Chemical Physics Letters 610–611 (2014) 62–69

Contents lists available at ScienceDirect

Chemical Physics Letters

journal homepage: www.elsevier.com/locate/cplett

The species- and site-specific acid–base properties of penicillamine and its homodisulfide

Arash Mirzahosseini[a,b], András Szilvay[a,b], Béla Noszál[a,b,*]

[a] Department of Pharmaceutical Chemistry, Semmelweis University, Budapest, Hungary
[b] Research Group of Drugs of Abuse and Doping Agents, Hungarian Academy of Sciences, Hungary

ARTICLE INFO

Article history:
Received 18 June 2014
In final form 10 July 2014
Available online 17 July 2014

ABSTRACT

Penicillamine, penicillamine disulfide and 4 related compounds were studied by ^{1}H NMR-pH titrations and case-tailored evaluation methods. The resulting acid–base properties are quantified in terms of 14 macroscopic and 28 microscopic protonation constants and the concomitant 7 interactivity parameters. The species- and site-specific basicities are interpreted by means of inductive and shielding effects through various intra- and intermolecular comparisons. The thiolate basicities determined this way are key parameters and exclusive means for the prediction of thiolate oxidizabilities and chelate forming properties in order to understand and influence chelation therapy and oxidative stress at the molecular level.

1. Introduction

(2*S*)-2-Amino-3-methyl-3-sulfanylbutanoic acid, or penicillamine (also known as 3-mercapto-D-valine, 3,3-dimethyl-D-cysteine) is a nonproteinogenic amino acid containing a thiol group. The three polar functional groups in penicillamine undergo characteristic chemical reactions and differ in their ability to participate in various chemical and biochemical reactions [1]. Penicillamine was at first solely of interest as a key substance in the structural elucidation of penicillins and as a central building block in their total synthesis [2]. Notwithstanding that its L-isomer is toxic; D-penicillamine is widely used in medicine to treat Wilson's disease [3], heavy metal poisoning [4], cystinuria [5] and rheumatoid arthritis [6]. The thiol group as a typical weak acid occurs mainly in neutral form in physiologic conditions, while the ammonium and carboxylate groups deliver a zwitterionic structure. However, it is the thiolate form that is directly engaged in thiol–disulfide redox equilibria [7]. It has also been reported that thiolate basicities are in correlation with the half-cell redox potentials [8]. Since most biomolecules bear multiple protonation sites which protonate in an overlapping fashion, site-specific characterization of acid–base equilibria helps untangle the interactions between the basic moieties. Such thiolate basicities are indirect indicators of the thiolate oxidizability in the particular protonation stage of the neighboring groups [9]. Penicillamine primarily metabolizes *via* its disulfide form, and the pharmacological and therapeutic actions are largely explained by its ability to take part in thiol–disulfide exchange reactions. For example the formation of the mixed disulfide from penicillamine and cysteine is decisive for the treatment of cystinuria as penicillamine disulfide and penicillamine-cysteine disulfide are much more soluble than cystine and are thus eliminated [2,10].

Although the acid–base properties of penicillamine have been reported in a few papers [11–15], no data appeared on the site-specific protonation constants of D-penicillamine and D-penicillamine disulfide. Here we report the determination of all the site-specific basicities for D-penicillamine, and its homodisulfide using ^{1}H NMR-pH titrations on the parent molecules and 4 synthesized auxiliary compounds. This is the first complete microspeciation of these molecules, providing *sine qua non* constituents for a comprehensive species- and site-specific characterization of the complex, codependent acid–base and thiol–disulfide equilibrium system, that is of crucial importance in maintaining the intracellular redox homeostasis.

2. Materials and methods

2.1. Materials

All chemicals (including D-penicillamine) were purchased from Sigma, and were used without further purification.

* Corresponding author at: Department of Pharmaceutical Chemistry, Semmelweis University, H-1092 Budapest, Hőgyes E. u. 9, Hungary.
E-mail address: noszal.bela@pharma.semmelweis-univ.hu (B. Noszál).

http://dx.doi.org/10.1016/j.cplett.2014.07.026
0009-2614/

2.2. NMR spectroscopy measurements

NMR spectra were recorded on a Varian 600 MHz spectrometer at 25 °C. The solvent in every case was an aqueous solution with H_2O:D_2O, 95:5, v/v (0.15 mol/l ionic strength), using DSS as the reference compound. The sample volume was 600 μl. In proton NMR experiments pH values were determined by internal indicator molecules optimized for NMR [16,17], and the water resonance was diminished by double pulsed field gradient spin echo (nt = 16, np = 64 000, acquisition time = 3.33 s, relaxation delay = 1.5 s).

