Microscopic Dissociation Processes of Alaninehydroxamic Acids

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A pH-metric and ^{13}C NMR study has been made of the proton complexes of L-α-alaninehydroxamic acid and β-alaninehydroxamic acid at 25 °C and I = 0.2 mol dm^{-3} and 1.0 mol dm^{-3} (KCl), to determine the macroscopic and microscopic dissocation constants of the ligand. It has been found that the NH_3^+ group is more acidic than the NHOH group for the α-derivative, while the acidity sequence is the opposite for the β-derivative.

Hydroxamic acids play an important role in living systems, as constituents of a number of natural and partly synthetic products, such as antibiotics, growth factors, tumour inhibitors, enzyme inhibitors, antifungal agents, *etc.*[1] In addition, some aminohydroxamic acids have found application in the design of metal chelates, as suitable sources of trace elements in animal nutrition.[2,3] Although many hydroxamate and aminohydroxamate complexes of transition-metal ions have been reported recently,[2,4] there are contradictions concerning the acid–base chemistry of these ligands. In the pH range 5–10, simple aminohydroxamic acids can liberate two protons in overlapping processes, one from the protonated amino group (NH_3^+) and one from the hydroxamic (NHOH) group. Kurzak *et al.*[5,6] and Paniago and Carvalho[7] have concluded that the NH_3^+ group is more acidic than the NHOH group, whereas Brown *et al.*[2,3] and Leporati consider the acidity sequence of the two groups to be the opposite. As the pK values of NHOH groups are similar to those and that of NH_3^+ groups,[9] it is reasonable to assume overlapping dissociation processes for aminohydroxamic acids, which contain both of these acidic groups within the same molecule.

In the present work, detailed pH-metric and ^{13}C NMR studies were carried out to determine the dissociation macro- and micro-constants of L-α-alaninehydroxamic acid (α-AHA) and β-alaninehydroxamic acid (β-AHA), to obtain an exact picture of their acid–base properties.

Experimental

The ligands were prepared *via* the methyl ester of the respective amino acid by the method described in ref. 10. The purity of the ligand and the exact concentrations of the solutions were checked and measured by the Gran method.[11]

The dissociation macroconstants were determined by pH-metric titration of 25 cm^3 samples in the pH range 4–10.5. The ligand concentration was 4×10^{-3}, 6×10^{-3} or 1×10^{-2} mol dm^{-3}. The titrations were performed with KOH solutions of known concentration (*ca.* 0.2 or 1.0 mol dm^{-3}). These measurements were made with a Radiometer PHM 84 instrument with a GK 2322 C combined electrode. The electrode system was calibrated by the method of Irving *et al.*,[12] so that the pH-meter readings could be converted into hydrogen-ion concentrations. In all cases the temperature was 25.0 ± 0.1 °C.

The dissociation microconstants were determined by pH–^{13}C NMR spectrometric titration of 2.5 cm^3 aqueous samples. The ligand concentration was 0.10 mol dm^{-3}. Sodium 3-(trimethylsilyl)propanesulphonate (TSS) served as internal reference for the shift measurements. At each titration point in the same pH range as for the pH-metric titrations, the pH and the chemical shifts of the α and β carbon atoms of the ligands were measured. The spectra were obtained on a Bruker WP 200 SY impulse FT NMR spectrometer.

Both the pH-metric and the pH-spectrometric titrations were carried out at two different ionic strengths, 0.2 and 1.0 mol dm^{-3} (KCl), since in the NMR measurements our usual value, 0.2 mol dm^{-3}, was not sufficient to keep the ionic strength constant in the whole titration range, due to the high ligand concentrations used.

The dissociation macroconstants were calculated from the pH-metric titration curves by means of the PSEQUAD computer program,[13] and the dissociation microconstants from the fractional dissociation curves by means of a non-linear least-squares curve-fitting method, as reported previously.[14,15]

Results and Discussion

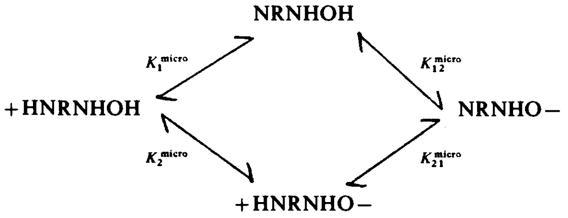
The acid dissociation constants of the ligands are given in the Table. In the pH range studied (4–10.5), the ligands contain two dissociable protons, one each on the NH_3^+ and NHOH groups. The reference compounds α-alanine (α-Ala) and β-alanine (β-Ala) have pK = 9.68 or 10.14, characteristic of the NH_3^+ group,[16] while propionohydroxamic acid has pK = 9.56, characteristic of the NHOH group.[3] The electron-withdrawing character of both the NH_3^+ and the NHOH groups increases the acidity of the other group in comparison with that in the related reference compounds, and thus the lower pK values for α-AHA and β-AHA are in accordance with expectation. These pK values, however, cannot be ascribed unambiguously to either of the acidic groups, because of the possible overlap of their dissociation processes. The dissociation microconstants characteristic of the acidity of the individual groups were determined by selective monitoring of the dissociation of the NH_3^+ group *via* the ^{13}C chemical-shift data relating to the closest carbon atom.

