate DHPG and acyclovir concentrations to therapeutically active levels (~5 mg/mL). For a 100-mL (500 mg) daily injection of DHPG, caffeine and nicotinamide doses (calculated from ligand concentrations needed to achieve a 5-mg/mL drug solubility) would be 0.6 and 4.5 g, respectively. For acyclovir, calculated caffeine and nicotinamide doses would reach 4.4 and 9.4 g, respectively. It seems unlikely that caffeine or nicotinamide would be well tolerated at the high doses necessary to effect useful DHPG or acyclovir solubility enhancement. Consequently, we consider that the ligands reported here have only limited value as additives to parenteral formulations containing DHPG or acyclovir.

With the objective of identifying molecular interactions that govern water soluble complex formation for DHPG, acyclovir, and related compounds, we examined correlations between complex formation constants and various parameters that reflect ligand and substrate hydrophobicity and donor–acceptor properties. For 41 substrate–ligand combinations, we established a significant (r^2 = 0.788) correlation between log K_1 and ligand log P values. Although the correlation between log K_1 and ligand log P values extended very well to two subsets of the data (namely complexes of DHPG and menadione with various ligands), two other subsets (adenine and deoxyguanine as substrates) provided poor correlations between K_1 and ligand log P values.

We conclude that hydrophobicity is a general determinant of complex formation but that secondary factors may significantly contribute to complex formation for some ligand–substrate combinations. The foregoing caveat notwithstanding, we consider it to be significant that our investigation reveals for the first time a quantitative relationship between ligand log P values and complex formation. Other workers[11,14,15,20,23] have recognized the primary role of hydrophobicity in complex formation, but the existing literature either fails to provide quantitative relationships or employs correlates of hydrophobicity (e.g., ligand–substrate overlap area) that are less straightforward to obtain than log P values. Thus, our own work extends the generality of the observation that hydrophobicity governs complex formation and identifies log P as a convenient correlate of the complex formation constant.

We have also clearly demonstrated that donor–acceptor interactions do not control complex formation for a variety of

substrates and ligands. Again, we cannot generalize our observations to include all complexes. It is noteworthy, however, that the literature[9,10,16] either explicitly or implicitly identifies donor–acceptor properties as strong or sole determinants of complex formation for some substrates that clearly fail to show significant dependence of K_1 on donor–acceptor properties.

Our findings suggest that reported significant donor–acceptor interactions in water-soluble complexes should be interpreted with some caution in the absence of significant quantitative structure–activity relationships between formation constants and parameters that reflect relative donor and acceptor strengths.

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