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Innershell Absorption Spectroscopy of Amino Acids at All Relevant Absorption Edges

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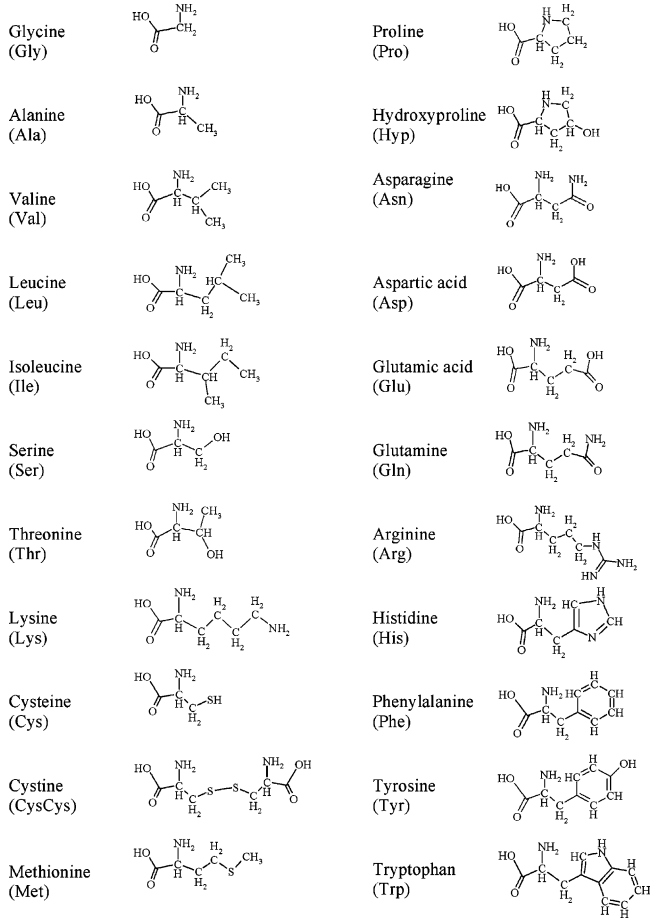
The C, N, and O K-edge near-edge X-ray absorption fine structure spectra of the 22 most common proteinogenic α-amino acids in the zwitterionic form collected from solvent-free polycrystalline powder films in the partial electron yield mode are reported. Spectral features common to all amino acids, as well as distinctive fingerprints of specific subgroups of these compounds, are presented and discussed.

NEXAFS (near-edge X-ray absorption fine structure) spectroscopy[1] and related synchrotron-based soft X-ray spectroscopic techniques[2] provide powerful tools for the elucidation of the electronic structure of organic substances in a variety of sample environments. One of the current challenges in this field is the application of NEXAFS to bioorganic objects. Such studies are difficult for various reasons, including the complexity of the molecular composition and radiation sensitivity of the target objects. Proteins, the most important class of biopolymers, are composed of amino acids. Thus, a careful spectral characterization of amino acids is a necessary first step in the application of NEXAFS to complex systems of biological significance. Although many NEXAFS studies of amino acids have been performed since the first published works by Boese et al. in the late 1990s,[3,4] no comprehensive spectral libraries have been reported to date.[5−13] So far, only the C K-edge spectra of the major 20 amino acids have been summarized by Kaznacheyev et al.[5] The spectra were measured in the transmission mode from thin polycrystalline films cast from solutions of the respective amino acids in trifluoroacetic acid onto thin silicon nitride substrates. The samples prepared in this way were shown to contain traces of the solvent, and thus, a correct analysis of high-lying σ^* resonances appeared difficult.[5] Meanwhile, the N and O K-edge NEXAFS spectra were reported only for a few selected amino acids.[6−14]

In this letter, we present a collection of carefully calibrated low-noise NEXAFS spectra of the 22 most common proteinogenic amino acids (i.e., 20 standard gene-encoded amino acids along with cystine and hydroxyproline) in the zwitterionic form measured at all relevant absorption edges, i.e., at the C, N, and O K-edges using the partial electron yield mode from solvent-free powder films. The molecular formulas of the studied amino acids (as neutral molecules) together with their full and abbreviated names are listed in Scheme 1.