The chemical shifts of the α- and β-carbons of the ligands as a function of pH are shown in Figure 1.

The microconstants can be calculated from the pH-dependence of the chemical-shift data if the dissociation of one of the acidic groups can be followed independently of the dissociation of the other.[14] If the two groups are not separated completely within the molecules so that the changes in chemical shift are affected by the dissociation of both groups, suitable model compounds can be useful.[15] The basic principle of independent monitoring is not valid for the chemical-shift data concerning either the α-carbon or the β-carbon of these molecules, because of the close proximity of the two acidic groups. In an attempt to allow for this effect of the dissociaton of one group on the

Table. Dissociation macroconstants (pK) and microconstants (pK^{micro}) of the ligand[5] at 25 °C.

$$H_2A^+ \overset{K_1}{\rightleftharpoons} HA \overset{K_2}{\rightleftharpoons} A^-$$



	α-AHA		β-AHA	
I/mol dm^{-3} =	0.2 (KCl)	1.0 (KCl)	0.2 (KCl)	1.0 (KCl)
pK_1	7.34 ± 0.01	7.40 ± 0.01	8.32 ± 0.01	8.37 ± 0.01
pK_2	9.16 ± 0.01	9.19 ± 0.01	9.59 ± 0.01	9.63 ± 0.01
K_1^{micro}/K_2^{micro}	2.7 ± 0.1	2.7 ± 0.1	0.42 ± 0.05	0.42 ± 0.05
pK_1^{micro}	7.48 ± 0.02	7.54 ± 0.02	8.85 ± 0.02	8.90 ± 0.02
pK_2^{micro}	7.90 ± 0.03	7.96 ± 0.03	8.47 ± 0.02	8.52 ± 0.02
pK_{12}^{micro}	9.02 ± 10.02	9.05 ± 0.02	9.06 ± 0.02	9.10 ± 0.02
pK_{21}^{micro}	8.60 ± 0.03	8.63 ± 0.03	9.44 ± 0.02	9.48 ± 0.02

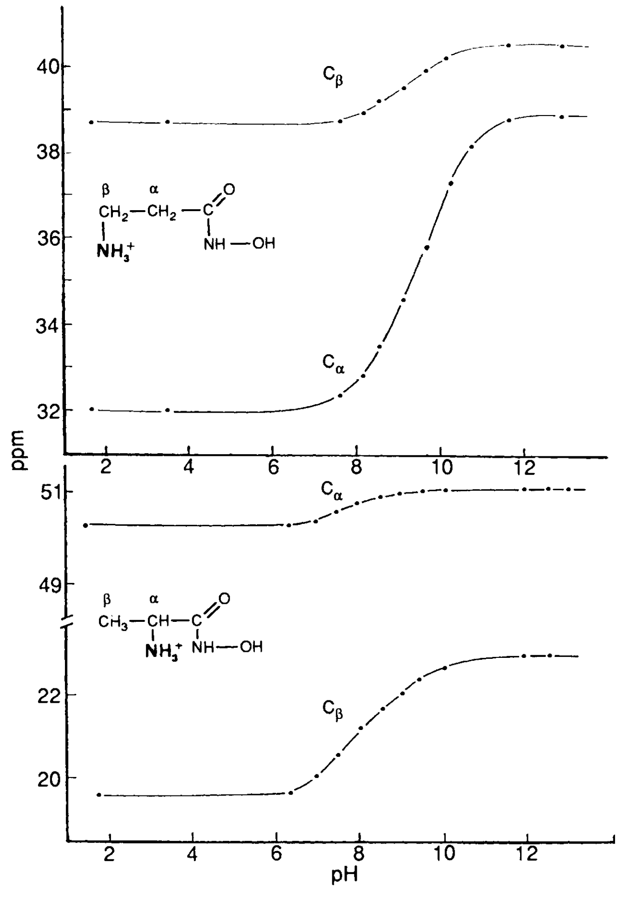


Figure 1. The pH dependence of chemical shifts of the α- and β-carbon atoms of L-α-alaninehydroxamic acid (α-AHA) and β-alaninehydroxamic acid (β-AHA).

chemical shift of the other group, the dissociation of propionohydroxamic acid (PHA) was also studied. In PHA, the effects of the dissociation of the NHOH group on the α- and β-carbons could be obtained (0.45 and 0.92 ppm, respectively) and was taken into account in the evaluation. The calculation procedure of the microconstants involved first calculating from the chemical shift data the fractional dissociation of the NH_3^+ group of the ligands as a function of pH with equation (1).

$$x_{NH_2} = \frac{[AH]+[A^-]}{[^+HAH]+[AH]+[^+HA^-]+[A^-]} = \frac{\nu^{obs}-\nu^{d}-x_{NHO}\,\Delta\nu_{PHA}}{\nu^{p}-\nu^{d}-\Delta\nu_{PHA}} \qquad (1)$$

