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Lyophilized histidine investigated using X-ray photoelectron spectroscopy and cryogenics: Deprotonation in vacuum

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Abstract

Lyophilized histidine samples were investigated using X-ray photoelectron spectroscopy (XPS). Lyophilized samples were prepared from aqueous solutions at a pH in the range between ∼1.5 and ∼10, and with no further addition of electrolyte. The use of cryogenics allowed the determination of protonated to unprotonated molar ratios of sites in L-histidine, which correlates well with the dissociation constants of the residual amino acid sites. When cryogenics was not used deprotonation of the lyophilized samples occurred, where the degree and the total concentration of deprotonated sites correlates well with the formation constants and the decrease in Cl concentration, respectively. This later relation clearly indicates a correlation between deprotonation and the desorption of HCl from lyophilized samples.

Keywords: Histidine; XPS; Formation constant; Deprotonation

1. Introduction

The concept of acidity is of fundamental importance in most chemical areas (inorganic to biochemistry). It relies on the degree of protonation of a given basic site B with respect to its conjugate acid BH, which is characterized by the molar ratio, *r*, where $r = BH/B$. The experimental approach to determine the acidity depends critically on the systems to be studied, although electrochemical and spectrophotometric measurements are not conceivable for concentrated or opaque solutions. Therefore, alternative strategies are needed. Recently, solid state NMR spectroscopy emerged as a promising technique to determine *r* of organic materials in solid non-aqueous matrices [1]. However, when organic species are present in aqueous phases, it is not longer possible to determine *r* directly by NMR due to the high rate of intermolecular proton exchange between protonated and basic species [2].

As shown here, the use of electron/X-ray radiation in combination with ultra high vacuum conditions (UHV) is potentially an ideal method to obtain the information about proton associated events on surfaces on a molecular level. However, decomposition often occurs under these conditions, in particularly for polymers [3], or polymer/metal interfaces [4]. Identifying the mechanism that drives or controls the transformation of the surface is often not possible except in certain rare attempts where model approaches could be applied [5–7]. In this respect, decomposition can be understood as a process of breaking bonds or dissociation of chemically bonded surface groups, e.g. the deprotonation of oxide surface sites, and not the desorption of physisorbed molecules from the surface. In recent years the use of cryo-samples (samples cooled before exposing them to UHV) and their preservation under UHV has been shown to reflect well the chemical state of hydrated surfaces [8,9]. Therefore, the question arises about the factors that control the degradation of volatile samples under UHV, in particular for biological samples in aqueous environments [10,11]. Here, we show that XPS in combination with cryogenics can be used to elucidate the mechanism of deprotonation of lyophilized histidine, which may serve as a reference system for what may occur (in vacuum) for biological macromolecules.

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2. Experimental

Lyophilized samples of histidine were produced at various pH values (1.5, 1.97, 2.24, 4.0, 7.4, 9.05, 10.0) as follows: 100 mg of L-histidine (Sigma, >99%) were dissolved in 9 ml water (Millipore) and adjusted to the desired pH value (pH meter with capillary electrode) by dropwise adding 5 M NaOH or 3 M HCl aqueous solution; subsequently, upon final volume adjustment (10 ml, 64 mM histidine) and pH measurement, samples were transferred to 100 ml round-bottom flasks where the solution was frozen under rotation in liquid nitrogen until a thin aqueous ice film was obtained. After freeze-drying (lyophilization, where excess water is removed by sublimation at a pressure of ∼0.1 Torr) the samples, they were kept in powder form in a desiccator prior to measurements. Upon mounting samples on an XPS sample holder, the material was shortly exposed to air and, therefore, potentially to adventitious carbon. Hence, a slightly higher C to N ratio than 2 can be expected.

X-ray photoelectron spectroscopy using monochromatic Al Kα X-rays was carried out on a Kratos Axis Ultra instrument with cooling facilities in the evacuation chamber and in the analysis chamber. The sample holder was fitted on the pre-cooled (to −110 °C) feedthrough in the evacuation chamber and allowed to thermally equilibrate before evacuating. The temperature of the sample was not directly monitored during the pumpdown, but only the temperature of the feedthrough, which reached a steady state temperature of ∼−140 °C within ∼3 min from the instant of initiated pumpdown; the pressure in the evacuation chamber was then 5×10^{-6} Torr. The temperature of the sample (when cryogenics was used) during measurements was ∼−60 °C or below if nothing else is mentioned in the text. Further, in general no changes in the spectra during measurements were observed when using cryogenics. However, when performing measurements at temperatures ∼−10 °C or above, the rate of sample degradation was significantly high to yield changes of the acid/base ratio as a function of the X-ray dose.

