International Journal of Pharmaceutics, 63 (1990) 167–172
Elsevier

IJP 02125

Aqueous solubility properties of a dibasic peptide-like compound

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(Received 15 September 1989)
(Modified version received 1 February 1990)
(Accepted 2 March 1990)

Key words: Solubility; Dibasic compounds; Renin inhibitors; Peptides

Summary

The effects of pH and chloride ion concentration on the aqueous solubility of a dibasic peptide-like compound (A-64662) were examined. The solubility-pH profile agreed well with the theoretical profile for a compound with two basic groups. Above pH 8, the solubility was limited by the intrinsic solubility of the basic form of the compound. Between pH 5 and 8, the solubility was found to be determined by the intrinsic solubility of a monohydrochloride salt and was highly dependent on the chloride ion concentration. Due to the presence of a second ionizable group, the solubility increased significantly below pH 5. Regression analyses of the data gave estimates of 6.7 and $2.5–3.5 \times 10^{-5}$ for pK_1 and the solubility product for the monohydrochloride salt (K_{sp1}), respectively. It was possible to derive equations to estimate the solubility of A-64662 in aqueous media of varying pH and chloride ion concentration. This study also provided results that will be helpful in understanding the solubility properties of other peptides that have multiple ionizable groups.

Introduction

Previous work has been reported on the solubility properties of carboxylic acids (Chowhan, 1978; Anderson and Conradi, 1985) and zwitterionic (Streng and Tan, 1985), monobasic (Kramer and Flynn, 1972; Bogardus and Blackwood, 1979) and tribasic (Bogardus and Palepu, 1979) nonpeptide compounds. The present work addresses the solubility properties of a dibasic peptide-like compound. A-64662 is a renin inhibitor with two ionizable functional groups; one is the imidazole group of a histidine residue and the other is a primary amine on the *N*-terminal dimethyl-β-alanine moiety. The ionization characteristics of these two functionalities and their potential for salt formation are important in determining the aqueous solubility of the compound under various conditions. A detailed knowledge of the solubility properties of A-64662 (Fig. 1) was required for formulation of intravenous solutions. Therefore, a careful examination of the solubility data in light of solubility theory was necessary to understand the solubility-pH relationship. Accordingly, a study of the effects of pH and chloride ion concentration on the solubility of A-64662 was undertaken.

Methodology

Solubility determinations were carried out by making a slurry of excess A-64662 in the ap-

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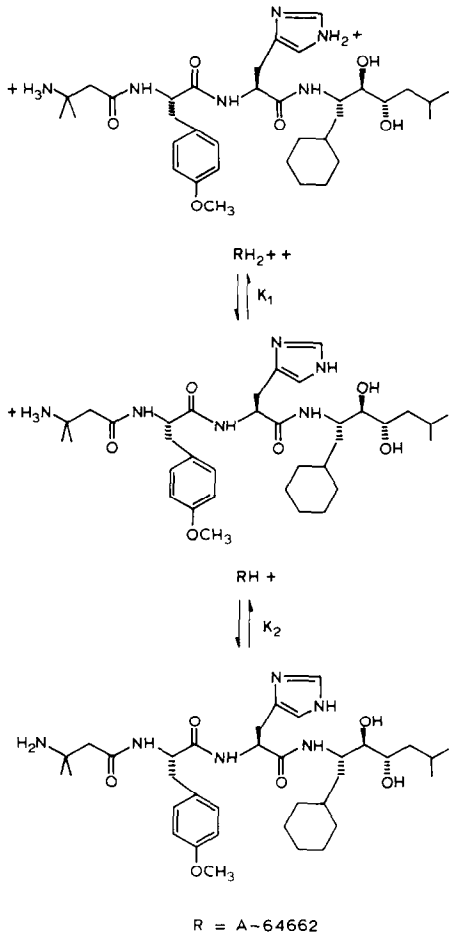

Fig. 1. Ionization scheme for A-64662.

propriate media (distilled water or sodium chloride solution). The pH of the slurry was adjusted to the appropriate value with glacial acetic acid, 0.1 N HCl or 0.1 N NaOH. At each pH value a 2–3 ml sample of the slurry was removed and placed in a small vial. The samples were then allowed to equilibrate by shaking at room temperature for 1–5 days. After equilibration the samples were filtered through 0.45 μm filters. Preliminary experiments showed adsorption of compound to the filters was insignificant. The equilibrium pH used for calculations was measured after filtration.