SCHEME 1: Molecular Formulas of 22 Studied Amino Acids (as neutral molecules)



For the sample preparation, the as-purchased powders of the respective amino acids (Sigma-Aldrich Chemie GmbH, stated purity >98%) were pressed into clean In foil and thinned by a brush to suppress charging effects.[10] Optically pure L-enantiomers of all the amino acids except for glycine (which is achiral) were used. Although the sample preparation procedure did not involve the use of a solvent, we cannot totally rule out a minor contamination of strongly hydrophilic amino acids (e.g., glycine, serine, aspartic, and glutamic acids) with traces of water. The measurements were performed at the bending magnet beamline

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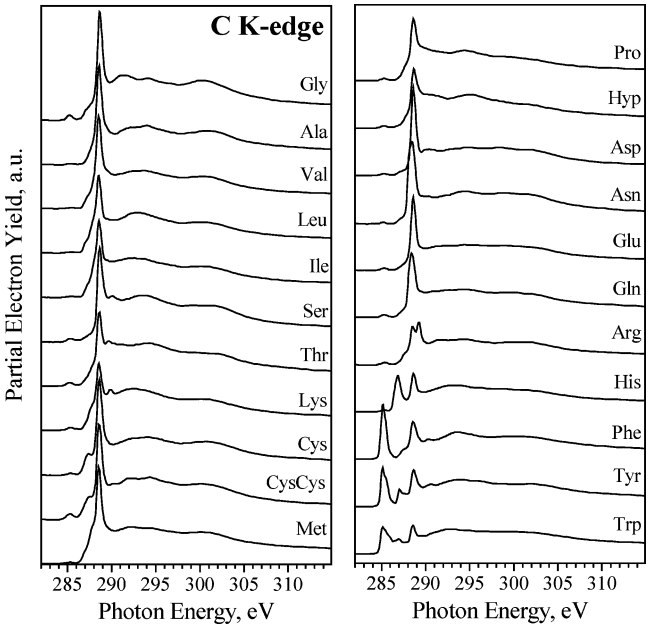

Figure 1. Experimental C K-edge NEXAFS spectra of the amino acids.

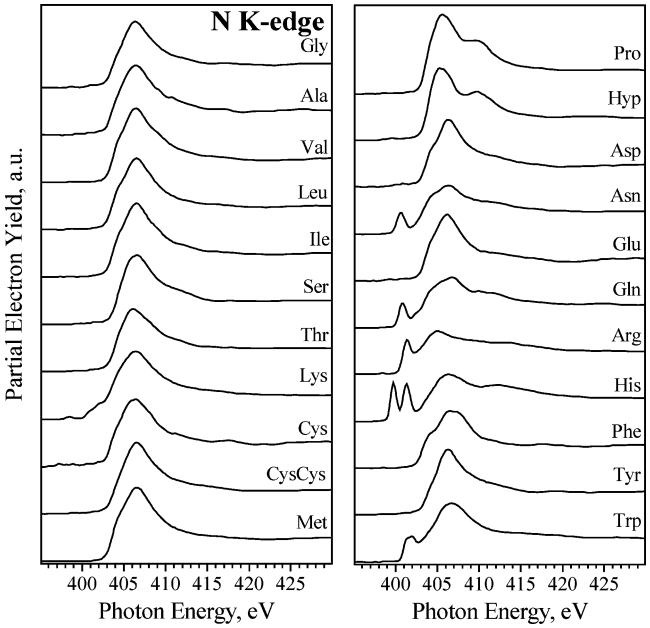

Figure 2. Experimental N K-edge NEXAFS spectra of the amino acids.

HE-SGM of the synchrotron radiation facility BESSY II in Berlin. The energy resolution of the setup was about 0.3 eV; the incident beam was kept at the "magic angle" of 55°. Due to a special procedure for the absolute energy calibration of the experimental spectra against the distinct π^* resonance of highly oriented pyrolytic graphite (285.38 eV),[15] the accuracy of the determined positions of the spectral features is estimated to ±0.05 eV. The spectra were fully reproducible over several independent runs. No spectral changes due to radiation damage within the data acquisition time were detected with the current setup (the photon flux on the order of 10^{11} photons/s at the spot size of about 1.2×0.5 mm^2). Even several successive spectra taken from the same place (as control measurements) did not exhibit any X-ray-induced changes.

