Solubility Enhancement of Nucleosides and Structurally Related Compounds by Complex Formation

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Water-soluble vitamins, amino acids, and nontoxic pharmaceutical excipients were studied as solubilizing agents for poorly water-soluble adenine (nucleic acid base), guanosine (nucleoside), and structurally related drugs (acyclovir and triamterene). The apparent solubility of the substrates (adenine, guanosine, acyclovir, or triamterene) was appreciably increased by forming complexes with the ligands (vitamins, amino acids, or other ligand). Apparent association constants (K_a) values were measured at 25°C in pH 7 phosphate buffer using phase solubility analysis. The effect of combination ligands on substrate solubility was also studied. Additive solubility enhancement was obtained for several ligand pairs.

KEY WORDS: molecular complex; niacinamide; pyridoxine; association constant; solubility.

INTRODUCTION

Nucleoside derivatives are common parent structures of therapeutic agents, such as antivirals and antimetabolites (1–3). Aqueous solubilities of these compounds are often so poor that formulation of injectable dosage forms is difficult (4). As an alternative to the use of surfactant, cosolvent, and pH modification, complexing agents (ligands) have been used occasionally as solubility enhancers for poorly water-soluble drugs (substrates) (5–7). Besides potential ligand toxicity (8), the substantial amount of ligand needed because of their weak solubility enhancing property often limits their use for iv formulation. In this study, vitamins, amino acids, and pharmaceutical excipients were screened as nontoxic ligands for their solubility enhancing property. To obtain greater solubility enhancement and reduce the amount of individual ligand needed, combinations of two ligands (ligand pairs) were explored. It was assumed that two ligands would complex with the substrate in parallel and therefore increase the substrate solubility additively. The effects of ligand type, mixing ratio, and total concentration on the additive solubility enhancement were studied. The classification of complexing agents proposed by Higuchi *et al.* (9) was used to account for the difference in the solubility enhancement by different ligand pairs.

MATERIALS AND METHODS

Acyclovir was obtained from Burroughs Wellcome, NC. Triamterene was obtained from SmithKline Beecham, NJ. Caffeine was USP grade. Most other chemicals used were purchased from either Sigma or Aldrich Chemical Co. All reagents had a purity of 97% or better and were used without further purification.

Phase Solubility Analysis. Phase solubility analysis involved adding excess amounts of solid substrate to six to eight ligand solutions of various concentration in a pH 7 phosphate buffer (GramPac, Fisher). The mixtures were than agitated for 48 to 72 hr at a constant temperature (25 ± 1°C). After equilibration, the mixture was filtered through a 0.45-μm Millipore filter (Bedford, MA). Substrate concentrations in filtered aliquots were measured by reversed-phase HPLC.

HPLC Assays. Reversed-phase HPLC analyses were carried out at ambient temperatures. The HPLC system consisted of a Waters pump (Model 450), a UV detector (LambdaMax), and a Novapac C-18 column (4.6 mm × 15 cm, 5-μm particle size; Waters). Chromatographic data obtained at a detection wavelength of 254 nm were analyzed using a chromatographic integrator (Hitachi D2000). The mobile phases were mixtures of methanol and water containing 0.004 *M* NaH_2PO_4. The ratio of methanol to water and final pH values of the mobile phases were adjusted depending upon the substrate and ligand in the sample solution.

Association Constants. The apparent association constant (K_a) values for 1:1 complexes were calculated using the method of Higuchi and Connors (7):

$$K_a = \frac{\text{slope}}{\text{intercept} * (1 - \text{slope})} \quad (1)$$

where the "slope" and "intercept" were obtained from the least-squares regression of the phase solubility diagram according to the following equation:

$$[S]_t = \text{intercept} + \text{slope} * [L]_t \quad (2)$$

where $[S]_t$ and $[L]_t$ represent the total molar concentrations of the substrate and ligand in solution, respectively.

