Reference XPS spectra of amino acids

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Abstract. In this report we present XPS data for five amino acids (AAs) (tryptophan, methionine, glutamine, glutamic acid, and arginine) with different side chain groups measured in solid state (powder form). The theoretically and experimentally obtained chemical structure of AAs are compared. Here, we analyse and discuss C 1 s, N 1 s, O 1 s and S 2p core level binding energies, FWHMs, atomic concentrations of the functional groups in AAs. The experimentally obtained and theoretically calculated ratio of atomic concentrations are compared. The zwitterionic nature of methionine and glutamine in solid state was determined from protonated amino groups in N 1s peak and deprotonated carboxylic groups in the C 1s spectrum. The obtained XPS results for AAs well correspond with previously reported data.

1. Introduction

Amino acids (AAs) could be considered as one of the smallest biomolecules which are defined as organic compounds with both an amino group and a carboxyl group in one molecule. The structure of AA includes carboxylic group, an amino group and a side chain. However, the most important is that AAs are the basic building blocks of more complex organic materials such as peptides and proteins which are the objects of numerous investigations in biomaterials science. Thus, it is important to have detailed and reliable experimental data for reference AAs (including the position of core level peaks, characterization of functional groups, atomic concentrations, etc.) for further investigation of more complex organic tissues.

X-ray photoelectron spectroscopy (XPS) is one of the most powerful methods for the characterization of the surface chemistry of a wide variety of materials. XPS is a quantitative non-destructive spectroscopic technique that measures the elemental composition, chemical state and electronic state of the elements that exist within a material. As the depth of analysis in XPS (< 10 nm) is limited by the escape depth of photoelectrons from solid materials it is successfully used as a surface-sensitive method.

In the case of powder samples, like amino acids, care should be taken during the mounting samples for XPS analysis. According to the D. R. Baer [1], the powder samples can be prepared in three ways: (i) by using of the double-sticky tape, (ii) by placing powder in some type of sample holder or containment device, (iii) compressing the powder onto a substrate or sample holder or forming a free-standing pellet. As for AAs, there are a lot of reports dedicated to the investigation of their surface chemical composition by XPS using double-side tape [2], AAs decomposition under irradiation with soft X rays in the gas phase [3] or in a powder form pressed to a uniform layer [4]. Nevertheless, there
is a lack of detailed information for high-resolution solid state reference data of AAs in the powder form purchased from various suppliers (including chemical composition and possible contamination).

In this report, we present a detailed XPS analysis of five amino acids (tryptophan, methionine, glutamine, glutamic acid, and arginine) in the solid state which differ in their structure, namely in side chains. Their detailed analysis of chemical atomic composition and high-resolution core levels XPS spectra (C 1s, O 1s, N 1s and S 2p) revealed the differences in theoretical and elemental atomic composition and characteristic functional groups of AAs. The availability of detailed reference data for AAs will be helpful for other researchers in providing of a quality interpretation of obtained XPS spectra.

2. Materials and methods

Different types of AAs in the powder form such as tryptophan (TRP, purity ≥ 98 %), methionine (MET, purity ≥ 98 %), glutamine (GLN, purity ≥ 99 %), glutamic acid (GLU, purity ≥ 99 %), arginine (ARG, purity ≥ 98 %) purchased from Sigma-Aldrich were analyzed by XPS. The chemical structure of AAs is shown in Figure 1. Prior to sample mounting, the sample holder was cleaned in an ultrasonic bath (60 Hz) in ethanol for 15 min and dried in air. The AAs powder was slightly ground immediately before XPS measurements and placed directly on the samples holder by pressing the powder to the surface of the sample holder with a nickel spatula (cleaned with ethanol) by hand. In such a way the pellets of AAs were produced. The sample was pumped in the spectrometer air-lock chamber for 1.5-2 hours before reaching 10⁻⁷ Torr needed for the transportation of the sample holder into an analytical chamber for XPS measurements.