The monohydrochloride salt of A-64662 was prepared by adding one equivalent of HCl to a solution of A-64662 in distilled water, evaporating the solvent, and isolating the remaining solid. For chloride ion quantitation, approx. 10-mg samples of this solid were accurately weighed and dissolved in 10 ml of water containing 4 drops of glacial acetic acid to facilitate solubility. The chloride ion concentrations of the resulting solutions were determined with a chloride ion-specific electrode (Orion, Model 96-17B).

A-64662 was quantitated by HPLC and chloride ion concentration was determined as above. The HPLC system consisted of a Spectra-Physics SP8800 pump and SP4100 integrator, Waters WISP 710B auto-injector, Regis Little Champ® column, and Kratos Spectroflow 773 detector with detection at 205 nm. The mobile phase was 0.01 M tetramethylammonium perchlorate in 0.1% trifluoroacetic acid, acetonitrile, and methanol at a volume ratio of 57 : 38 : 5. The flow rate was 1.0 ml/min and the injection volume was 25 μl. Under these conditions, A-64662 eluted at 6–7 minutes.

Theory

The ionization scheme of A-64662 is shown in Fig. 1. The ionization constants, K_1 and K_2, are described by Eqns 1 and 2:

$$K_1 = [RH^+] \cdot \{H^+\} / [RH_2^{2+}] \quad (1)$$

$$K_2 = [R] \cdot \{H^+\} / [RH^+] \quad (2)$$

The total solubility, S_t, is the sum of the concentrations of each species in solution (Eqn 3):

$$S_t = [RH_2^{2+}] + [RH^+] + [R] \quad (3)$$

Depending on the conditions, S_t will be limited by the intrinsic solubility of the unionized form or a salt of one of the ionized species. The three possible limiting cases are described below. Activity coefficients were not included in the development of the equations.

Case 1

If the solid in equilibrium with the solution is R, the concentration of R is fixed at its saturation solubility (S_0) and Eqn 4 is obtained:

$$S_t = [RH_2^{2+}] + [RH^+] + S_0 \quad (4)$$

Rearrangement of Eqns 1 and 2 with substitution into Eqn 4 gives:

$$S_t = S_0 \cdot \left(1 + \{H^+\}/K_2 + \{H^+\}^2/K_1 \cdot K_2\right) \quad (5)$$

Case 2

If the solid in equilibrium with the solution is a salt of the singly ionized species, the concentration of RH^+ is fixed at the saturation solubility of the salt (S_{HX}) and Eqn 6 is obtained:

$$S_t = \left[RH_2^{2+}\right] + S_{HX} + [R] \quad (6)$$

Rearrangement of Eqns 1 and 2 with substitution into Eqn 6 gives:

$$S_t = S_{HX} \cdot (1 + \{H^+\}/K_1 + K_2/\{H^+\}) \quad (7)$$

The solubility of the salt (S_{HX}) will be dependent upon the counterion concentration, $[X^-]$, through the solubility product, K_{sp1} (Eqn 8)

$$RHX_{(s)} \overset{K_{sp1}}{\rightleftharpoons} RH^+ + X^-$$

$$K_{sp1} = [RH^+] \cdot [X^-] \quad (8)$$

Rearrangement of Eqn 8 with substitution into Eqn 7 gives:

$$S_t = \left(K_{sp1}/[X^-]\right) \cdot (1 + \{H^+\}/K_1 + K_2/\{H^+\}) \quad (9)$$

Case 3

If the solid in equilibrium with the solution is a salt of the doubly ionized species, the concentration of RH_2^{2+} is fixed at the saturation solubility of the salt (S_{2HX}) and Eqn 10 is obtained:

$$S_t = S_{2HX} + [RH^+] + [R] \quad (10)$$

Rearrangement of Eqns 1 and 2 with substitution into Eqn 10 gives:

$$S_t = S_{2HX} \cdot \left(1 + K_1/\{H^+\} + K_1 \cdot K_2/\{H^+\}^2\right) \quad (11)$$

