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Near-edge X-ray absorption and dichroism in amino acids

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Results from calculations of near-edge X-ray absorption and X-ray circular dichroism spectra of amino acids are reviewed and discussed.

Keywords: amino acids; x-ray absorption; x-ray circular dichroism.

1. Introduction

Nitrogen-containing organic compounds form a special group of compounds that have become of interest for spectroscopic research owing to their potential technical use and biological importance. Near edge X-ray absorption (NEXAFS) and circular dichroism (XCD) spectroscopies constitute two spectroscopies with capability of giving information on composition and structure of such samples. In the present work we address a particular set of organic compounds with special relevance for biochemistry, namely the amino acids, and explore in what way NEXAFS and XCD spectra can fingerprint such compounds. We briefly review and discuss recent theoretical results on spectra at the K edges of C, N and O, that were presented in a series of papers (Plashkevych *et al.*, 1998; Carravetta *et al.*, 1998) These studies were also motivated by the observations (Kirtley *et al.*, 1992) that the nitrogen edge NEXAFS spectrum of DNA can be understood as a weighted sum of the polynucleotide spectra, and by the suggestion that differences in the NEXAFS spectra might be used for mapping proteins which differ in their amino acid content (Boese *et al.*, 1997).

Except for glycine, all the amino acids investigated - alanine, cysteine, serine, valine, glycine, phenylalanine, histidine, tyrosine and tryptophan - exist in two optically active forms, namely as D- and L- isomers that are mirror images of one another. The rotatory X-ray absorption intensities have been computed and XCD spectra for this class of molecules are predicted (Plashkevych *et al.*, to be published). The direct atomic orbital static exchange approach STEX is employed. It is based on a separate channel description of the absorption process, which allows an easy analysis of the spectra in terms of contributions from different molecular subunits. The STEX technique is implemented for fully relaxed core hole potentials of the different sites, and for using the double basis set algorithm, allowing for close to basis set limit results.

2. C1s edge spectra

The simulated NEXAFS spectra of the nine amino acids investigated are collected in Fig.1 and compared to the presently available experimental spectra (Boese *et al.*, 1997). Two good building blocks in the investigated series can be clearly identified and it is observed that their spectral features remain largely unperturbed in the different molecules. The first building block is the carboxyl group which is present in all the molecules and which is responsible for the strong peak in the high energy discrete part of the spectra. The second one is the phenyl ring in phenylalanine, histidine, tyrosine and tryptophan, giving origin to the intense spectral feature in the low energy discrete part of the spectra. The discrete spectrum for each phenyl carbon is dominated by one $C1s \rightarrow \pi^*$ transition. The π^* transitions corresponding to the different phenyl carbon atoms bound to H are grouped in a sub-eV energy range and give then origin to a practically unresolved band at 285 eV irrespective of number, strength and character of substitution. The core excitations at the connected carbon are shifted by roughly an eV and typically appear as a high energy slope of the main phenyl band, or, if the substituent is strong enough, as a weaker separate peak the position of which is evidently dependent on the character of the substituent(s).

The third feature of the considered amino acids NEXAFS spectra enters at the left of the carboxylic bands and varies more between the different species. In histidine and tryptophan, for instance, it receives intensity from the nitrogen containing 5-ring, but it can get some intensity also from the $-CH_2-$ carbon and the NH_2 bonded carbon. The latter contributions are though quite small, in line with the observation that sp^3 carbons bound to heavy atoms produce most of the intensity beyond the edge.

The XCD spectra collected in Fig.2 appear more sensitive than the ordinary NEXAFS spectra and could pose good prospects to be used as fingerprints. Due to the almost spherical shape of the core orbitals, the circular dichroism in the X-ray region is much weaker than in the optical region and its detection therefore requires experiments of high sensitivity. However, the detection of the natural CD effect in the X-ray region could add a very selective feature to the photon absorption process that already is chemically selective.