Figure 1. Chemical structure of AAs: a) tryptophan, b) methionine, c) glutamine, d) glutamic acid, e) arginine.

XPS spectra were acquired using Axis Ultra DLD XPS electron spectrometer (Kratos Analytical Ltd., UK) equipped with a monochromated Al Kα X-ray source (1486.6 eV) and hemispherical energy analyzer. The XPS survey spectra were collected at a pass energy of 160 eV at a constant take-off angle of 90°. The high resolution spectra of photoelectron lines were acquired at the pass energy of 20 eV. The charge neutralization system was used during XPS measurements. The Hybrid mode providing the analysis area of 0.3 × 0.7 mm² was applied for collecting XPS spectra. Peak fitting of the measured high resolution spectra was performed by the CasaXPS software using Shirley background and Gaussian/Lorentzian line shapes (GL(30), 70% Gaussian/30% Lorentzian) without fixing peak parameters (binding energies (BE), full width at half maximum (FWHM) and area/intensity). Binding
energies (BE) of photoelectron lines were determined with an accuracy of ± 0.1 eV. BE scale was referenced to C 1s component of aliphatic carbon, set at 285.0 eV [2].

3. Results and discussion

3.1. XPS analysis of the total atomic concentration of AAs

The theoretical (calculated from chemical structure shown in Figure 1) and experimental atomic concentrations (AC) for five AAs calculated from core-level XPS peaks are given in Table 1. For measured AAs, except MET, the higher concentration of carbon for experimentally obtained AC can be attributed to adventitious carbon contamination of the samples surface. In the case of MET, the presence of a sulphur signal was detected. The highest experimental AC (~ 30 at. %) of nitrogen was detected on the surface of ARG when other AAs had nitrogen content between 9.8-13.7 at. %. As for oxygen concentration, GLU had 37.6 at. % of oxygen whereas other analyzed AAs had the content of oxygen atoms in the range from 13.4 to 28.1 at. %. XPS analysis of ARG revealed the low chlorine contamination (~ 0.8 at. %) (excluded from experimental AC shown in Table 1). Experimental ACs are in good agreement with theoretical ones within the error of XPS quantification (~ 10% rel.)

Table 1. The theoretical and experimental atomic concentrations of chemical elements calculated from XPS spectra

| Amino acid powder | Theoretical atomic concentration, at. % | Experimental atomic concentration, at. % |
|-------------------|-----------------------------------------|------------------------------------------|
|                   | O     | C     | N     | S     | O     | C     | N     | S     |
| Tryptophan (TRP)  | 13.3  | 73.3  | 13.3  | -     | 13.4  | 72.9  | 13.7  | -     |
| Methionine (MET)  | 22.2  | 55.6  | 11.1  | 11.1  | 21.3  | 55.2  | 10.9  | 12.6  |
| Glutamine (GLN)   | 30.0  | 50.0  | 20.0  | -     | 28.1  | 52.9  | 19.0  | -     |
| Glutamic acid (GLU)| 40.0  | 50.0  | 10.0  | -     | 37.6  | 52.6  | 9.8   | -     |
| Arginine (ARG)    | 16.7  | 50.0  | 33.3  | -     | 17.1  | 52.5  | 30.4  | -     |

3.2. XPS data for tryptophan (TRP)

Table 2 and Figure 2 show the C 1s peak of TRP fitted with five components in accordance with its chemical structure (Figure 1). The dominant component at 285.0 eV was attributed to carbon atom bound only to other carbon atoms and hydrogen atoms. The component at 286.2 eV corresponds to sp² carbon singly bound to nitrogen, –C-NH. The components at 286.9 eV and 289.1 eV correspond to carbon in H₂N-C-COOH bond and carboxylic group, respectively. The C 1s spectrum of TRP exhibits π-π* satellite at 291.8 eV. This indicates the presence of aromatic rings, sp³ state of carbon, in agreement with the chemical structure of TRP (Figure 1). This carbon has BE at 284.7 eV [5]. In spite of the difference between binding energies of C=C and C-(C, H) bonds is 0.3 eV, BE scale was calibrated using C-C/C-H component at 285.0 eV. The obtained atomic concentrations for C-(C, H) ~ 35.6 at. %, C-N ~ 30 at. %, H₂N-C-COOH ~ 6.9 at. %, O=C-OH - 6.1 at. % lead to the calculation of atomic ratio 5.8:4.9:1.1:1 (the resulted atomic ratio is almost the same as the theoretical one).