S_{2HX} will be dependent upon $[X^-]$ through the solubility product, K_{sp2} as shown in Eqn 12:

$$RH_2X_{2(s)} \overset{K_{sp2}}{\rightleftharpoons} RH_2^{2+} + 2X^-$$

$$K_{sp2} = \left[RH_2^{2+}\right] \cdot [X^-]^2 \quad (12)$$

Rearrangement of Eqn 12 with substitution into Eqn 11 gives:

$$S_t = \left(K_{sp2}/[X^-]^2\right) \cdot (1 + K_1/\{H^+\} + K_1 \cdot K_2/\{H^+\}^2) \quad (13)$$

Results and Discussion

A theoretical solubility-pH profile for a dibasic compound that forms mono- and divalent salts is shown in Fig. 2. The structure of the profile is determined by Eqns 5, 7 and 11. Values for S_0, S_{HX} and S_{2HX}, of 0.5, 5 and 50, respectively, were arbitrarily chosen for construction of the graph. The values used for pK_1 and pK_2 were 6.0 and 9.3, which had been determined (Oheim, personal

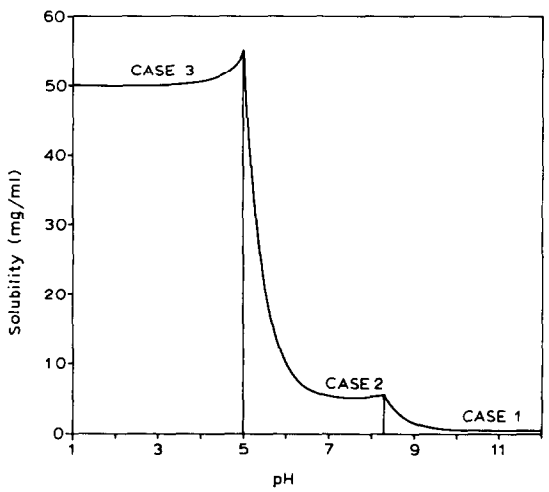

Fig. 2. Theoretical solubility-pH profile for a dibasic compound that forms mono- and divalent salts. Lines are calculated according to Eqns 5, 7 and 11. See text for discussion of cases 1–3.

communication) for A-64662 by potentiometric titration. At higher pH values the solubility is determined by S_0 and $\{H^+\}$ as in Eqn 5 (case 1). In the pH range 5–9 the solubility is determined by S_{HX}, and $\{H^+\}$ as in Eqn 7 (case 2). Below pH 5 the solubility is determined by S_{2HX}, and $\{H^+\}$ as in Eqn 11 (case 3). The situation depicted in Fig. 2 is simplified in that common ion effects accounted for by introducing solubility product terms are not considered. S_{HX} and S_{2HX} can be affected by $[X^-]$ through common ion effects, as shown in Eqns 8 and 12.

A typical A-64662 solubility-pH profile where pH was adjusted with HCl is shown in Fig. 3. Above pH 7 the solubility is relatively low (< 3 mg/ml) and then increases gradually as the pH decreases below 7. The data resemble a profile that might be expected for a monobasic compound with very high salt solubility. However, careful inspection of the data and comparison with theoretical curves demonstrated that this was not the case. The data are plotted in Fig. 4 with lines which represent theoretical solubility curves obtained from Eqns 5 and 7, using $S_0 = 0.20$, $S_{HX} = 3.0$, $pK_1 = 6.0$ and $pK_2 = 9.3$. Values for S_0 and S_{HX} were obtained from preliminary experiments, and pK_a values were from Oheim (personal communication). Examination of the theoretical curves suggests that the observed profile is

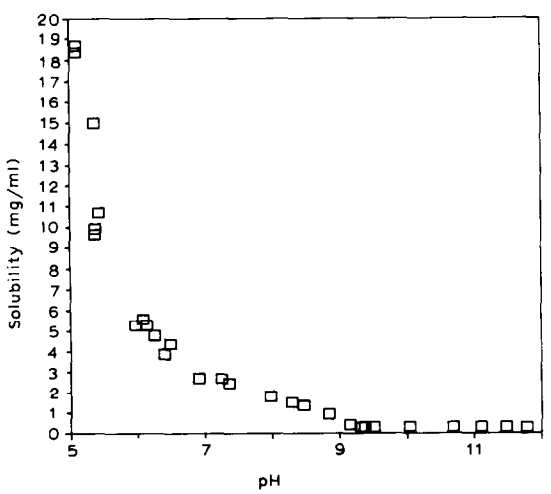

Fig. 3. Solubility-pH profile for A-64662. pH adjustment was made with 0.1 N HCl.