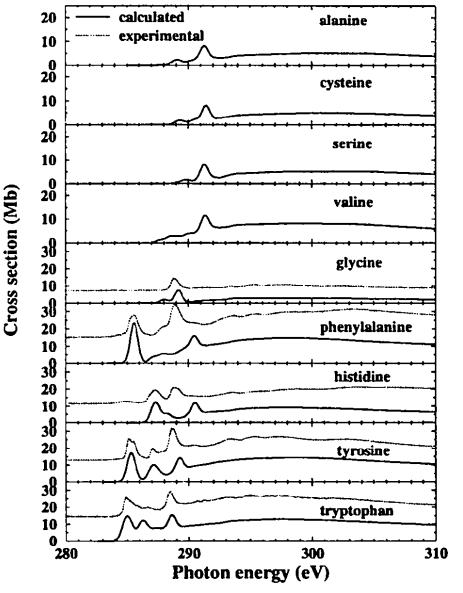

Figure 1
Calculated C_{1s} NEXAFS spectra of the nine amino acids investigated (Plashkevych *et al.*, 1998; Carravetta *et al.*, 1998), compared to the available experimental (Boese *et al.*, 1997) spectra. All the theoretical spectra have been convoluted with a Gaussian function (FWHM=0.7 eV) and those compared to experimental data have also been energy shifted for a more convenient comparison.

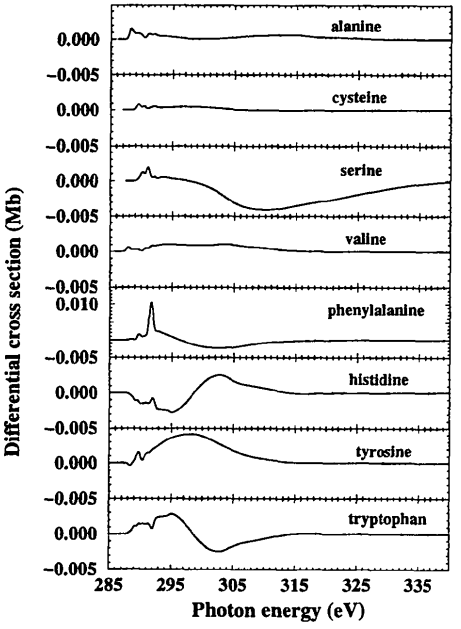

Figure 2
Calculated XCD spectra at the C K-edge for the chiral amino acids.

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3. N1s edge spectra

An obvious complication of the carbon spectra analyzed above is that they are formed by the superposition of spectra originating from several core sites. The chemical shifts between the C1s ionization thresholds and between the main discrete absorption transitions are relatively small, and it can be difficult to separate out special features by resonant excitation even with a well monochromatized beam of photons. Using spectra of unique atoms, like nitrogen in the amino acid series, the possibility to fingerprint the molecule should be better. However, in the amino acids the nitrogen atom has similar surroundings and according to the building block principle one cannot anticipate large changes in the ordinary NEXAFS spectra.

Fig.3 collects the computed N1s edge spectra of the amino acids here considered. The convoluted total spectra show two close peaks in the discrete energy region, one below 405 eV and one above, followed by a third structure which is a collection of Rydberg like transitions closer to the ionization threshold. The relative intensities of these three features are predicted to vary between the compounds, however, while the change in intensity is appreciable, the position of the peaks shows small variations. This could make the distinction of these amino acids by ordinary NEXAFS spectra not so straightforward. On the other hand, the computed XCD spectra at the N K-edge, collected in Fig.4, suggest that the different amino acids could more easily be fingerprinted by the circular dichroism effect in measurements with relatively moderate resolution.

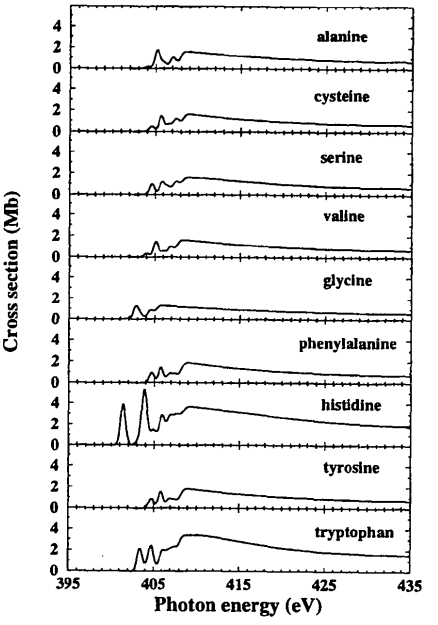

Figure 3
Calculated N_{1s} NEXAFS spectra of the nine amino acids investigated (Plashkevych *et al.*, 1998; Carravetta *et al.*, 1998) after convolution with a Gaussian function (FWHM=0.7 eV).

