Journal of Inorganic Biochemistry 105 (2011) 75–83

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

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

Effect of fluorine position on the coordinating ability of fluorosalicylic acids — An experimental study complemented with computations

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ARTICLE INFO

Article history:
Received 14 April 2010
Received in revised form 23 September 2010
Accepted 24 September 2010
Available online 20 November 2010

Keywords:
Electron spin resonance
Copper(II) complexes
Fluorosalicylic acids
Speciation
Coordination modes

ABSTRACT

The complexation of 3-, 4-, and 6-fluorosalicylic acids (HL) with copper(II) was investigated in aqueous solution by pH-potentiometry combined with UV–visible spectrophotometry, and in 50 v/v % water–methanol mixture by the two-dimensional ESR simulation method. Both methods showed the formation of $[CuLH_{-1}]$ and $[CuL_2H_{-2}]^{2-}$ of high stabilities, and, at low excess of ligand, the ESR-silent mixed hydroxido complex $[Cu_2L_2H_{-3}]^{-}$. Further species were also identified by the two-dimensional ESR simulation method: $[CuL]^{+}$ in the acidic region, the minor dimer $[Cu_2L_2H_{-2}]$, and the *cis* and the *trans* isomers for $[CuL_2H_{-2}]^{2-}$. The position of the fluorine atom in the aromatic ring had significant effect on the coordination abilities of the ligands, in good correlation with their reported biological activities. It was 3-fluorosalicylic acid, which formed the most stable complexes $[CuLH_{-1}]$ and $[CuL_2H_{-2}]^{2-}$, while the mononuclear complexes with 6-fluorosalicylic acid were found to be the least stable. For the other ligands (including 5-fluorosalicylic acid studied recently), complexes of medium stabilities were formed. For the interpretation of these findings, *ab initio* and semi-empirical quantum chemical calculations were carried out for the ligand molecules, isolated and surrounded by water molecules, respectively.

1. Introduction

Salicylic acid and its derivatives (*e.g.* aspirin, *i.e.* acetylsalicylic acid) are widely used in human medicine. Many copper(II) complexes of salicylic acid derivatives (*e.g.* the complex of 3,5-diisopropylsalicylic acid) have anti-inflammatory, anti-ulcer, antidiabetic, antimutagenic, analgesic, antineoplastic, anticonvulsant, and radioprotective activity [1–4]. The active form of aspirin was also suggested to be a copper complex, formed *in vivo* [5]. It has been established that the metal complexes are generally more active than the drugs themselves [1,6–8]. Moreover, there are inactive ligands that are activated by copper(II) [8]. Synthesis and characterization of solid copper(II)–salicylato complexes have been carried out in our laboratories as well [9–16].

Since the first synthesis of 5-fluorouracil, an antineoplastic agent [17], fluorine substitution has become a commonly used method in drug development, and by now 20–25% of drugs contain at least one fluorine atom [18]. In many cases, fluorine improves bioavailability and metabolic stability of the drug, and affects protein–ligand interactions [18,19]. Owing to its strong electronegativity, it may alter the acid–base character of the proximal functional groups [20,21], may have impact on the lipophilicity [22]; may induce conformational changes.

Fluorosalicylic acids (FSAs) induce systemic acquired resistance [23,24] in tobacco plants, similarly to salicylic acid (as a plant hormone) playing a key role in the pathogen defense in plants [25,26]. An interesting observation is that their biological effect is influenced significantly by the fluorine position on the aromatic ring: 3-FSA is much more effective than the non-substituted compound (the activity doubled), while the efficacy of 6-FSA is much lower [27]. The full mechanism of action is unclear yet, but it is known that salicylates induce the accumulation of pathogenesis-related (PR) proteins, with copper-containing enzymes among them [28–32]. Thus, a number of observations prove the common effect of copper(II) ions and salicylate in the above process [33–36]. In an environment containing both FSAs and copper(II) ion it is likely that the interaction between them results in complex formation to a certain extent. There are iron(III)-coordination chemistry based methods for determination of total salicylic acid content in plants [37], but the speciation of the

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doi:10.1016/j.jinorgbio.2010.09.009

different forms of the complexes may provide deeper insight into biological processes.

The promising results of the biological screening of halogenosalicylato copper(II) complexes [27,38–40] inspired solution studies of complex equilibria between copper(II) and FSAs. As a continuation of our work on copper(II)–5-FSA system [41], a combination of pH-potentiometry, UV–Visible (UV–Vis), NMR and ESR spectroscopies, especially the so-called two-dimensional (2D) ESR simulation method [42] have been applied for the investigation of the equilibrium properties of copper(II) complexes of 3-, 4-, and 6-fluorosalicylic acids in aqueous solution and in methanol/water mixture. The experimental work was complemented with quantum chemical calculations, too. The main aim of our work was to elucidate the effect of fluorine substituent in various positions on the complexing abilities of the ligands.

2. Experimental

2.1. Materials

The ligands 3-, 4- and 6-FSA were all of analytical grade, and were purchased from Aldrich Chemical Co. Their numbering scheme is shown later in Scheme 1. The ligands are symbolized by HL in their neutral form when giving the composition of various complexes. Doubly de-ionized water and freshly distilled methanol were used as solvents. The other reagents used were also of analytical grade and supplied by Aldrich or Sigma. They were used as received.