The C K-edge spectra of the 22 amino acids are shown in Figure 1. The majority of spectra are dominated by three features, viz., a narrow resonance at around 288.6 eV attributable to the π^*(COO) transition[5] and broader σ^* resonances at ca. 293.0 and 300.0 eV. The former dominantly comprises σ^* (C−C) components, whereas the latter corresponds to transitions to σ^* states associated with the carboxylate group.[6] The spectral feature related to the σ^*(C−NH_3^+) transition is most probably located around 290.3−291 eV (it is well-resolved only for glycine). In the case of amino acids with the amide group (i.e., asparagine and glutamine), the major π^* resonance is broadened and shifted to 288.3 eV in agreement with the results reported by Kaznacheyev et al.[5]

The amino acids arginine, phenylalanine, tyrosine, histidine, and tryptophan manifest characteristic patterns of narrow π^* transitions (see Table 1). The amino acids containing −OH or −NH_2 substituents in the side chains (i.e., serine, threonine, and lysine) manifest distinct peaks at 289.6−290.0 eV attributable to the σ^*(C−OH/NH_2) transitions. The sulfur-containing amino acids (cysteine, cystine, and methionine) exhibit distinct σ^*-(C−S) peaks just below the main π^*(COO) feature at ca. 287.3 eV.

It is of note that the C K-edge spectra of some amino acids (in particular, glycine, serine, threonine, cystine, and glutamine) reveal a weak but distinct peak at ca. 285.2 eV, which has not been reported in earlier studies and cannot be assigned within the simple building-block principle.[5,9] Although it can arise due to a number of extrinsic factors, such as defficiencies of the normalization procedure, contamination of the samples with aromatics, or radiation damage effects, we have strong evidence that this feature is intrinsic to the amino acids. Probably, it is due to the minute contribution of the aminic carbon atom to the π system of the adjacent carboxylate group. In such a case, the transition from the aminic carbon atom to the π^*(COO) orbital should be located 2−3 eV below the main π^*(COO) feature due to the respective difference in the core levels of the aminic and carboxylate carbon atoms.[16,17]

The N K-edge NEXAFS spectra of the amino acids are shown in Figure 2. The spectra of the majority of amino acids are

TABLE 1: Experimental Positions and Suggestive Assignments of the Main Spectral Features Observed in the C K-edge NEXAFS Spectra of the Amino Acids

assignment	energy position, eV	occurrence
π^*(COO)/(COOH)	288.6	All/Asp, Glu
π^*($CONH_2$)	288.3	Asn, Gln
π^*(C-substituted benzene)	285.1, 285.4	Phe, Tyr
π^*(OH-substituted benzene)	287.0	Tyr
π^*(C-substituted imidazole)	286.8, 288.6	His
π^*(C-substituted indol)	285.1, 285.4, 285.9, 285.5, 287.0	Trp
π^*(guanidine)	289.2	Arg
σ^*(C−C) (alkyl, amine, carboxyl)	293.0−297.0	All
σ^*(C−N) C−NH_3^+	290.3−291	All
C−NH_2	289.9	Lys
σ^*(COO)/(COOH)/($CONH_2$)	298−305	All/Asp, Glu/Asn,Gln
σ^*(C−OH) (protonated carboxyl, alcohol)	289.6−290.6	Asp, Glu, Ser, Thr, Tyr
σ^*(C−S)	287.3	Cys, CysCys, Met