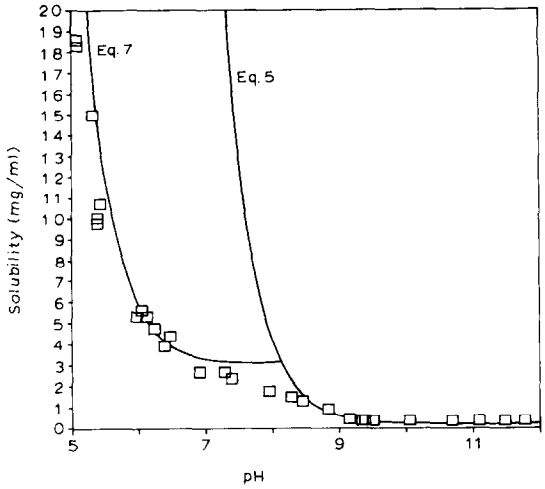

Fig. 4. Solubility-pH profile for A-64662 compared to theoretical equations. The solid lines are calculated according to Eqns 5 and 7 with $S_0 = 0.20$ mg/ml, $S_{HX} = 3.0$ mg/ml, $pK_1 = 6.0$ and $pK_2 = 9.3$.

composed of two regions: $pH > 8.5$ where the solubility of the unionized form is limiting (Eqn 5); and $pH < 8.5$ where the solubility of a monohydrochloride salt is limiting (Eqn 7). An interesting observation is that the solubility does not reach a plateau at low pH values as shown in Fig. 2; it continues to increase, presumably due to the ionization of the histidine side chain and a very high value for the solubility product of a dihydrochloride salt.

Since the results suggested that a monohydrochloride salt was formed, the effect of added chloride ion on the solubility-pH profile was examined. Slurries of A-64662 in water or 0.15 N NaCl were titrated with glacial acetic acid. Earlier work (Pyter and Leveque, personal communication) demonstrated that acetate salts had extremely high solubility products. Fig. 5 shows that in the presence of 0.15 N $[Cl^-]$ at $pH < 9$, the total solubility is dramatically depressed. The lines represent theoretical solubility curves obtained from Eqns 5 and 7, using $S_0 = 0.20$, $S_{HCl} = 3.0$, $pK_1 = 6.0$ and $pK_2 = 9.3$. The reduced solubility provided further evidence that a monohydrochloride salt forms in the presence of chloride ion, and thus, solubility is likely to be affected through a common ion effect. The solubility curve in the

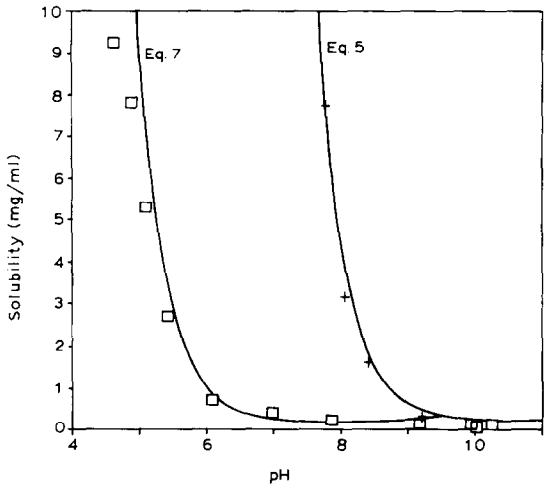

Fig. 5. Solubility-pH profile of A-64662 in 0.15 N NaCl (□) and distilled water (+). pH was adjusted with glacial acetic acid. The solid lines are calculated from Eqns 5 and 7 with $S_0 = 0.20$ mg/ml, $S_{HX} = 3.0$ mg/ml, $pK_1 = 6.0$ and $pK_2 = 9.3$.