The O 1s spectrum of TRP was fitted with two components (see Table 2 and Figure 2). The component at 531.9 eV corresponds to doubly bonded oxygen in the carboxylic group (O**=C-OH) when the other component at 532.9 eV was assigned to an oxygen atom in C-OH bond (O=C-O**H) there.

The N 1s spectrum of TRP consists of two components (see Table 2 and Figure 2). The low BE component at 400.5 eV is assigned to nitrogen in C-NH-C group while the second one at 401.9 eV
corresponds to the protonated, NH$_3^+$, amino group. All XPS results are in agreement with data reported in [6, 7].

| Table 2. The binding energies (BE), full width at half maximum (FWHM) and atomic concentration (AC) for fitted components of high resolution XPS peaks of TRP |
|-------------------------------------------------|
| Chemical bonds | BE, eV | FWHM, eV | AC, at. % | C-(C,H) |
|-----------------|---------|----------|-----------|---------|
| C 1s            | 285.0   | 0.85     | 35.6      | =C-NH   |
|                 | 286.2   | 0.97     | 30        | H$_2$N-C-COOH |
|                 | 286.9   | 0.97     | 6.9       | O=C-OH  |
|                 | 289.1   | 0.98     | 6.1       | $\pi-\pi^*$ satellite |
| O 1s            | 531.9   | 1.15     | 11.4      | O**=C-OH |
|                 | 532.9   | 1.52     | 2.0       | O=C-O**H |
| N 1s            | 400.5   | 0.92     | 5.9       | C-NH$_2$ |
|                 | 401.9   | 1.60     | 7.8       | NH$_3^+$ |

**chemical state of oxygen.

3.3. XPS data for metionine (MET)

The C 1s core level spectrum of MET was fitted with three components corresponding to three chemically different carbon atoms in the molecule (Figure 1). The main component at 285.0 eV corresponds to C-(C, H) bonds; the component at 285.8 eV represents the sum of signals from two carbon atoms bound to the sulphur atom (C-S-C) and amino group (C-NH$_2$); the component at 288.0 eV arise from carboxylic group (see Table 3 and Figure 3). Table 3 shows the following atomic concentrations for MET: C-(C, H) – 25.7 at. %, C-S-C, C-N – 18.2 at. %, O=C-H – 11.3 at. %. The corresponding atomic ratio for those atomic concentrations is 2.3:1.6:1 which is almost equal to the theoretical value.

The fitting of O 1s core level peak of MET revealed the presence of two contributions. Same as for TRP, oxygen in (O**=C-OH) bond was fitted at 530.9 eV while minor O 1s peak component at 532.0 eV correspond to the O atom in (O=C-O**H) bonds.

The N 1s spectrum of MET demonstrates one component at 401.1 eV which is attributed to protonated NH$_3^+$ groups. Observation of fully protonated amino group together with dominating O 1s component at 530.9 eV indicates a proton transfer from the carboxylic group of MET to amino group, i.e. zwitterionic form of the molecule in the solid state.
In the case of MET, the S 2p core level spectrum shown in Figure 3 consists of one spin-orbital doublet with BE of S 2P\(^{3/2}\) components at 163.0 eV corresponding to the sulphur atom and C-S-C bonds. Our XPS data are in agreement with the results presented in [8, 9].