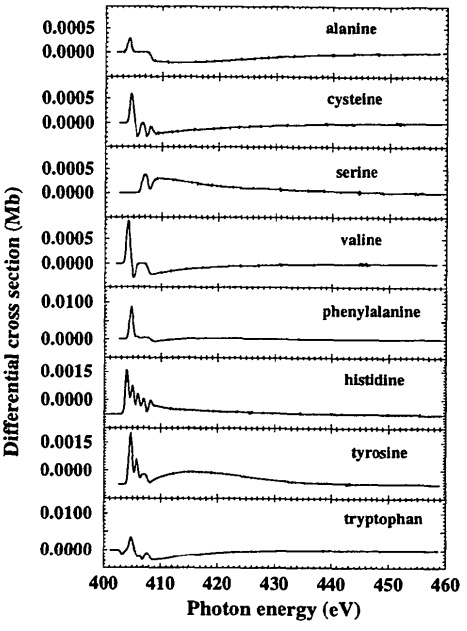

Figure 4
Calculated XCD spectra at the N K-edge for the chiral amino acids.

The calculations confirm anyway that the main experimental problem remains in the low values of the rotatory strengths; the anisotropy ratio g is predicted to be in the range of 10^{-4} - 10^{-3}, which, however, should still be measurable with a modern equipment.

4. O1s edge spectra

The oxygen content of the amino acids are explored by computations of the NEXAFS spectra (Plashkevych *et al.*, 1998; Carravetta *et al.*, 1998) and of the XCD spectra as shown in Fig.5. Both types show much less structure than the corresponding C1s spectra, and they are even sparser than the nitrogen spectra. Giving signals at the same energy, the O1s spectra could eventually be used to fingerprint an amino acid content, but would obviously not provide evidence of which type of amino acid (Plashkevych *et al.*, 1998; Carravetta *et al.*, 1998; Plashkevych *et al.*, to be published).

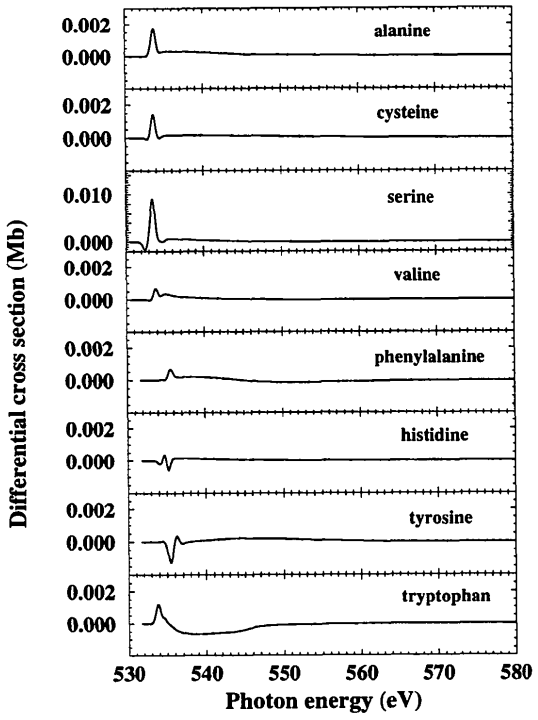

Figure 5
Calculated XCD spectra at the O K-edge for the eight chiral amino acids investigated (Plashkevych *et al.*, 1998 and Plashkevych *et al.* to be published).

5. Discussion

By large, the building block principle works well for the amino acids. One finds especially the phenyl ring and the carboxyl group to act as good fingerprints in the absorption spectra. The XCD spectra are more sensitive and appear as more specific for each particular amino acid. In the case of spectra at the C1s edge the scrambling due to the superposition of slightly chemically shifted spectra reduces the possibility to use them as fingerprints. The nitrogen XCD spectra in the amino series appear quite sparse and different for the different molecules.

The amino acid spectra indicate some of the capabilities of NEXAFS; the use of building blocks, and for fingerprinting the type of bonds and substitution. Substitution that give chemical shifts in the order of 1 eV are easily detected by NEXAFS. The position of the substituent peak is evidently dependent on the character of the substituent, but, on the other hand, not much on the actual number of substitutions and the isomeric forms (Plachkevytch *et al.*, 1997). So NEXAFS seems to be too blunt tool for assigning isomers of organic compounds. Owing to the building block character, the calculations of NEXAFS spectra show good convergence with respect to the size of the system, for instance various model molecules and repeat units of polymers can be appropriate to understand the spectrum of the full compound (Ågren *et al.*, 1995; Magnuson *et al.*, 1998).

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