MXAN: new improvements for potential and structural refinement

Maurizio Benfatto¹ and Stefano Della Longa²
¹Laboratori Nazionali di Frascati, INFN CP13, 00044 Frascati, Italy
²Dipartimento di Medicina Sperimentale, Università dell’ Aquila via Vetoio, loc. Coppito II 67100 L’Aquila Italy
¹maurizio.benfatto@lnf.infn.it; ²dlonga@caspur.it

Abstract. Multiple Scattering (MS) theory, via the MXAN package, is able to reproduce the experimental XANES data of aqua ions, small molecules, and metal sites in biomolecules from the edge up to 150-200 eV. In this paper we present the last advances of the method, including the potential description and optimization. Improved parameterization also includes new routines to handle the structural determinants via generalized degrees of freedom. Examples will be presented to illustrate the method.

1. Introduction

The MXAN procedure [1] has been utilized in the last years to obtain structural information on numerous compounds [ions in solution [2], small molecules [3], crystals, metal sites in proteins [4, 5]] using the low energy part of a XAS spectrum from the edge up to about 200 eV. This procedure works in the standard Multiple Scattering (MS) theoretical environment using the muffin-tin (MT) approximation for the shape of the potential and the so-called “extended continuum” scheme to calculate both the bound states and the continuum part of the XAS spectrum.

In the MT approximation there is the need to define the radii of the MT spheres surrounding all the atoms in the cluster used in the calculation. MT radii are thought to be proportional to the ionic atomic radii, however they are almost free parameters of the theory; the Norman criterion is usually applied to choose them, with inclusion of some overlap (from this point forward called OVL) between the MT spheres. In this way just one parameter, OVL, determines the choice of all the MT radii of the atomic cluster. Moreover the MT potential in the interstitial volume between the MT spheres is constant and equal to V0. Its value is calculated by averaging the potential over the interstitial volume contained between an “outer sphere” (centred on the absorbing atom and encompassing the cluster) and each individual MT sphere. The interstitial volume is minimized by choosing the outer sphere tangent to the farthest sphere from the centre. However, in the “extended continuum” scheme the outer sphere is not used, and the interstitial potential (that we call now V0_imp) can also be considered as a free parameter of the theory.

The overcoming of the MT approximation, and the use of a self-consistent (SCF) procedure for the whole cluster to generate the potential is the way to remove such arbitrariness. However their practical use in a fitting procedure is complicated and too heavy time consuming. Moreover during a fit
procedure, the calculation of a SCF charge density for a geometry far from the “true one” could be even harmful as it could select wrong excited electron configurations. For these reasons, in a number of previous publications on heme-proteins [4, 6] we have calculated the SCF potential related to the starting structure, chosen on the basis of the information derived by diffraction data available on the protein data bank, but we were unable to recalculate it at each step of structural optimization.

Although the use of a full SCF potential is the main goal for a future quantitative XANES analysis, in this work we have tested the possibility to use refined OVL and $V_{0_{\text{imp}}}$ values to mimic both the SCF calculation and the overcoming of the MT approximation. This idea is based on the theoretical consideration that the scattering atomic $t$-matrixes depend on these two parameters via the Wronskians calculated at the boundary of the MT spheres for a given interstitial potential. On the other hand, the $t$-matrixes depend on both the charge density and the non-MT corrections. Hopefully, a judicious choice of OVL and $V_{0_{\text{imp}}}$ could minimize errors in the potential determination due to the present approximations, giving a better agreement with the experiment and an accurate structural recovery.

The main concern is expected to arise from the statistical correlation between OVL and $V_{0_{\text{imp}}}$ and the structural parameters, leading to systematic errors. A previous application to known hemes and hemeproteins in solution [7] showed that the final precision of this method is approximately comparable to that of a 1.4 Å resolved XRD, giving a 0.02 Å accuracy on the Fe-N$_\text{pyrrol}$ distance and 0.04-0.07 Å accuracy on the heme axial distances. This result suggested that the statistical correlation between potential and structural parameters is low and the induced systematic errors can be minimized by calibrating the potential parameters on known model structures. In this work we have modified the MXAN program to have a complete statistical analysis including potential parameters, with correlation between potential and structural parameters.

2. Methods

Details on the MXAN method were described previously [1, 4]. It is based on the full MS approach in the framework of the MT potential approximation. It also takes into account inelastic processes by way of a phenomenological Lorentzian broadening function.