RESULTS AND DISCUSSION

Effect of Ligands on Solubility. The effect of various vitamins, amino acids, and pharmaceutical excipients on the solubility of the model substrates (adenine, guanosine) was examined. Many of these molecules were able to complex with either adenine or guanosine at a neutral pH and consequently increase the apparent solubility of the substrate. Typically, according to phase solubility, the apparent solubility of the substrate increased linearly with an increase in ligand concentration, with no evidence of higher-order complexes (7) formed over the ligand concentration ranges employed. Representative data for adenine complexes with various water-soluble vitamins and amino acids are shown in Fig. 1. The association constant K_a of each complex was calculated from the slope and the intercept of the fitted line. Some pharmaceutical excipients, e.g., methylparaben, propylparaben, vanillin, benzoate, saccharin, etc., also formed

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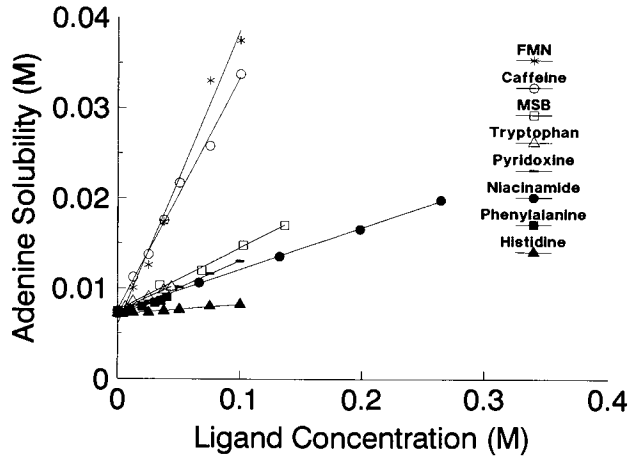

Fig. 1. Effect of ligand concentration on adenine solubility in pH 7 buffer at 25°C.

complexes with either adenine or guanosine. The K_a and the linear regression statistics for each adenine and guanosine complex investigated are summarized in Tables I and II.

Adenine and Guanosine Complexes. A saturated solution of adenine has a pH value between 6.2 and 6.4 in purified water and a value of 6.7 to 6.8 in 0.05 *M*, pH 7, phosphate buffer. In both cases, adenine exists predominantly in the nonionized form at saturation. The average intrinsic solubility of adenine is $(7.7 \pm 0.08) \times 10^{-3}$ *M* in water and $(7.2 \pm 0.07) \times 10^{-3}$ *M* in pH 7 phosphate buffer at 25°C. The average intrinsic solubility of guanosine in the phosphate buffer is $(4.3 \pm 0.2) \times 10^{-4}$ *M*.

Among the vitamins studied, flavin mononucleotide (FMN), menadione sodium bisulfite (MSB), niacinamide, and pyridoxine were found to form complexes with either adenine or guanosine. Ascorbic acid, thiamine hydrochloride, and biotin did not show an appreciable complexing tendency. The association constants K_a for the adenine–vitamin complex were in the order $K_{a(FMN)} > K_{a(MSB)} > K_{a(pyridoxine)} > K_{a(niacinamide)}$. Some amino acids studied also showed a remarkable complexing tendency with respect to adenine or guanosine, yielding K_a values in the order $K_{a(L\text{-tryptophan})} > K_{a(L\text{-phenylalanine})} > K_{a(L\text{-histidine})}$.

Table I. Association Constants (K_a) Adenine Complexes with Various Ligands in pH 7 Phosphate Buffer at 25°C

