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Report

Complexation of Nifedipine with Substituted Phenolic Ligands

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The bioavailability of nifedipine in man is highly variable. This may be partly due to its poor aqueous solubility (5–6 μg/ml over pH 2.2–10.0, as determined in this laboratory). We initiated this study to examine the enhancement of aqueous nifedipine solubility via complexation. A series of substituted aromatic ligands was studied to identify those structural features important for complexation with nifedipine. The studies were performed at 25°C employing the solubility technique, using pH 2.2 or 7.0 buffers at an ionic strength of 0.25 *M*. The apparent equilibrium complexation constants for the 1:1 and/or 1:2 complexes were determined, where appropriate. A linear free-energy approach was used to relate $K_{1:1}$ with Hammett's sigma (σ) and fractional partition coefficient (π) parameters. The following correlation was obtained: $\log (K_{1:1}/K_o) = 0.31\sigma + 0.10\pi + 0.36$ ($r^2 = 0.86$, $P < 0.003$, $N = 9$), where K_o is the complexation constant for phenol. Statistical analyses showed that σ was more important than π in affecting nifedipine complexation. The exact location of this interaction on the nifedipine molecule is undefined at present.

KEY WORDS: nifedipine; 1,4-dihydropyridines; linear free-energy relationships; nifedipine solubility; complexation.

INTRODUCTION

The 1,4-dihydropyridines are an important class of calcium channel blockers. Many compounds of this group hold good promise for the clinical management of a number of cardiovascular diseases such as essential hypertension, congestive heart failure, and cerebral ischemia. Nifedipine is the first member of this series to be approved for the treatment of angina pectoris.

A major pharmaceutical problem with nifedipine and other uncharged 1,4-dihydropyridine analogues (e.g., felodipine, nitrendipine, nimodipine) is their poor aqueous solubility. Dissolution of the tablet dosage forms of these drugs appears to be the rate-limiting step in drug absorption. Hence, flip-flop (i.e., absorption rate-limited) pharmacokinetics are often observed with the tablet preparations (1,2). Nifedipine is commercially available in a soft gelatin liquid capsule. The oral bioavailability of the capsule is quite variable in man (mean ± SD, 56 ± 25%; range, 31–91%) (1). This variability could be attributed to interindividual differences in presystemic metabolism and absorption. Incomplete absorption of nifedipine could result from *in vivo* precipitation of the drug in the gastrointestinal tract followed by its slow redissolution.

Our interest in complexation as a means to enhance nifedipine dissolution and/or aqueous solubility was sparked by a report that an aqueous formulation containing ethanol and sodium salicylate enabled the preparation of a 22 mg/ml nifedipine solution (3). Since the ethanolic cosolvent could not account for the large apparent increase in nifedipine solubility, it appeared that sodium salicylate could have enhanced nifedipine solubility through a complexation mechanism.

The X-ray crystallographic spatial conformation of the nifedipine molecule (4) suggests that an aromatic ligand could complex with either the 1,4-dihydropyridine or the nitrobenzene ring system (Fig. 1). Additionally, a hydrogen-donating ligand could interact with the nitrogen or the methyl ester substituents of the dihydropyridine ring.

We initiated this study, therefore, to examine the structural features in phenolic ligands that might maximize nifedipine solubility via complex formation. We also sought to understand the nature of the molecular interactions involved in these complexation reactions through the use of linear free-energy relationships (LFER).

MATERIALS AND METHODS

Nifedipine was purchased from Sigma Chemical Co., St. Louis, Mo. The substituted phenolic ligands were used as supplied by Eastman Kodak Co., Rochester, N.Y. Polyvinylchloride (PVC) filters (0.45-μm pore size) were purchased from Gelman Instrument Co., Ann Arbor, Mich. Nimodipine was a generous gift from Miles Laboratories.

Various buffers of low ionic strength (0.01 *M*) were prepared as described by Perrin (5) for the pH–solubility studies. A U.S.P. phosphate buffer (6) of pH 7.0, $\mu = 0.25$ *M*, was used for the hydroxybenzoate ligand solutions. A U.S.P. HCl–KCl buffer (6) of pH 2.2, $\mu = 0.25$ *M*, was used for all other ligand solutions. A series of substituted phenols (Table 1) was used as ligands in the complexation studies.