2.3. Data analysis

For the analysis of NMR titration curves of proton chemical shifts *versus* pH, the software Origin Pro 8 (OriginLab Corp., Northampton, MA, USA) was used. In all regression analyses the non-linear curve fitting option was used with the following function [18]:

$$\delta_{obs}(pH) = \frac{\delta_L + \sum_{i=1}^{n} \delta_{H_iL} \times 10^{\log\beta_i - i\times pH}}{1 + \sum_{i=1}^{n} 10^{\log\beta_i - i\times pH}} \quad (1)$$

where δ_L is the chemical shift of the unprotonated ligand (L), δ_{H_iL} values stand for the chemical shifts of successively protonated ligands, where n is the maximum number of protons that can bind to L, and β is the cumulative protonation macroconstant, as exemplified in Eq. (1). The standard deviations of log β values from the regression analyses were used to calculate the Gaussian propagation of uncertainty to the other equilibrium constants derived in Section 3.

2.4. TOF MS measurements

The exact mass of the synthesized and isolated compounds was determined with an Agilent 6230 time-of-flight mass spectrometer equipped with a JetStream electrospray ion source in positive ion mode. JetStream parameters: drying gas (N_2) flow and temperature: 10.0 l/min and 325 °C; nebulizer gas (N_2) pressure: 10 psi; capillary voltage: 4000 V; sheath gas flow and temperature: 325 °C and 7.5 l/min. TOF MS parameters: fragmentor voltage: 170 V; skimmer potential: 170 V; OCT 1 RF Vpp: 750 V. Samples were introduced (0.1–0.3 μl) by the Agilent 1260 Infinity HPLC system (flow rate = 0.5 ml/min, 70% methanol–water mixture 0.1% formic acid). Reference masses of *m/z* 121.050873 and 922.009798 were used to calibrate the mass axis during analysis. Mass spectra were acquired over the *m/z* range 100–1000 at an acquisition rate of 250 ms/spectrum and processed using Agilent MassHunter B.02.00 software.

2.5. Synthetic protocols

D-*Penicillamine methyl ester hydrochloride* (**2**) was synthesized by dissolving 0.10 g (0.67 mmol) D-penicillamine (**1**) in 5 ml methanol and bubbling dry HCl gas into the solution for 15 min at room temperature [19]. After stirring overnight at room temperature the reaction mixture was evaporated *in vacuo* to yield a white solid (0.13 g, 98%). Mp: 187–188 °C; ^{1}H NMR (600 MHz, H_2O:D_2O, 95:5, v/v) δ (ppm) 1.42 (3H, s, βCH_3), 1.45 (3H, s, βCH_3), 3.68 (1H, s, αH), 3.79 (3H, s, OCH_3); HRMS *m/z* $[M+H]^+$ Calc 164.0745 Found 164.0724.

S-Methyl D-*penicillamine* (**3**) was synthesized from 0.10 g (0.67 mmol) D-penicillamine (**1**) using 54 μl (1.3 equiv.) methyl iodide and 0.02 g (1.3 equiv.) sodium hydride in 5 ml methanol under N_2 atmosphere [20]. After stirring overnight at room temperature the reaction mixture was evaporated *in vacuo*. The residual oil was purified by column chromatography on silica gel (ethyl acetate–hexane, 1:5, v/v) to afford compound **3** as a white solid (0.11 g, 99%). Mp: 253–260 °C. ^{1}H NMR (600 MHz, H_2O:D_2O, 95:5, v/v) δ (ppm) 1.31 (3H, s, βCH_3), 1.52 (3H, s, βCH_3), 2.07 (3H, s, SCH_3), 3.67 (1H, s, αH); HRMS *m/z* $[M+H]^+$ Calc 164.0745 Found 164.0687.