**Table 3.** The binding energies (BE), full width at half maximum (FWHM) and atomic concentration (AC) for fitted components of high resolution XPS peaks of MET

| Methionine (MET) | Chemical bonds | BE, eV | FWHM, eV | AC, at. % |
|------------------|----------------|--------|----------|-----------|
| C 1s             | C-(C,H)        | 285.0  | 0.94     | 25.7      |
|                  | 285.8          | 1.33   | 18.2     |
|                  | 288.0          | 1.04   | 11.3     |
| O 1s             | O=C-OH         | 530.9  | 1.19     | 19.5      |
|                  | O**=C-OH       | 532.0  | 1.21     | 1.8       |
| N 1s             | NH\(_3^+\)     | 401.1  | 1.48     | 10.9      |
| S 2p\(^{3/2}\)   | C-S-C          | 163.0  | 0.86     | 12.6      |

**Figure 3.** Fitted C 1s, O 1s, N 1s and S 2p peaks for MET.

### 3.4. XPS data for glutamine (GLN)

According to the chemical structure of GLN (Figure 1, b), there are three chemically different carbon atoms: C-(C, H), C-NH\(_2\), and “carboxylic type” carbon - O=C-OH and NH\(_2\)-C=O with corresponding atomic ratio 2:1:2. Correspondingly, C 1s core level peak was fitted with three components (see Table 4 and Figure 4). The assignment of chemical bonds is the same as earlier. Observed atomic concentrations for C-(C, H) – 29.1 at. %, C-N – 11.3 at. %, and COOH/NH\(_2\)-C=O – 19.7 at. % produce corresponding atomic ratio 2.6:1:1.7 which is close to the theoretical one.
The O 1s spectrum is very similar to ones for TRP and MET, and N 1s spectrum to TRP. Two components in N 1s spectrum indicate that only the “pure” amino group, C–NH$_2$, is protonated, but not nitrogen in the NH$_2$–C=O. XPS data for GLN presented above agree with the previous study [2].

**Table 4.** The binding energies (BE), full width at half maximum (FWHM) and atomic concentration (AC) for fitted components of high resolution XPS peaks of GLN

|          | BE, eV  | FWHM, eV | AC, at. % | Chemical bonds          |
|----------|---------|----------|-----------|-------------------------|
| C 1s     | 285.0   | 1.10     | 21.9      | C–(C, H)                |
|          | 286.1   | 1.19     | 11.3      | C–N                    |
|          | 288.1   | 1.14     | 19.7      | O=–C–O, O=–C–NH$_2$     |
| O 1s     | 531.2   | 1.36     | 24.9      | O$^{**}$=C–OH, O$^{**}$=C–NH$_2$ |
|          | 532.5   | 1.70     | 3.2       | O=C–O$^{**}$H           |
| N 1s     | 399.6   | 1.21     | 8.7       | NH$_2$                 |
|          | 401.2   | 1.55     | 10.3      | NH$_3^+$                |

$^{**}$chemical state of oxygen.

**Figure 4.** Fitted C 1s, O 1s and N 1s peaks for GLN.

### 3.5. XPS data for glutamic acid (GLU)

The C 1s core level spectrum of GLU was fitted in accordance with the chemical structure shown in Figure 1 with three components: C–(C, H) bonds at 285.0 eV, C–N bonds at 286.1 eV and O=–C–O bonds at 288.6 eV (see Table 5 and Figure 5). The atomic concentrations of chemical bonds in fitted C 1s spectrum of GLU are shown in Table 5. In the case of GLU, the atomic ratio in the C 1s spectrum is 1.61:1:1.6 which is only slightly different from the theoretical ratio 2:1:2.

The O 1s core level spectrum of GLU was fitted with two components same as for TRP: oxygen in (O$^{**}$=C–OH) bond at 531.6 eV and O atom in (O=C–O$^{**}$H) bond at 533.0 eV.

In contrast to GLN, the N 1s spectrum of GLU demonstrated only one component at 401.4 eV which was attributed to protonated NH$_3^+$ groups (see Figure 5). Notice, the presence of a fully protonated amino group together with O 1s component at 531.6 eV revealed a proton transfer from the carboxylic group of GLU to the amino group. Thus, GLU has a zwitterionic form of the molecule in the solid state same as MET. The XPS results obtained for GLU corresponds with previously reported data in [2].