To initiate these studies, an excess of solid nifedipine was added to a screw-capped test tube containing the appro-

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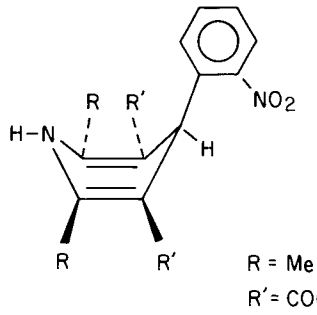

Fig. 1. Three-dimensional representation of nifedipine.

priate buffer of ligand solution and tumbled in a 25 ± 0.5°C water bath for a minimum of 48 hr. Successive samples were collected at least 24 hr apart. Each sample was obtained by filtering a 2-ml aliquot of the equilibrated solution through a 0.45-μm PVC filter. Previous studies showed that nifedipine adsorbed to PVC materials. It was established that saturation of the filter was complete after filtration of 1 ml of a 5.7 μg/ml aqueous nifedipine solution. Hence, the first 1.5 ml of filtrate was discarded and the last 0.5 ml was reserved for high-performance liquid chromatographic (HPLC) analysis. The filtered, saturated nifedipine solutions were diluted with an appropriate volume of HPLC-grade methanol followed by addition of the internal standard, nimodipine. The samples were analyzed by HPLC with ultraviolet detection at 254 nm. The mobile phase, 62/38% (v/v) methanol/0.01 *M* phosphates buffer (pH 4.8), was pumped through a Waters μ-Bondapak C-18 column (30 cm × 3.9-mm i.d.) at a flow rate of 1.0 ml/min. We defined apparent equilibrium to be attained when the coefficient of variation of quadruplicate concentration measurements over 4 days was less than 10%. All work was performed under sodium vapor (yellow) light to prevent nifedipine photodegradation (7).

Both differential scanning calorimetry (DSC) and HPLC techniques were used to define the stoichiometric ratio of those complexes which appeared to precipitate out of solution. DSC scans were performed using a Perkin Elmer differential scanning calorimeter (Model DSC-2) programed to heat at 10°K/min from 273.0 to 480.0°K.

Mathematical analysis of the complexation phase diagrams provided estimates of the apparent equilibrium complexation constants. It was assumed that a 1:1 nifedipine-ligand complex was formed at those ligand concentrations where the total solubility versus ligand concentration curve was linear. The apparent 1:1 equilibrium complexation constant, $K_{1:1}$, was estimated by regression analysis (8) using Eq. (1) (9).

$$S_t = \frac{K_{1:1}S_0}{1 + K_{1:1}S_0} L_t + S_0 \quad (1)$$

where S_t is the observed molar solubility of nifedipine, $K_{1:1}$ is the 1:1 equilibrium complexation constant, S_0 is the intrinsic molar solubility of nifedipine, and L_t is the total molar ligand concentration.

When the total solubility versus ligand concentration curve was parabolic, it was assumed that higher-order complexes were formed. These data were analyzed via nonlinear regression analysis using Eq. (2), assuming that only 1:1 and 1:2 complexes were present and that the concentration of the complexed ligand was negligible relative to the total concentration of the added ligand (10).

$$S_t = S_o + S_oK_{1:1}L_t + S_oK_{1:2}L_t^2 \quad (2)$$

where $K_{1:2}$ is the 1:2 equilibrium complexation constant. A stepwise multiple linear regression program (11) was used to establish a linear free-energy relationship between the observed 1:1 equilibrium complexation constants and various physicochemical parameters.

Table I. Equilibrium Complexation Constants (1:1 and 1:2) for Various Ligands with Nifedipine[a]

Ligand	Substituent	$K_{1:1}$ (M^{-1})	$K_{1:2}$ (M^{-2})
Phenol	H	7.2 ± 0.6	
Pyrocatechol	*o*-OH	11.2 ± 0.9	
Resorcinol	*m*-OH	13.2 ± 0.6	
Hydroquinone	*p*-OH	9.9 ± 0.3	
Phloroglucinol	3,5-diOH	17.4 ± 0.6	
Pyrogallol	2,3-diOH	7.2 ± 0.7	
Benzenetriol	2,4-diOH	10.6 ± 1.5	
o-Cresol	*o*-CH_3	25.4 ± 4.3	
m-Cresol	*m*-CH_3	17.0 ± 1.8	
p-Cresol	*p*-CH_3	16.3 ± 2.7	
Salicylate	*o*-COO^-	8.6 ± 2.4	39.0 ± 15
m-OH benzoate	*m*-COO^-	1.3 ± 1.9	282 ± 31
p-OH benzoate	*p*-COO^-	0.2 ± 0.3	280 ± 31

[a] Values are regression estimates ± SD.

