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The influence of temperature and X-ray dose on the deprotonation of lyophilized phenylalanine during X-ray photoelectron spectroscopy

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Abstract

Lyophilized phenylalanine (LP) samples were prepared from aqueous solutions at pH ∼ 1.3 and subsequently analysed using X-ray photoelectron spectroscopy (XPS) in combination with cryogenics. When samples are measured at temperatures above ∼0 °C deprotonation occurs, which gradually proceeds with X-ray bombardment. In addition, deprotonation scales linearly with the difference between the Cl and the Na concentration, which strongly suggests that HCl sublimates from the sample.
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Keywords: Phenylalanine; XPS; Deprotonation

1. Introduction

Surface science techniques appears at first consideration to have strong disadvantages for characterising specimen of biological relevance due to the use of ultra high vacuum (UHV) and X-ray bombardment. Indeed, decomposition often occurs under these conditions, in particularly for polymers [1], or polymer/metal interfaces [2]. Identifying the mechanism that induces the transformation of the surface is often not possible except in certain rare attempts where model approaches could be applied [3–5]. In recent years, the use of cryo-samples (rapid cooled samples before exposing them to UHV where the cryogenic conditions are maintained during experiments) has been shown to reflect well the chemical state of hydrated surfaces [6,7]. Therefore, the question arises about the factors that control the degradation of volatile (hydrated) samples under UHV and X-ray bombardment, in particular for biological samples in aqueous environments [8,9]. Recently, we showed that lyophilized histidine deprotonate due to X-ray bombardment most probably resulting in desorption of molecular HCl from the samples [10]. Here, we show that carboxyl groups in LP show similar behaviour and that the effect depends on analysis temperature and X-ray bombardment time.

2. Experimental

Lyophilized samples of phenylalanine were produced as follows: 94 mg of L-phenylalanine (>99%, Sigma UK) were dissolved in 9 ml water (Millipore, US) and adjusted to the desired pH-value (pH meter with capillary electrode) by dropwise adding concentrated NaOH or HCl solution (with no further addition of salt). Upon final volume adjustment (10 ml, 57 mM phenylalanine) and pH determination (pH ∼ 1.3), the solution was transferred to a 100 ml round-bottom flask where it 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), samples were kept in powder form in a desiccator prior to measurements.

X-ray photoelectron spectroscopy was carried out on a Kratos Axis Ultra Instrument (using monochromatic Al Kα X-rays) with cooling facilities in the evacuation chamber and in the analysis chamber. The sample holder was fitted on the pre-cooled (to a temperature below the one used during measurement in the analysis chamber) feedthrough in the evacuation chamber and allowed to thermally equilibrate (∼90 s) before evacuating.

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Table 1
Molar fraction of elements and components (where the binding energy, BE, is given in the second column) for L-phenylalanine measured at different temperatures and X-ray doses

	BE (eV)	70 °C (1)	70 °C (2)	30 °C (1)	30 °C (2)	30 °C (3)	0 °C (1)	0 °C (2)	0 °C (3)	−135 °C
Na 1s	1072.5	5.20	4.57	6.05	5.52	4.57	1.77	2.02	2.00	3.57
O 1s CO	531.5	9.18	9.44	7.63	9.69	9.42	7.14	7.94	9.35	7.44
O 1s COH	533.0	4.32	2.81	5.65	4.01	3.04	6.98	6.77	4.83	5.74
N 1s	401.6	7.66	7.07	7.42	7.27	7.02	8.00	8.27	8.22	7.40
C 1s CC	285.0	52.89	52.91	49.12	48.54	52.36	55.28	55.78	56.06	51.67
C 1s COH	286.7	7.73	10.51	9.49	10.93	12.45	7.30	8.24	8.63	8.28
C 1s COO	288.6	4.21	4.42	3.04	3.77	3.80	1.11	1.77	1.74	0.00
C 1s COOH	289.5	0.00	0.71	1.18	0.85	0.00	3.95	2.38	2.81	5.04
Cl 2p	201.8	8.81	7.55	10.43	9.42	7.33	8.47	6.83	6.37	10.85

For a particular sample, the number in parentheses indicates the sequential order of the measurement (and the X-ray dose), which were performed every ∼0.5 h.

