

Using linear combination of NEXAFS spectra to characterize radiation damage in insulin

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Synopsis The sulfur *K*-edge absorption spectra of cysteine (cys), cystine (cyscys) and insulin were recorded before and after irradiation with 0.8 keV electrons. As we know, NEXAFS technique can be used to investigate relative intensities using the contribution from different species of sulfur for quantitative analysis. Considering the shape and relative intensities of the S 1s spectra in the cysteine and cystine molecules, two different methodologies were used to perform linear combination spectra of these species in order to characterize the structural modifications observed in irradiated insulin spectrum.

The samples studied in this work are sulfur-containing biomolecules. In the amino acid cysteine, sulfur is present in a reduced form as a thiol (-S-H). In molecules like cystine and insulin, sulfur is present in an oxidized form: a disulfide bond (-S-S-). Due to its different sulfur oxidation forms, cysteine spectra present only one peak of S1s $\rightarrow$ σ^* (S-C) transition while cystine and insulin spectra present two peaks attributed to S1s $\rightarrow$ σ^* (S-S) and S1s $\rightarrow$ σ^* (S-C) transitions [1].

As insulin is known to contain three disulfide bridges, it seems reasonable to suppose that the differences observed in the irradiated insulin spectrum can be related to disulfide bridges breaking due to the irradiation process: when disulfide bridges break apart, the oxidized form of sulfur (-S-S-) in cystine gives rise to thiol redox status (-S-H).

As NEXAFS technique can also be used to investigate relative intensities using the contribution from different species of sulfur for quantitative analysis [2] and considering the shape and relative intensities of the S 1s spectra in the cysteine and cystine molecules, we used linear combination (LC) of these spectra to reproduce the spectrum of insulin irradiated.

Two methodologies were used for data treatment. The first method involved the use of BGAUSS [3] for peaks deconvolution and ORIGIN [4] for linear combination spectra, whereas the second method involved the use of ATHENA [5] software for both deconvolution and spectra combination. Figure 1 shows insulin experimental spectrum as well the results of fittings.

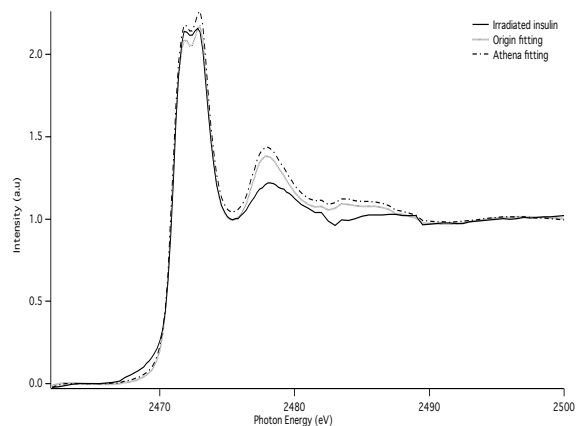


Figure 1. Irradiated insulin and linear combination fitting.

The combination of 33% cys and 66% cyscys spectra was found for Origin fitting and for Athena method, the LC best fit gives 32,9% cys and 67,1% cyscys. The two different methodologies used to calculate the LC presented concordant results. These results suggest that only one from the three disulfide bridges is broken.

Our results show that NEXAFS spectra can also be used for quantitative proposes.

Referências

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