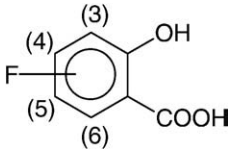
2.2. Combined pH-potentiometric and spectrophotometric titrations

Titrations were carried out with NaOH (Sigma) standard solution ($I = 0.1$ M $NaClO_4$, and $T = 298.0 \pm 0.1$ K, under argon atmosphere) in the 2–11 pH range. Copper(II) perchlorate (Fluka) solutions were standardized by complexometry. The metal-to-ligand ratios were varied between 1:1 and 1:4 with the total metal ion concentrations (T_{Cu}) between 4.5×10^{-4} and 1.8×10^{-3} M. Both the protonation and the complex formation constants were determined from three independent titrations (100–120 data points per titration). An automatic titration setup including a PC controlled Dosimat 665 (Metrohm) autoburette and an Orion 710A precision digital pH-meter was applied. The Metrohm Semimicro pH glass electrode was calibrated *via* the modified Nernst equation [43].

$$E = E_0 + K \cdot \log\left[H^+\right] + J_H \cdot \left[H^+\right] + \frac{J_{OH} \cdot K_w}{[H^+]}$$

where J_H and J_{OH} are fitting parameters in acidic and alkaline media, respectively, for the correction of experimental errors caused mainly by the liquid junction, and the alkaline and acidic errors of the glass electrode; $K_w = 10^{-13.75}$ is the autoprotolysis constant of water [44]. The parameters were calculated by the non-linear least squares method. The protonation equilibria were also studied in 1:1 methanol–water mixture.

Spectrophotometric measurements were carried out with the same solutions as above using an Ocean Optics PC2000 fiber optic dip-in spectrophotometer with 1 cm path length.



Scheme 1. Substituent positions in the fluorosalicylic acids.

The equilibria were characterized by the following general process:

$$pCu^{2+} + qL^- + rH^+ \overset{\beta_{Cu_pL_qH_r^{(2p-q+r)+}}}{\rightleftharpoons} Cu_pL_qH_r^{(2p-q+r)+}$$

$$\beta_{Cu_pL_qH_r^{(2p-q+r)+}} = \frac{\left[Cu_pL_qH_r^{(2p-q+r)+}\right]}{\left[Cu^{2+}\right]^p[L^-]^q[H^+]^r}$$

where Cu^{2+} denotes the metal ion and L^- the non-protonated ligand molecule. The corresponding formation constants ($\beta_{CupLqHr} \equiv \beta_{pqr}$) were calculated using the PSEQUAD computer program [45].

2.3. ESR titrations

Titrations were carried out in an air-conditioned room at 298 ± 0.2 K. The solutions were kept under argon atmosphere, the pH was varied in the 2–11 range at a total (analytical) copper(II) concentration of $T_{Cu} = 0.002$ M and a total ligand concentration of $T_L = 0.003$ M, $T_{Cu} = 0.010$ M and $T_L = 0.015$ M (Cu:L = 1:1.5), $T_{Cu} = 0.0025$ M and $T_L = 0.0125$ M (Cu:L = 1:5), and $T_{Cu} = 0.0008$ M and $T_L = 0.0205$ M (Cu:L = 1:25). The solvent was a 50 v/v % methanol–water mixture, since the solubility of the ligands in water proved to be too low for the ESR measurements. (Apart from pH-potentiometry, the solutions where one or another species is predominant, are also informative, therefore, it was necessary to record ESR spectra at high ligand concentration, too.) 0.2 M KCl was applied as background electrolyte, the pH was adjusted with HCl (0.2 M) and then NaOH (0.2 M) to an accuracy of 0.01 pH unit using a Metrohm 765 Dosimat apparatus equipped with a Metrohm LL combined microelectrode. A Masterflex CL peristaltic pump ensured the circulation of the solution through the capillary tube in the cavity. The ESR spectra were recorded after 2 min of circulation at the chosen pH values using an X-band Bruker EleXsys E500 instrument.

2.4. ESR measurements at 77 K

Samples of 0.15 mL volume, taken at various pH values from the mixtures titrated at 298 K, were frozen in liquid nitrogen. Then, the ESR spectra were recorded at 77 K with the same spectrometer as above.

2.5. NMR measurements

^{1}H NMR spectra of FSAs were recorded in $CDCl_3$ at 298 K with a Varian 600 VNMRS spectrometer at 600 MHz.

2.6. Evaluation of ESR spectra in fluid solution

In the course of 2D analysis, a series of experimental ESR spectra were simulated in the same optimization procedure, fitting the magnetic parameters and formation constants of each ESR-active species simultaneously with the 2D_EPR program [42]. In other words, the intensity was treated as the function of two variables, the field and the concentration (that is why the evaluation method is called "two-dimensional").

Analysis of the spectra was preceded by eliminating the background signal and a numerical field shift in order to obtain the spectra at a common frequency. The ESR spectra of the various species were described by the parameters g_0, the copper hyperfine coupling constant A_0, and the relaxation parameters α, β and γ relating to the line-widths of the copper hyperfine multiplet as $W_{M_I} = \alpha + \beta M_I + \gamma M_I^2$ (M_I is the magnetic quantum number of copper nuclei). Since a natural mixture of copper isotopes was used, the spectra were calculated as the sum of the curves of molecules containing isotope