RESULTS

Nifedipine aqueous solubility is 5.8 ± 0.31 μg/ml (mean ± SD, N = 20) over the pH range of 4–10. Solubility increased slightly, to 6.6 ± 0.24 μg/ml (N = 4), at pH 2.2, suggesting that nifedipine may be a weak base with a pK_a less than 2.2.

Three types of complexation curves were observed (Figs. 2–4). According to Higuchi and Connors (9), these curves can be classified as A_L (linear), A_P (parabolic), and

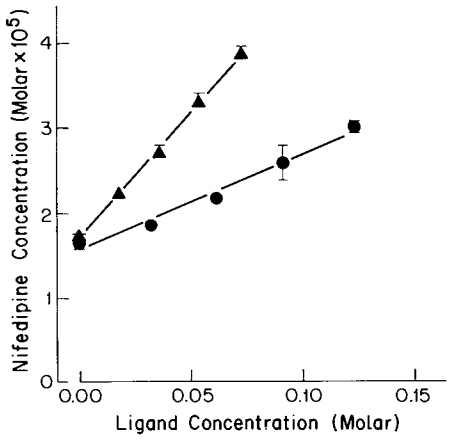

Fig. 2. Nifedipine solubility as a function of ligand concentration. The data are mean ± SD. (●) Phenol; (▲) 3,5-dihydroxy phenol.

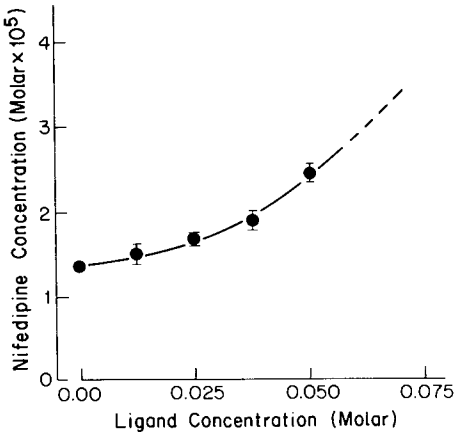

Fig. 3. Nifedipine solubility as a function of *m*-hydroxybenzoate concentration. The data are mean ± SD.

B_S (plateau). For phenol and the various hydroxy-substituted phenols, nifedipine solubility was linearly related to the added ligand concentration (Fig. 2, A_L curve). Nifedipine complexation with hydroxy-substituted benzoates showed a parabolic increase in solubility with added ligand concentration (Fig. 3, A_P curve). A B_S-type profile (Fig. 4) was observed for the cresol ligands. In the last case, nifedipine solubility was linearly related to cresol ligand concentrations up to 0.05 *M*, but plateaued out at cresol concentrations between 0.05 and 0.10 *M*. A decrease in nifedipine solubility was observed over a ligand concentration of 0.10–0.15 *M*; this phenomenon is classically interpreted as a result of depletion of nifedipine in solution due to precipitation of the complex.

The complexation pattern between nifedipine and cresol thus indicated the possible formation of an insoluble complex. Accordingly, DSC and HPLC techniques were used to characterize the solid material isolated from the 0.03 and 0.13 *M* *m*-cresol ligand solutions. The control thermogram of the material isolated from the 0.03 *M* solution showed a sharp endothermic melting peak at 445°K, which corresponded with the published melting point of crystalline nifedipine (12). The DSC scan of the insoluble material isolated from the 0.13 *M* solution was similar to the control scan, an indication that the precipitate was composed essentially of nifedipine. HPLC analysis of the solid revealed a stoichiometric ratio of 1 mol of *m*-cresol/10–12 mol of nifedipine.