S-Methyl D-*penicillamine methyl ester hydrochloride* (**4**) was synthesized in a similar fashion to compound **2** from 0.05 g (0.31 mmol) *S*-methyl D-penicillamine (**3**) to yield 0.06 g (98%) yellow oil. ^{1}H NMR (600 MHz, H_2O:D_2O, 95:5, v/v) δ (ppm) 1.48 (3H, s, βCH_3), 1.56 (3H, s, βCH_3), 1.92 (3H, s, SCH_3), 3.71 (3H, s, OCH_3), 3.88 (1H, s, αH); HRMS *m/z* $[M+H]^+$ Calc 178.0902 Found 178.0930.

D-*Penicillamine disulfide* (**5**) was synthesized *in situ* by dissolving 0.10 g (0.67 mmol) D-penicillamine (**1**) into a 5% H_2O_2 aqueous solution and adjusting the pH to 8. After stirring overnight the aqueous solution was used directly for NMR measurements. ^{1}H NMR (600 MHz, H_2O:D_2O, 95:5, v/v) δ (ppm) 1.44 (3H, s, βCH_3), 1.55 (3H, s, βCH_3), 4.01 (1H, s, αH); HRMS *m/z* $[M+H]^+$ Calc 297.0943 Found 297.1017.

D-*Penicillamine disulfide dimethyl ester* (***6***) was synthesized in a similar fashion to compound **5** from 0.05 g (0.25 mmol) D-penicillamine methyl ester hydrochloride (**2**). ^{1}H NMR (600 MHz, H_2O:D_2O, 95:5, v/v) δ (ppm) 1.50 (3H, s, βCH_3), 1.57 (3H, s, βCH_3), 3.66 (3H, s, OCH_3), 4.19 (1H, s, αH); HRMS *m/z* $[M+H]^+$ Calc 325.1256 Found 325.1296.

3. Results

Figure 1 shows the formulae of the molecules studied. Figure 2 represents the microscopic protonation schemes of penicillamine (A) and penicillamine disulfide (B). Macroequilibria (top lines) indicate the stoichiometry of the successively protonated ligand and the stepwise macroscopic protonation constants. In the microspeciation schemes the 8 and 16 microspecies with their one-letter symbols (a, b, . . ., h for penicillamine, and *a*, *b*, . . ., *p* [in italics] for penicillamine disulfide), and the 12 and 32 microscopic protonation constants are depicted (k^O, $k_O{}^N$, $k_{ON}{}^S$, . . .), for sake of consistency penicillamine disulfide protonation constants are depicted in italics. The superscript of k indicates the protonating group while the subscript (if any) shows the site(s) already protonated. N, S and O symbolize the amino, thiolate and carboxylate sites, respectively. Some examples of macro- and microconstants of penicillamine are:

$$K_1 = \frac{[HL^-]}{[L^{2-}][H^+]} \quad K_2 = \frac{[H_2L]}{[HL^-][H^+]} \quad K_1K_2 = \beta_2 = \frac{[H_2L]}{[L^{2-}][H^+]^2} \quad (2)$$

$$k^O = \frac{[d]}{[a][H^+]} \quad k_O^N = \frac{[f]}{[d][H^+]} \quad k_{ON}^S = \frac{[h]}{[f][H^+]} \quad (3)$$

Concentrations of the various macrospecies comprise the sum of the concentration of those microspecies that contain the same number of protons. For example:

$$[HL^-] = [b] + [c] + [d] \quad (4)$$

$$[H_2L] = [e] + [f] + [g] \quad (5)$$

The following equations show the relationships between the micro- and macroconstants of penicillamine [21]:

$$K_1 = k^N + k^S + k^O \quad (6)$$

$$K_1K_2 = k^Nk_N^S + k^Nk_N^O + k^Sk_S^O = k^Sk_S^N + k^Ok_O^N + k^Ok_O^S = \ldots \quad (7)$$

$$K_1K_2K_3 = k^Nk_N^Sk_{SN}^O = k^Sk_S^Nk_{SN}^O = \ldots \quad (8)$$

Eqs. (7) and (8) can be written in 2 and 6 different, equivalent ways depending on the path of protonation. To characterize all of the microscopic bacisities, the introduction and utilization of auxiliary compounds are necessary.