Ligand	K_a $(M^{-1})^a$	Slope[b]	Intercept $(M) \times 10^{3b}$	Conc. range $(M)^c$
Flavin mononucleotide	52	0.33	6.0	0–0.1
Caffeine	46	0.26	7.6	0–0.1
Vanillin	15	0.10	7.2	0–0.1
Propylparaben	11	0.07	7.0	0–0.005
Menadione sodium bisulfite	9.8	0.07	7.6	0–0.1
Pyridoxine	9.0	0.06	7.1	0–0.1
L-Tryptophan	8.5	0.06	7.1	0–0.05
Niacinamide	6.5	0.05	7.5	0–0.3
Saccharin, sodium	5.3	0.04	7.1	0–0.1
Methylparaben	5.0	0.03	7.2	0–0.01
L-Phenylalanine	3.4	0.02	7.2	0–0.1
Benzoate, sodium	2.1	0.01	7.1	0–0.1
L-Histidine	1.5	0.01	7.1	0–0.1
L-Alanine	—	0.001	7.2	0–0.1
L-Ascorbate, sodium	—	—	7.2	0–0.1

[a] Calculated according to Eq. (1).
[b] By least squares linear regression according to Eq. (2) using the average intercept (7.2×10^{-3} M), $r^2 > 0.95$.
[c] Concentration ranges employed in phase solubility analyses.

Table II. Association Constants (K_a) of Guanosine Complexes with Various Ligands in pH 7 Phosphate Buffer at 25°C

Ligand	K_a $(M^{-1})^a$	Slope $\times$ 10^{4b}	Intercept $(M) \times 10^{4b}$	Conc. range $(M)^c$
Flavin mononucleotide	70	291	4.0	0–0.1
Caffeine	65	276	4.4	0–0.1
Vanillin	20	89	4.5	0–0.1
L-Tryptophan	12	43	4.9	0–0.05
Pyridoxine	8.8	23	4.6	0–0.4
Niacinamide	5.2	21	4.0	0–0.1
L-Phenylalanine	4.3	18	4.2	0–0.1
Benzoate, sodium	2.5	11	4.3	0–0.1
L-Histidine	0.9	3.9	4.3	0–0.1

[a] Calculated according to Eq. (1).
[b] By least-squares linear regression according to Eq. (2) using the average intercept (4.3×10^{-4} *M*), $r^2 > 0.95$.
[c] Concentration ranges employed in phase solubility analyses.

Solubilization Using Ligand Pairs. Although the ligands studied were considered to be relatively nontoxic, the weak solubility enhancing property and the considerable amount of ligand needed make their use as solubility enhancers impractical (4). One approach was to develop a multiligand system. By using more than one ligand, it was assumed that the ligands selected could increase the substrate solubility in an additive manner. Thus, individual ligand concentrations could be reduced to more practical levels. The simplest form of a multiligand system is the combination of two ligands in a ligand pair. In this particular study, the possibility of using ligand pairs as solubility enhancers was explored, and the effect of varying the ligand pair composition, i.e., *ligand type, ratio,* and *total concentration,* on solubility enhancement was also evaluated.

Selection of Ligands. The ligands used were categorized according to a classification of complexing agents first proposed by Higuchi *et al.* (9), i.e., complexing agents of similar structure were grouped into the same class. For example, Class A is uncharged xanthines and Class B is benzene derivatives. It was also suggested that ligands of one class tend to form stronger complexes with ligands of the other class rather than with members of their own class (9). In other words, the interclass complexation is usually stronger than the intraclass complexation. In the present study, the ligand pairs were made using ligands from the same class as well as from different classes. Their solubility enhancing properties were compared with the individual ligands at the same concentrations. The differences in the solubility enhancing characteristics by different ligand pairs can be explained by the "inter-, intraclass" complexation theory with respect to the structure of each particular ligand chosen for study.

Effect of Ligand Pairs of Different Class on Solubility Enhancement. Ligand pairs containing niacinamide (Class B) and caffeine (Class A) were prepared at various concentrations. The affect on adenine solubility was determined using phase solubility analysis. Figure 2 shows the increase in adenine solubility produced by this particular ligand pair. In this case, the ligand concentration ratio was kept constant (1:1) and the total ligand concentration varied. Each ligand was also studied individually at the same concentration as in the ligand pair, and the solubility enhancement is also shown for purposes of comparison. The solubility enhancement by ligand pairing was more than in the case of individual niacinamide but less than in the case of individual caffeine, i.e., the addition of niacinamide to caffeine had no additional enhancement upon adenine solubility. The results in this particular case were not encouraging. Since niacinamide is structurally unrelated to caffeine, according to Higuchi theory, such ligand pairs should show a strong interligand complexation effect. Thus, hypothetically, the ligands were inactivated with respect to the substrate by forming a complex with each other. As a result, a less than additive effect on solubility enhancement was observed.