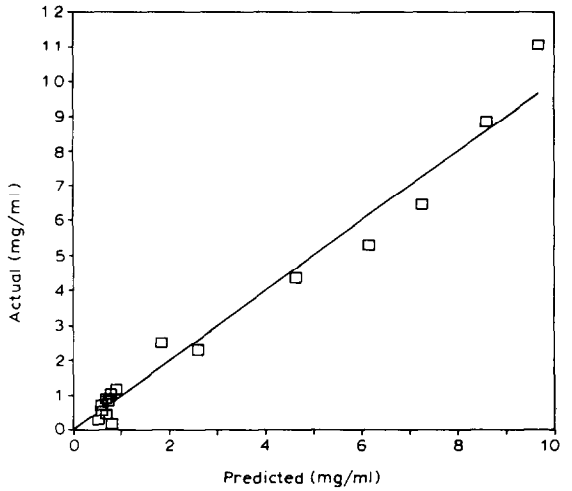

Fig. 6. Plot of actual vs predicted values obtained from nonlinear regression analysis using Eqn 10. The solid line is the line of unity.

absence of chloride ion was used to estimate values of K_2 and S_0 using nonlinear regression analysis. The values obtained for K_2 and S_0 were 6.4×10^{-10} and 0.20, respectively.

It was attempted to confirm the formation of a monohydrochloride salt by isolation as described in Materials and Methods. The chloride ion concentrations of solutions of the monohydrochloride salt were determined with a chloride ion-specific electrode and compared to the theoretical chloride ion concentrations based on the weight of sample dissolved. The average ($n = 2$) observed chloride ion concentrations were 95% of the theoretical values, indicating that the solid was a monohydrochloride salt. Also, the melting point of the solid was 174–178°C, while that of A-64662 is 190.3–191°C (Oheim, personal communication).

To explore the common ion effect, solubility was determined over the pH range 5–9 in the presence of 0.01–0.10 N [Cl^-]. The pH, total solubility and [Cl^-] of each sample were measured and the experimental data were fitted to Eqn 9 by nonlinear regression analysis (Statgraphics, 1987). A good fit of the data to the model was observed ($r^2 = 0.974$). Fig. 6 shows a plot of the actual vs predicted values obtained from the regression analysis. Estimates for K_{spl} and K_1 of 2.50×10^{-5} and 1.70×10^{-7}, respectively, were obtained.

According to Eqn 9, it should be possible to estimate K_{spl} by determining the total solubility at fixed pH and varying [Cl^-]. A plot of S_t vs $1/[Cl^-]$ should give a straight line with an intercept of 0 and a slope of K_{spl} $(1 + \{H^+\}/K_1 + K_2/\{H^+\})$. Knowing the values of K_1, K_2 and $\{H^+\}$ allows calculation of K_{spl} from the slope.

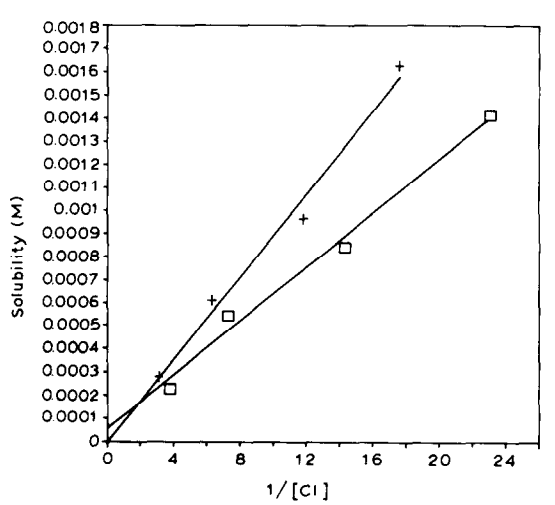

Fig. 7. Plots of solubility vs $1/[Cl^-]$ at pH 6.5 (+) and pH 7.0 (□).

Fig. 7 shows plots of S_t vs $1/[Cl^-]$ at pH 6.5 and 7.0. Good fits of the data to straight lines were observed ($r^2 > 0.98$). Use of the previously obtained values of 6.77 and 9.3 for pK_1 and pK_2, respectively, yielded $K_{sp1} = 3.13 \times 10^{-5}$ at pH 6.5 and 3.66×10^{-5} at pH 7.0. Considering the errors expected to be associated with these analyses, these values are in good agreement with each other and with $K_{sp1} = 2.50 \times 10^{-5}$ as determined by the nonlinear regression analysis. Using the estimated parameters, the predicted solubility at potential formulation and physiological values of pH and chloride ion concentration can be calculated according to Eqns 5 and 9.

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