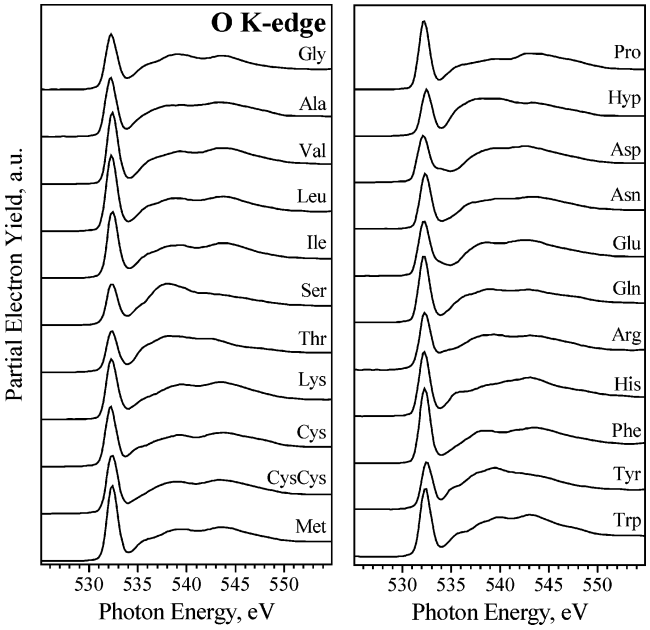

Figure 3. Experimental O K-edge NEXAFS spectra of the amino acids.

TABLE 2: Experimental Positions and Suggestive Assignments of the Main Spectral Features Observed in the N K-edge NEXAFS Spectra of the Amino Acids

assignment	energy position, eV	occurrence
$\pi^*(CONH_2)$	400.7	Asn, Gln
π^*(imidazole)	399.8, 401.3	His
π^*(indol)	401.7	Trp
π^*(guanidine)	401.4	Arg
σ^*(C−N)	406.2−406.6	All

TABLE 3: Experimental Positions and Suggestive Assignments of the Main Spectral Features Observed in the O K-edge NEXAFS Spectra of the Amino Acids

assignment	energy position, eV	occurrence
$\pi^*(COO)/(COOH)/(CONH_2)$	532.3−532.5	All
π^*(C−OH)	534.0	Asp, Glu
$\sigma^*(COO)/(COOH)/(CONH_2)$	542−549	All
σ^*(C−OH)	538−539.4	Ser, Thr, Hyp, Tyr

dominated by a relatively broad σ^*(N−C) peak at 406.2−406.6 eV. In the cases of proline and hydroxyproline, this peak is apparently split into two components at ca. 405.6 and 409.7 eV due to the saturated nitrogen heterocycle. This peak is broadened in the case of phenylalanine and has a diffuse pre-edge structure (400.1−402.8 eV) in the case of lysine, probably due to the additional nonprotonated amino group present in this amino acid. Some amino acids show distinct π^* resonances. In particular, asparagine and glutamine exhibit a peak at 400.7 eV due to involvement of the amide nitrogen atom into the π system of the carbamide group, similar to the situation observed for amide groups in peptides.[9−11] Arginine, histidine, and tryptophan exhibit narrow π^* resonances at 401.3−401.7 eV, while histidine shows another narrow feature at 399.8 eV due to respective aromatic π systems (see Table 2). In the case of glycine, pre-edge features of variable intensities were sometimes observed in the N K-edge spectra.[10] The possible origin of this variation will be the subject of a dedicated paper.

The O K-edge NEXAFS spectra of the amino acids are shown in Figure 3. All these spectra are quite similar to each other. They exhibit a dominant π^* peak associated with the carboxylate group at 532.3−532.5 eV and the respective σ^* component at ca. 543.5 eV in agreement with earlier observations.[6,8] No substantial shifts in the position of the main resonance due to introduction of additional COOH or $CONH_2$ groups is observed, although the amino acids containing protonated carboxy groups (i.e., aspartic and glutamic acids) show a minor feature at 534.0 eV, which can be assigned to the transition of the electron from the protonated oxygen atom to the π^* orbital of the carboxy group (see Table 3).[8] The amino acids containing the hydroxyl group (serine, threonine, hydroproline, and tyrosine) manifest broad features at 538.0 (Ser and Thr), 539.0 (Hyp), and 539.4 eV (Tyr), which can be thus attributed to the σ^*(O−C) transitions. The probable contribution from the adventitious water is expected in the similar energy range, i.e., 537−540 eV.[14]

In summary, we have presented and analyzed the C, N, and O K-edge NEXAFS spectra of the 22 most common proteinogenic α-amino acids in the zwitterionic form. The respective spectral library is an important step to establish such a powerful technique as NEXAFS spectroscopy to study complex systems of biological significance.

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Supporting Information Available: The calibrated and normalized NEXAFS spectra of 22 amino acids at the C, N, and O K-edges in the ASCII text format (WinZip archive). This material is available free of charge via the Internet at http://pubs.acs.org.

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