Effect of Ligand Pairs of the Same Class on Solubility Enhancement. Ligand pairs containing two ligands from the same class were also prepared. Here the ligands selected were structurally related, e.g., caffeine–theophylline (Class A), niacinamide–pyridoxine (Class B), and sodium benzoate–sodium salicylate (Class B). The solubility enhancement of these ligand pairs was studied by phase solubility analyses using adenine as the substrate. Figure 3A shows the increase in adenine solubility by the caffeine–theophylline ligands at various molar ratios. It was observed that the increase in solubility by the ligand pairs was about the same as the combined solubility increase by individual caffeine and theophylline at the same concentrations, i.e., an additive solubility enhancement was obtained. Similarly, Class B ligand pairs also showed additive solubility enhancement (Figs. 3B and 3C). Since the ligands in these pairs were structurally similar, a weak interligand complexation was anticipated. Ligands were thought to form complexes with adenine in a parallel manner, which resulted in additive solubility enhancement.

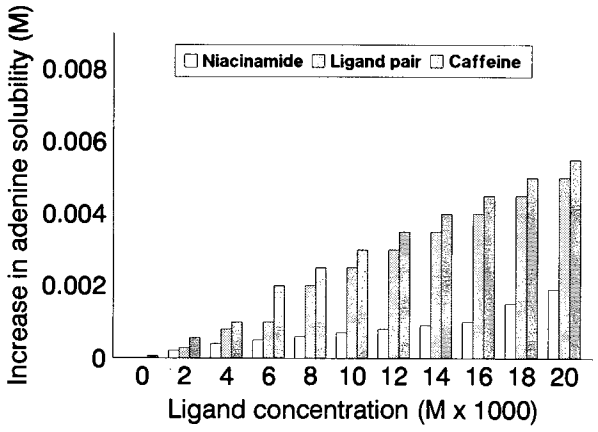

Fig. 2. Effect of caffeine–niacinamide ligand pair on adenine solubility in pH 7 buffer at 25°C. Ligand concentration ratio = 1:1.