In equation (1), x_{NH_2} and x_{NHO^-} are the fractional dissociations of the functional groups, ν^{obs} is the chemical shift of the monitoring carbon atom observed at a particular pH, ν^{p} and ν^{d} are the chemical shifts of the monitoring carbon atom in the fully protonated and deprotonated forms of the molecule, and $\Delta\nu_{PHA}$ is the total change in the chemical shifts of the carbon atoms of PHA due to dissociation of the hydroxamic acid function. For α-AHA the chemical-shift data relating to the β-carbon, and for β-AHA those relating to the α-carbon, were used (see Figure 1) in the calculations.

The dissociation microconstants were then determined in the usual way[15] by non-linear least-squares curve-fitting of the fractional dissociation values to equation (2). The results are

$$x_{NH_2} = \frac{K_1^{micro}[H^+] + K_1K_2}{[H^+]^2 + (K_1^{micro} + K_2^{micro})[H^+] + K_1K_2} \qquad (2)$$

listed in the Table. The fractional dissociation curves obtained at two different ionic strengths (0.2 and 1.0 mol dm^{-3}) were practically identical within experimental error (see K_1^{micro}/K_2^{micro} values in the Table). As an illustration, the concentration–distribution curves of the macro- and microspecies of α-AHA are depicted as a function of pH in Figure 2.

It can be seen from the Table that in α-AHA the NH_3^+ group is more acidic than the NHOH group (K_1^{micro}/K_2^{micro} = 2.7), whereas the acidity sequence is reversed in β-AHA (K_1^{micro}/K_2^{micro} = 0.42). The overlap between the two dissociation processes is quite considerable for both ligands. The first proton dissociates to the extent of *ca.* 73 or *ca.* 30% from the NH_3^+ group, and *ca.* 27 or *ca.* 70% from the NHOH group, respectively. The electron-withdrawing effect of the NHOH group on the NH_3^+ group seems to be stronger than that of the NH_3^+ group on the NHOH group, since the acidity of the NH_3^+ group is increased to a greater extent than that of the NHOH group, as compared with the respective reference compounds ($\Delta pK = pK_{\alpha-Ala} - pK_1^{micro} = 2.20$, and $\Delta pK = pK_{PHA} - pK_2^{micro} = 1.42$ for α-AHA; and $\Delta pK = pK_{\beta-Ala} - pK_1^{micro} = 1.29$, and $\Delta pK = pK_{PHA} - pK_2^{micro} = 0.85$ for β-AHA).

In accordance with expectation, the electronic effect of each group on the dissociation of the other is the stronger, the closer the two groups are to each other. The ratios of these electronic effects in α-AHA and β-AHA are very similar for both acidic groups (2.20:1.29 ~ 1.42:0.85 ~ 1.7), and agree very well with the analogous ratios for other α- and β-substituted acids. For example, the effect of the presence of the NH_3^+ group in α-Ala on the acidity of the carboxylic group as compared with the acididity of propionic acid[9] is $\Delta pK - 4.86 - 2.35 = 2.51$, while the effect of the NH_3^+ group in β-Ala on the acidity of the carboxylic group is $\Delta pK = 4.86 - 3.42 = 1.44$, and their ratio is again *ca.* 1.7.

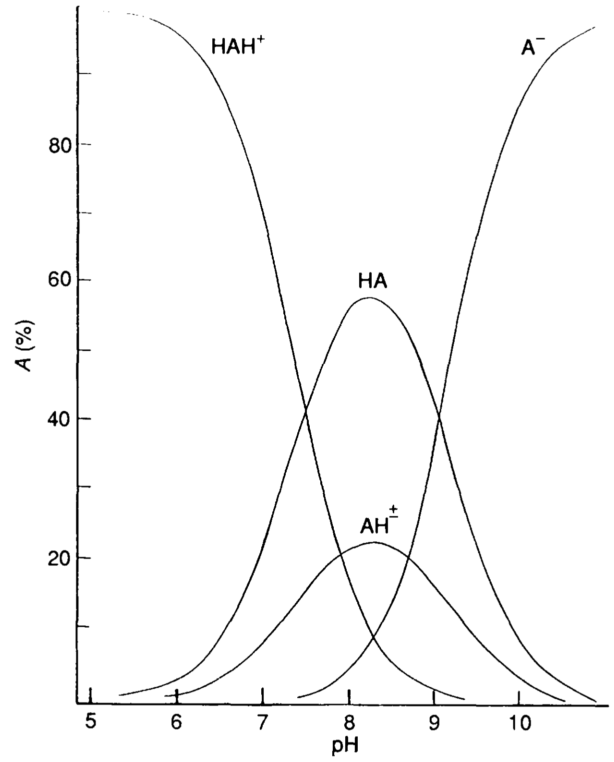


Figure 2. The concentration distribution curves of the proton complexes of α-AHA as a function of pH.

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