The core-level lines are all referenced to the main carbon peak (assumed to have a binding energy, BE, ∼285 eV). Experiments were performed using a passing energy of 20 eV. The analysis area was ∼0.5 mm × 0.5 mm. The samples were flooded with electrons having a kinetic energy of less than 3 eV to maintain charge equilibrium. Element quantification was performed using Kratos's sensitivity factors.

3. Results

The identification of the different components in the core level spectra were performed assuming that purely electrostatic terms dominate the chemical shifts in the BE of the components [12]. Hence, e.g. if C_{COO} and C_{COOH} denote the unprotonated and the protonated form of the carboxyl group, respectively, then the former is associated with a lower BE. The quantification of the N 1s core level was more complicated since four different components were present with a significant overlap. However, quantification was simplified since the BEs of the different components were nearly constant, i.e. a specific component had approximately the same BE (within ∼0.1 eV) in all spectra, and stoichiometric constraints were applied.

In Fig. 1, the N 1s core levels are shown for L-histidine samples prepared at pH ∼10, 7.4, 4.0 and 1.5. All the N 1s core level spectra can be described by four components. Totally, five components were used to describe all five spectra, where the relative intensities are shown in Table 1 with associated binding energies. Hence, two components are associated with imidazolium (denoted N_{INH} and N_{IN}), one with imidazolium cation (N_{I2NH}), and two with the amino group (denoted N_{AH} and N_A). The respective binding energies are shown in Table 1. During the fitting of the components to the spectra the intensities of the components were constrained by stoichiometric relations; therefore, the description of the N 1s core level line is consistent with the intensity (I) ratios (within ∼0.1%): $I_{INH}/I_{IN} = 1$ and $(I_{AH} + I_A)=(I_{I2NH} + I_{INH} + I_{IN})/2$. In addition the width of the N_{IN} component was constrained by the value extracted from the spectra measured at pH ∼7.4. At pH ∼1.5 and ∼4.0 the N 1s core level is (mainly) described by the N_{AH} and N_{I2NH} components where their intensity ratio I_{I2NH}/I_{AH} is ∼2.0 and ∼1.8, respectively, confirming the fact that below pH ∼4 only LH_2^+ and LH_3^{+2} forms dominate; Surprisingly, there appears to be a small N_{IN} component observable at pH ∼4.0. At pH ∼7.4 it is evident that the peak

Table 1
Element quantification (in at.%) and fraction of components (in % of the total element amount) present in lyophilized histidine samples prepared from solutions at different proton concentration (pH) and measured under cryogenic (cryo) or room temperature (RT) conditions

	pH	N	C	O	Cl	Na	COOH	COO	AH	A	INH	IN	I2NH
							288.3	287.3	401	399	400	398.5	400.5
Cryo	1.5	21.0	49.6	15.1	13.6	0.7	14.7	0.0	33.5				66.5
RT	1.5	22.4	51.4	15.3	10.2	0.8	8.5	5.9	33.2		7.7	7.5	51.7
Cryo	1.97	19.8	52.1	11.3	16.5	0.4	7.5	8.2	33.3				66.7
RT	1.97	21.1	53.9	11.1	13.6	0.2	3.8	12.4	32.7		7.3	7.4	52.6
Cryo	2.24	20.5	53.4	12.0	14.2	0.0	5.7	11.2	33.3				66.7
RT	2.24	20.7	56.8	10.9	11.6	0.0	3.5	13.5	33.5		7.7	7.7	51.1
Cryo	4.0	20.6	47.3	21.4	7.9	2.8	0.0	17.0	33.2		3.7	3.4	59.7
Cryo	7.4	26.2	57.0	16.2	0.7		0.0	17.0	33.0		28.7	28.6	9.7
Cryo	9.05	20.9	59.5	14.6	0.0	5.1	0.0	17.5	23.3	11.0	32.9	32.8	0.0
Cryo	10	21.3	49.4	18.7	1.7	8.8		18.7	6.2	26.8	33.5	33.6	0.0