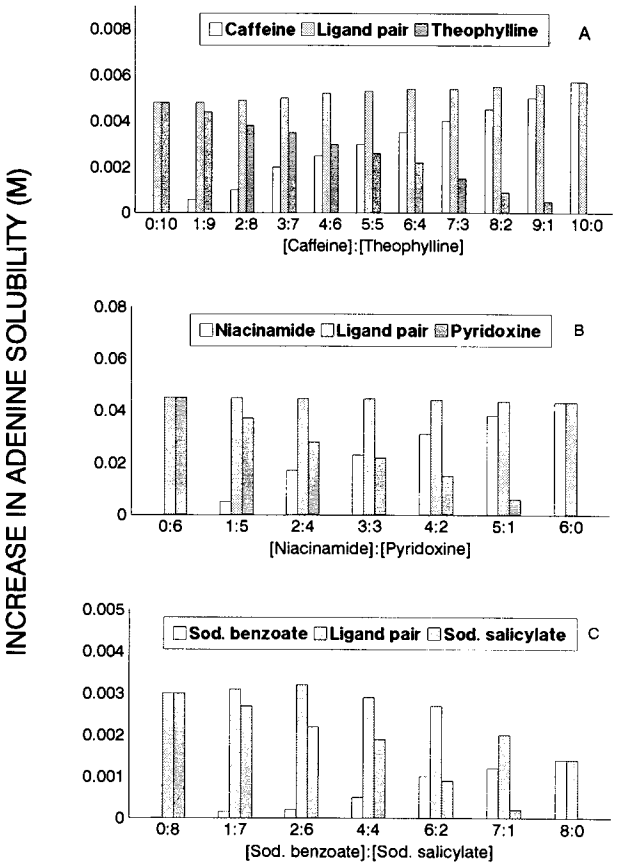

Fig. 3. Effect of ligand pairs of the same class on adenine solubility in pH 7 buffer and at 25°C. Total ligand concentration: 0.02 *M* for the caffeine–theophylline ligand pair (A), 0.1 *M* for the niacinamide–pyridoxine and sodium benzoate–sodium salicylate ligand pairs (B, C).

Effect of Ligand Ratio on Solubility. The effect of the ligand molar ratio in ligand pairs of the same class on solubility was examined. For example, as shown in Fig. 3A, when the total concentration of ligand pairs ([caffeine] + [theophylline]) was kept constant, the molar ratio varied as [caffeine]:[theophylline] = 10:0, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10. It is evident that the extent of additive solubility enhancement obtained at all molar ratios studied was similar. The ligand ratios, therefore, had little effect on the solubility enhancement by this particular ligand pair system. Class B ligand pairs (niacinamide–pyridoxine, sodium benzoate–sodium salicylate) also showed similar results.

Effect of Total Ligand Concentration on Solubility. Class B ligand pairs (niacinamide–pyridoxine) were used to study the effect of total ligand concentration on solubility enhancement. Such ligand pairs were prepared by varying the total ligand concentration ([niacinamide] + [pyridoxine]) while keeping the ligand molar ratio constant. Figure 4 shows that, initially, the increase in adenine solubility was linear with the increase in total ligand concentration, then the rate started decreasing, and eventually the solubility reached a plateau. Thus, increasing the total ligand concentration enhanced solubility only up to a limiting concentration. Once that limiting concentration was reached, further increases in total ligand pair concentration had no effect on

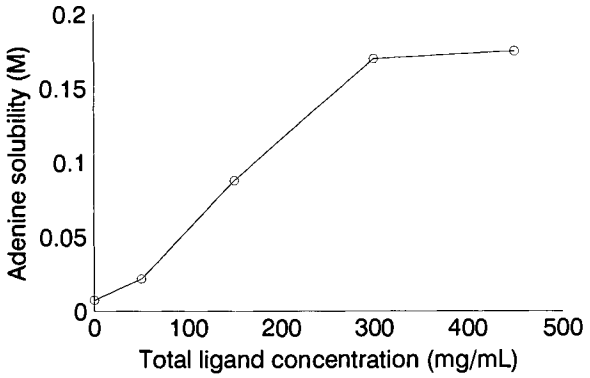

Fig. 4. Effect of total ligand concentration on adenine solubility. Ligand concentration ratio: [niacinamide]/[pyridoxine] = 3.75/1.

adenine solubility (Fig. 4). To establish the usefulness of the ligand pair as solubility enhancers, acyclovir and triamterene, two water-insoluble drugs with heteroaromatic structures related to adenine were also selected as substrates. Using niacinamide and pyridoxine as a ligand pair, at concentrations of 301 and 330 mg/mL, respectively, 19- and 129-fold increases in solubility were observed for acyclovir and triamterene, respectively. Such an increase in solubility was not possible if niacinamide or pyridoxine was used alone. In the case of acyclovir and triamterene, the dramatic increase in aqueous solubility by ligand pairing suggested a synergistic effect. Such synergism was also evident in the increase in adenine solubility reported in the presence of the weak Class B salicylate:benzoate ligand pairs (Fig. 3C), where the increase in adenine solubility in the presence of the ligand pair was greater than the sum of the solubility increase in the salicylate and benzoate interactions separately.

In conclusion, a ligand pair consisting of ligands from the same class (either Class A or Class B) showed additive solubility enhancement with respect to the water-insoluble base, nucleoside, and other structurally related drugs. Using such ligand pairs, it is possible to obtain much greater solubility enhancement.

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