**Table 5.** The binding energies (BE), full width at half maximum (FWHM) and atomic concentration (AC) for fitted components of high resolution XPS peaks of GLU

|          | BE, eV  | FWHM, eV | AC, at. % | Chemical bonds  |
|----------|---------|----------|-----------|----------------|
| Glutamic acid | C 1s   | 285.0    | 1.14      | 20.1           | C–(C, H)       |

[Image of fitted C 1s, O 1s, and N 1s peaks for GLU]
Table 6 and Figure 6 show the C 1s core level spectrum of ARG fitted with four components in accordance with chemical structure (see Figure 1). The bonds at 285.0 and 285.8 eV were attributed to C-(C, H) and C-N bonds, respectively. In contrast to previous AAs, in the C 1s spectrum of ARG the presence of HN=C-(NH, NH$_2$) bonds results in the spectral component at 287.7 eV. The component at 288.7 eV corresponds to the carboxylic group. Observed atomic concentrations for C-(C, H) – 20.2 at. %, C-N – 15.6 at. %, HN=C-(NH, NH$_2$) – 7.1 at. % and O=C-OH – 9.6 at. % produce corresponding atomic ratio 2.8:2.2:1:1.3 which is almost the same as theoretical (2:2:1:1).

Same as for MET and GLU, the O 1s core level spectrum of ARG was fitted with two components: doubly bonded oxygen in the carboxylic group (O**=C-OH) at 530.8 eV and the oxygen atom in C-OH bond both in carboxylic groups (O=C-O**H) at 531.9 eV.

The N 1s core level peak of ARG is very similar to ones for MET and GLU. Two components at 399.3 and 400.0 eV were attributed to nitrogen in NH$_2$ and non-protonated C=NH bonds, and C-NH-C bond together with protonated C=NH$_2^+$ group, respectively. The XPS data for ARG are in agreement with results from the previous study [2].

**Table 6.** The binding energies (BE), full width at half maximum (FWHM) and atomic concentration (AC) for fitted components of high resolution XPS peaks of ARG

|                | BE, eV | FWHM, eV | AC, at. % | Chemical bonds            |
|----------------|--------|----------|-----------|---------------------------|
| **Arginine**   |        |          |           |                           |
| (ARG)          |        |          |           |                           |
| C 1s           | 285.0  | 1.16     | 20.2      | C-(C, H)                 |
|                | 285.8  | 1.24     | 15.6      | C-N                      |
|                | 287.7  | 1.08     | 7.1       | HN=C-(NH, NH$_2$)        |
|                | 288.7  | 1.21     | 9.6       | O=C-OH                   |
| O 1s           | 530.8  | 1.25     | 15.1      | O**=C-OH                 |
|                | 531.9  | 1.59     | 2.0       | O=C-O**H                 |
| N 1s           | 399.3  | 1.33     | 18.5      | NH$_2^+$, C=NH           |

**Figure 5.** Fitted C 1s, O 1s and N 1s peaks for GLU.
4. Conclusion

We report on a detailed XPS analysis of five AAs in their solid state (powder form) with different side chain groups. Experimentally determined chemical compositions were in good agreement with theoretical values with some possible surface adventitious carbon contaminations. Insignificant chlorine impurity (~0.8 at.%) was detected in the case of ARG. High-resolution XPS spectra of C 1s, O 1s, N 1s and S 2p core levels were fitted and analyzed for each of AA in terms of binding energies, FWHMs, atomic concentrations and related to AAs chemical structures. In contrast to other AAs, amino groups of MET and GLU are fully protonated. This makes it possible to conclude that both MET and GLU are present in the zwitterionic form with deprotonated carboxylic groups. We suppose that our XPS data for AAs could be useful for XPS analysis of more complex organic substances (proteins, peptides), biomaterials and tissues. Reference spectra reported in this publication will be deposited in the Open Spectroscopy Database “Spectroscopy Hub” [10].

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