The apparent equilibrium complexation constants (1:1 and 1:2) for the various ligands are reported in Table I. A linear free-energy approach (LFER) was used to relate the ratio, $K_{1:1}/K_o$ (where K_o represents the apparent 1:1 equilibrium complexation constant for phenol, the reference compound), to Hammett's sigma (σ) and fractional partition coefficient (π) parameters (13,14). The best correlation found,

$$\log (K_{1:1}/K_o) = 0.31\sigma + 0.10\pi + 0.36 \quad (3)$$

was statistically significant, with $N = 9$, $r^2 = 0.86$, and $P < 0.003$. The $K_{1:1}$ values from the hydroxybenzoate ligands were excluded from the LFER analysis since these ligands formed predominantly higher complexes and the parameter estimates for their $K_{1:1}$ were less reliable when judged from the nonlinear regression statistics.

Figure 5 shows the relationship between the log $K_{1:1}/K_o$ values predicted from the LFER relationship and the corresponding experimental values. The correlation was quite satisfactory. Although both electronic (σ) and hydrophobic (π) factors are necessary to describe the molecular interaction(s) between nifedipine and a ligand, analysis of the multiple linear regression statistics suggests that σ is a more important factor that π (simple $r^2 = 0.68$ vs 0.51, respectively).

DISCUSSION

The aim of the present study was to understand the effect of complexing ligands on the solubility behavior of nifedipine, a model 1,4-dihydropyridine compound. Substituted aromatic phenolic ligands were chosen as complexing agents to gain a better understanding of the molecular interactions involved in the reported nifedipine–salicylate formulation (3). It can be postulated that nifedipine complexation with salicylate is facilitated by an electron donor–acceptor interaction involving the π orbitals of the ligand aromatic nucleus with those of either the nifedipine dihydropyridine or the nitrobenzene ring system (15,16). Additionally, the complexation mechanism could involve hydrogen

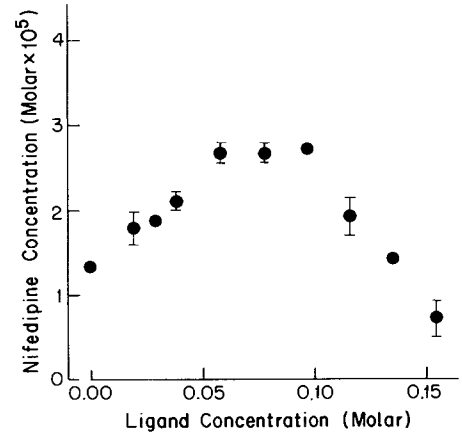

Fig. 4. Nifedipine solubility as a function of *m*-cresol concentration. The data are mean ± SD.

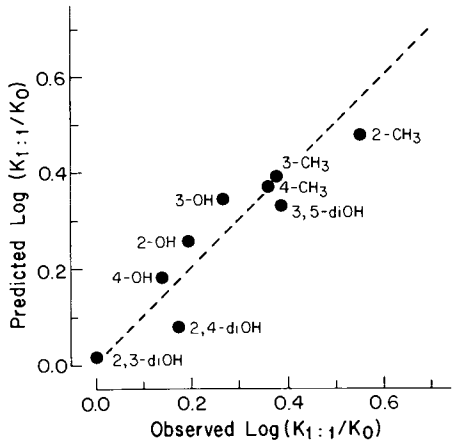

Fig. 5. Correlation of the log $K_{1:1}/K_o$ values predicted from the LFER relationship with the corresponding experimental values.

bonding between the ligand and the nitrogen atom of the dihydropyridine ring. In a preliminary study with L-ascorbic acid, a good hydrogen-donating molecule lacking an aromatic nucleus, we observed a $K_{1:1}$ of 3.0 M^{-1} with marginal increases in nifedipine solubility. This result suggests that the presence of an aromatic nucleus is important for ligand complexation with nifedipine.

Different types of complexes were apparently formed with the phenolic ligands studied. Soluble complexes were formed with the hydroxy-substituted phenols (primarily 1:1 complexes) and the hydroxy-substituted benzoates (primarily multiple complexes). The solubility curves with cresol ligands suggested the formation of insoluble complexes. DSC and HPLC analyses of the material isolated from these systems suggested that the precipitate was primarily nifedipine. Thus, the decrease in nifedipine solubility at high concentrations of cresol was not apparently due to precipitation of a simple complex of cresol-nifedipine per se. Rather, it is postulated that the ligand–drug complex might have precipitated out of solution with (or around) other nifedipine crystals. This process would hinder further nifedipine dissolution and cause an apparent nonequilibrium condition with respect to nifedipine saturated solubility.