3. Results

In Figure 1 we show the MXAN best fit of the Fe K-edge XANES spectrum of MbCO single crystal. The experimental spectrum (dotted line) is taken by orienting the X-ray photon vector almost parallel to the Fe-CO bond. For this orientation, the strong MS signal from the porphyrin macrocycle is suppressed, and the spectral features are extremely sensitive to the position and orientation of the axial ligands, i.e. the proximal histidine and the bound CO molecule. We have already successfully reproduced this spectrum [4] by using SCF-X$_a$ atomic potentials in the MXAN procedure. The SCF potential were calculated for the starting structure only. As the starting structure was the very accurate 1.1 Å resolved X-ray structure [8], we obtained an excellent reproduction of the spectrum by refining the structural parameters using the same (not-recalculated) SCF potential of the starting structure. Of course the closer the difference between the starting structure and the best fitting one, the more accurately this procedure works. Now in Figure 1 we show that by using non-SCF potentials, recalculating them at each step of optimization, and refining OVL and $V_{0_{\text{imp}}}$, we still get a nice fit of the spectrum: all the experimental features 1-7 are reproduced.
In Table 1 the MXAN results obtained with SCF, not recalculated and non-SCF, recalculated with OVL, V₀imp optimization potentials are compared. The two MXAN analyses provide identical (within the errors) values for the Fe-Nₚ and Fe-His distances and the Fe-C-O bending angle. A 0.02 Å discrepancy (out of errors) concerns the Fe-CO distance, whereas the C-O distance was not refined in the previous study. By comparing the new MXAN results with the 1.1 Å resolved X-ray structure [8] we still have an agreement on the Fe-Nₚ and Fe-His distances, and a discrepancy of 0.03 Å and 5° (out of errors) on the Fe-CO distance and the Fe-C-O bending angle, respectively. As far as it concerns the C-O distance, we measure 1.23(2) Å, larger than what found by XRD (0.1 Å out of errors), and consistent with a double C-O bond in a bent Fe-C-O coordination. However the 1.09 Å value of the X-ray structure is even shorter than the triple C-O bond found in literature for gaseous CO (1.13 Å) thus we believe the C-O bond length in MbCO to be underestimated by XRD.

### Table 1. Comparison between MXAN analyses of MbCO using different potential calculation methods.

| Method                        | Fe-Nₚ (Å) | Fe-His (Å) | Fe-CO (Å) | Bend (°) | C-O (Å) |
|-------------------------------|-----------|------------|-----------|----------|---------|
| SCF Potential [4]             |           |            |           |          |         |
| Not recalculated              | 2.00(2)   | 2.06(3)    | 1.83(2)   | 14(4)    | 1.07*   |
| V₀imp, OVL optim. Recalculated|           |            |           |          |         |
| XRD 1.1 Å [8]                 | 1.98(2)   | 2.06(2)    | 1.82(2)   | 9(3)     | 1.09(2) |

*data not refined

The MXAN fit of the XANES spectrum of nitrosyl-iron(II)-tetraphenyl-porphyrin (Fe(II)TPP-NO) in solution, is shown in Figure 2. The Fe(II) is penta-coordinated with a NO molecule as the only axial ligand. Its structure is precisely known by XRD [9]. Two fits have been performed, the former keeping fixed the structure, and varying only OVL and V₀imp, the latter leaving free to vary also the structure, along the parameters given in Table 2. Our full statistical analysis confirms the previously reported result [7]: in solution, that due to either the correlation between potential and structural parameters or the convolution of heme axial and heme planar signals, a systematic error (0.05-0.1 Å) affects the XANES determination of the axial Fe-heme distance.
Figure 2. Fe K-edge XANES spectrum of penta-coordinated Fe(II) in Fe(II)TPP-NO. Circles: experimental data; solid line: MXAN best fit obtained by potential and structure refinement; dotted line: unbroadened theoretical spectrum. All the features from the rising edge are reproduced. The experimental spectrum exhibits also a strong pre-edge peak. that is outside the MXAN energy range.

| Method                      | Fe-N_p (Å) | Fe-NO (Å) | Bend (°) | N-O (Å) |
|-----------------------------|------------|-----------|----------|---------|
| Fixed structure             | 2.00       | 1.71      | 30.9     | 1.12    |
| OVL=0.15 V0_imp=-5.94 eV    |            |           |          |         |
| Structural optimization     | 2.02(2)    | 1.82(2)   | 29       | 1.07    |
| OVL=0.05 V0_imp=-6.85 eV    |            |           |          |         |

4. Conclusions
The present work shows that it is possible to apply the method of refining the OVL and V0_imp MT parameters to obtain an excellent reproduction of spectra of hemes and hemeproteins. The systematic error introduced by these parameters when left free to vary is low, however it can be further reduced by a judicious choice of them, when model coordination systems are available.

References
[1] Benfatto M and Della Longa S 2001 J Synchrotron Radiat 8 1087
[2] D’Angelo P, Benfatto M, Della Longa S and Pavel N V 2002 Physical Review B 66
[3] Hayakawa K, Hatada K, D’Angelo P, Della Longa S, Natoli C R and Benfatto M 2004 J Am Chem Soc 126 15618
[4] Della Longa S, Arcovito A, Girasole M, Hazemann J L and Benfatto M 2001 Phys Rev Lett 87 155501
[5] Arcovito A, Benfatto M, Cianci M, Hasnain S S, Nienhaus K, Nienhaus G U, Savino C, Strange R W, Vallone B and Della Longa S 2007 Proc Natl Acad Sci USA 104 6211
[6] Arcovito A, Lamb D C, Nienhaus G U, Hazemann J L, Benfatto M and Della Longa S 2005 Biophys J 88 2954
[7] D’Angelo P, Lapi A, Migliorati V, Arcovito A, Benfatto M, Roscioni O M, Meyer-Klaucke W and Della Longa S 2008 Inorg. Chem. 47 9905
[8] Vojtechovsky J, Chu K, Berendzen J, Sweet R M and Schlichting I 1999 Biophys. J. 77 2153
[9] Scheidt W R and Frisse M E 1975 J. Am. Chem. Soc 97 17