The core level lines are all referenced to the main carbon peak (assumed to have a binding energy, BE, ∼285 eV). Experiments were performed on a sample specimen at a specific temperature several times in a consecutive order using a pass energy of 20 eV. Normally the measurements were initiated within ∼10 min after loading into the analysis chamber. The analysis area was ∼0.3 mm × 0.7 mm. The samples were flooded with electrons having a kinetic energy of less than ∼3 eV to maintain charge stability. Element quantification was performed using Shirley signal backgrounds and Kratos's sensitivity factors.

3. Results and discussion

Fig. 1 show C 1s core level spectra measured in a consecutive order with a time interval of ∼0.5 h between each (X-ray maintained between the measurements) for a L-phenylalanine sample (pH ∼ 1.3) measured at ∼0 °C, which provide evidence that deprotonation (and composition alterations as will be shown below) occurs gradually during measurements. Evidently the C 1s core level is described by four components where their binding energies are shown in Table 1. The binding energies of deprotonated (C_{COO}) and protonated (C_{COOH}) carboxyl acid groups are in agreement with previous results [11]. It was assumed that the C_{COO} and the C_{COOH} component had the same peak width.

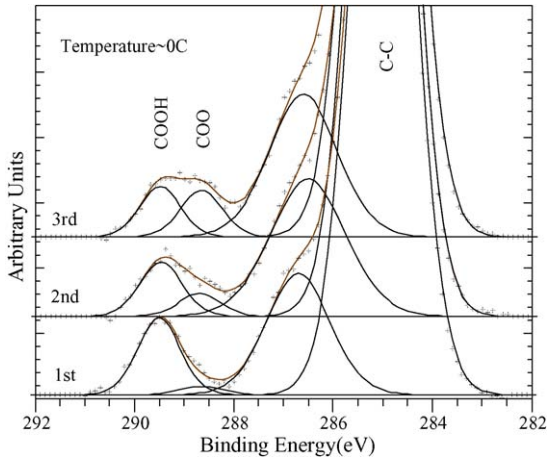

Fig. 1. C 1s core level spectra of a L-phenylalanine sample (prepared at pH ∼ 1.3) measured (at ∼0 °C) in a consecutive order with ∼0.5 h interval.

The observed deprotonation can be due to several reasons: bond breakage due to X-rays and/or photoelectrons/secondary electrons or X-ray induced heating (which may lead to desorption of volatile molecules). For example, it has been observed that irradiation of polyvinyl chloride results in chlorine–carbon and hydrogen–carbon bond breakage and the formation of HCl [12]. However, if the degradation process is sensitive to the analysis temperature one may conclude that the two first mechanisms are of less importance than the later. Hence, the deprotonation was investigated at different analysis temperatures. In Fig. 2, the (initial) C 1s core level spectra of LP (prepared at pH ∼ 1.3), measured at ∼−135 °C (the bottom one), ∼0, ∼30, and ∼70 °C, is shown and reveal that the abundance of –COOH groups decreases with increasing analysis temperature where no deprotonation is observed at the lowest temperature; hence, the degree of deprotonation, $[C_{COO}]/([C_{COO}] + [C_{COOH}])$, increases with temperature (T). Hence, deprotonation depends on X-ray bombardment time and measurement temperature, where the rate is most probably thermally activated. However, since the analysis

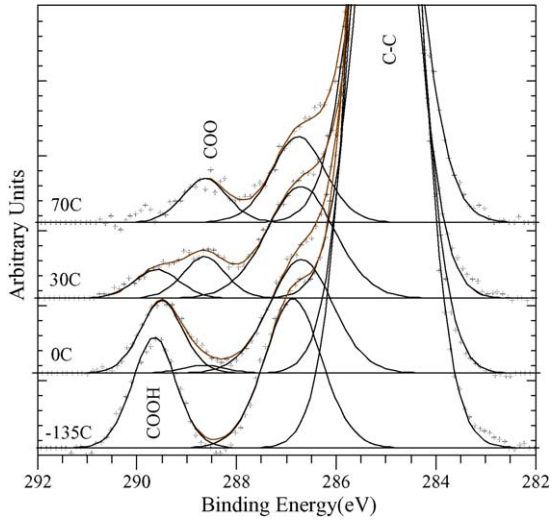

Fig. 2. C 1s core level spectra of a L-phenylalanine samples (prepared at pH ∼ 1.3) measured at different temperatures. The lines represent fitted components to the XPS data (crosses). The spectra shown are the initial spectra (acquired during ∼0.5 h), which depict the lowest degree of deprotonation at a specific temperature. The time that the sample spent in the spectrometer before measurements is less than 10 min for all temperatures.

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