Few investigators have employed a LFER approach in characterizing complexation phenomena. Lopata *et. al.* (17) established, for cyclodextrin–barbiturate complexes, several quantitative structure–stability relationships based on hydrophobic-group contributions and molar refractivities. The Hammett equation was used to describe the complexation of α-cyclodextrin with *p*-substituted benzoic acids (18). A LFER based on the interaction energies predicted by frontier molecular orbital calculations was employed to characterize the interaction of niacinamide with heteroaromatic ligands (19). Donbrow and Jan (20) observed a correlation between log $K_{1:1}$ and pK_a values for the complexation of caffeine with substituted benzoic acids. LFER have been observed for complexation phenomena in nonaqueous solvents: Higuchi and Connors (9) have used Taft's polar and steric substituent constants to describe complexation systems of salicylic acid/alcohols and catechol/alcohols in carbon tetrachloride.

We show that a linear free-energy approach utilizing published σ and π values can mathematically characterize the complexation phenomenon between nifedipine and substituted phenolic ligands. Electronic factors appear to be more important than hydrophobic interactions. The positive dependency on the sigma substituent value in the LFER suggests that an electron-withdrawing substituent facilitates complexation interaction with nifedipine. The observed LFER is consistent with the following mechanistic interpretations: (i) an electronegative substituent favors complexation not only by enhancing the hydrogen-donating strength of the phenol but also by increasing the electrophilicity of the aromatic nucleus and (ii) an increased lipophilicity of the ligand substituent facilitates complexation via hydrophobic interactions.

The observed LFER can be used, in principle, to examine whether ideal ligand structures exist for the optimum enhancement of nifedipine solubility in water. The fractional increase in substrate solubility is dependent on S_o, $K_{1:1}$, and L_T as shown by the rearranged form of Eq. (1):

$$\frac{S_t - S_0}{S_0} = \frac{K_{1:1}L_t}{1 + K_{1:1}S_0} \quad (4)$$

Thus, an increase in substrate solubility is a function of the product of $K_{1:1}$ and L_t (21). Based on the LFER analysis, an ideal phenolic ligand with a large $K_{1:1}$ would have substituents having both large σ and π values. However, substituents with large σ values usually have negative or small, positive π values, and *vice versa*. Additionally, phenolic substituents with large π values usually have limited aqueous solubility. It is possible to select ligands with large $K_{1:1}$ values, but usually the predicted maximal increase in nifedipine solubility is marginal due to the limited saturated solubility of the ligand. An example of this type of ligand is *p*-nitrophenol. The predicted $K_{1:1}$ is 42.6 M^{-1} with a ligand saturated solubility of 0.072 *M*. However, the predicted maximum nifedipine solubility with this ligand is only 23.5 μg/ml. Alternatively, one may select a ligand with a larger intrinsic solubility and smaller predicted $K_{1:1}$ to arrive at a greater enhancement in nifedipine solubility. Resorcinol is an example. Computations using the LFER data predicted a nifedipine solubility of 42.5 μg/ml at a resorcinol ligand concentration of 0.48 M. However, the experimentally observed nifedipine solubility was 65.7 μg/ml under these conditions. This discrepancy between the predicted and the observed values is most likely due to the formation of higher-order complexes (type A_P curve) at resorcinol concentrations greater than 0.24 *M*. Thus, the predictive ability of the LFER would be severely compromised at high ligand concentrations where the 1:1 complex is no longer the predominant molecular species.

In conclusion, nifedipine complexation with substituted phenolic ligands results in an approximately 10-fold increase in nifedipine solubility. At low ligand concentrations, nifedipine complexation with substituted phenols can be described by a LFER utilizing Hammett's σ and fractional partition coefficient parameters. While the precise molecular nature of the complexation interactions remains undefined, the LFER data indicate that hydrogen bonding, aromatic electronic, and hydrophobic processes are involved.

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