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Inner shell excitation of glycine, glycyl-glycine, alanine and phenylalanine

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Abstract

Oscillator strengths for C 1s excitation spectra of gaseous glycine (Gly), alanine (Ala), phenylalanine (Phe) and glycyl-glycine (Gly-Gly), plus the N 1s and O 1s spectra of Gly and Gly-Gly, have been derived from inner shell electron energy-loss spectroscopy recorded under scattering conditions where electric dipole transitions dominate (2.5 keV residual energy, $\theta :\sim 2^{\circ}$). X-ray absorption spectra of solid glycine and glycyl-glycine were recorded in a scanning transmission X-ray microscope. Complications in solid state measurements associated with sample crystallinity and the benefits of spectroscopy in a microscope to resolve them are illustrated. Inner shell excitation spectral features characteristic of the peptide bond, readily identified by comparison of the spectra of glycine and glycyl-glycine, include: a ∼0.3 eV shift of the C 1s → $\pi^*_{C=O}$ peak, and introduction of a new pre-edge feature in the N 1s spectrum. These effects are due to 1s → π^*_{amide} transitions introduced with formation of the peptide bond. The concept of spectral additivity (building block model) is tested by a comparison of the C 1s spectrum of phenylalanine with those of benzene and alanine.
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Keywords: ISEELS; NEXAFS; Peptide bond; Amino-acid; Glycine; Glycyl-glycine; Gly-Gly-Gly; Alanine; Phenylalanine; Oscillator strengths; Inner shell excitation; Benzene

1. Introduction

Inner shell electronic excitation spectroscopy is a useful tool to investigate the electronic structure of complex molecules [1–3]. It is a site-specific probe with well understood selection rules. In this study we use comparisons of the inner shell excitation spectra of glycine (Gly) and glycyl-glycine (Gly-Gly) in the gaseous and solid phases to probe the links between inner shell spectral transitions and molecular structure, specifically those changes that relate to formation of the peptide bond. In addition, molecular additivity concepts—the "building block principle" [2]—are investigated for the amino acid phenylalanine, by comparison to the spectra of alanine and benzene.

This investigation is part of a general study of fundamental aspects of inner shell excitation of amino acids and peptides, which is motivated by increasing analytical use of near edge X-ray absorption spectroscopy (NEXAFS) for the analysis of biological systems. The C 1s X-ray absorption spectra of the 20 common amino acids (as solids deposited from acidic solution) was reported recently [4]. The C1s, N1s and O 1s X-ray absorption spectra of surface-adsorbed glycine [6,7]; all 20 of the common amino acids as thin films [4]; the O 1s spectra of thin films of selected amino acids [8]; and the C 1s spectra of selected amino acids and several small peptides (Gly-Tyr, His-Phe, Gly-Trp, Arg-Gly and Gly-Gly-Gly) [9] have been investigated. Quantum chemical calculations have been reported for surface-adsorbed glycine [6,7], and all 20 common amino acids [4,10,11]. The present work provides highlights of an extensive study of inner shell excitation of gaseous glycine, and glycyl-glycine by inner shell electron energy-loss spectroscopy (ISEELS), near edge X-ray absorption spectroscopy and Gaussian self-consistent field type 3 (GSCF3) ab initio computation [5]. Here we explore in greater depth the use of comparisons of single amino acids and peptides as a tool to identify the spectral features characteristic of peptide bonds. Since this approach relies heavily on the validity of the building block model

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[2], we also report and compare the C 1s spectra of alanine and phenylalanine, in order to test the validity of molecular additivity for the case of phenylalanine. In general one might expect only weak agreement between the electronic spectrum of phenylalanine and the sum of those of benzene and alanine. However, in core excitation there is significant distortion of the optical (ground state unoccupied valence) orbital by the core hole, which is generally considered to be localized at a specific atom in the molecule. This distortion can dominate both the energy and transition matrix element for those transitions, resulting in a strong similarity of the transition in a complex molecule with its counterparts in fragment molecules. This typically holds except in cases of partial or full delocalization between fragments.

2. Experimental

Glycine, glycyl-glycine, alanine (Ala) and phenylalanine (Phe) were obtained commercially from Sigma Aldrich in the form of crystalline powders, each with a stated purity better than 99%. They were used without further purification. Electron energy-loss spectra of gaseous glycine, alanine, phenylalanine and glycyl-glycine in the regions of C 1s, N 1s and O 1s excitation were acquired with a gas phase ISEELS spectrometer that has been described in detail previously [1]. All samples were placed inside a small aluminium tube attached directly to the collision cell of the spectrometer. The cell was then heated with an internally mounted quartz–halogen bulb, while the end plates of the gas cell were water cooled to trap the vaporized sample and avoid insulating deposits on sensitive parts of the spectrometer. The spectrometer was operated at small scattering angle ($\sim 2^\circ$) and high electron impact energy (2.5 keV + energy loss), conditions where electric dipole transitions dominate. The energy resolution was 0.5–0.8 eV depending on beam current. Energy scales were calibrated from of mixture of the analyte and a reference compound—CO_2 for C 1s and O 1s, N_2 for N 1s. The spectrum associated with a particular core edge was isolated from the underlying valence-shell and core ionization continua by subtracting a smooth curve determined from a fit of the function $a(E-b)^c$ to the pre-edge experimental signal. The background subtracted spectra were converted to absolute oscillator strength scales using previously described methods [1].

NEXAFS spectra were recorded using the bending magnet scanning transmission X-ray microscope at the Advanced Light Source [12,13]. For glycyl-glycine an amorphous deposit was produced by solvent casting from dilute aqueous solution onto an X-ray transparent Si_3N_4 window. The same procedure for glycine resulted in a highly crystalline film. An amorphous gel sample was subsequently prepared by partial dehydration of a glycine solution in a wet cell composed of two Si_3N_4 windows. Although the pH was not controlled with buffers to avoid any background, the pH of the dilute glycine solution would be ~8, a value where the species is predominantly in the zwitterion form. The results for these two samples are compared in detail below. Given the propensity of many small molecules to form single crystals which often exhibit strong polarization effects, this experience is of value to demonstrate methods of dealing with this challenging situation and obtaining the desired spectrum of the randomly oriented material. The NEXAFS spectra were calibrated by measuring the spectrum simultaneously with the spectra of gaseous CO_2, N_2 and O_2, introduced into the scanning transmission X-ray microscopy (STXM) chamber.

3. Results and discussion

3.1. Inner shell spectra of the peptide bond

Fig. 1 compares the C 1s, N 1s and O 1s spectra of gaseous glycine with that of glycyl-glycine, along with the difference spectra computed as 2(Gly-Gly-Gly) for C 1s and N 1s, but as 1.5(Gly-Gly-Gly) for the O 1s spectrum, to account for correct number of atoms of each type. This procedure isolates the spectral signature of the amide C 1s contribution, assuming there is very little difference in peak positions or relative intensities of the α-C or the terminal COOH carbon signals. These are considered reasonable assumptions given that these sites are isolated from the peptide bond by at least one saturated C–C bond. The difference spectra are the signature of peptide bond formation. The C 1s difference signal is dominated by a narrow strong band at 288.2 eV, which is assigned as the C 1s → $\pi^*_{C=ONH}$ amide transition. Note that this peak is energy shifted by ~0.35 eV to lower energy when compared with the position of the C 1s → $\pi^*_{C=OOH}$ peak in glycine (288.55 eV). It might be thought that the energy shift arises principally from a core level shift associated with change of the carbonyl carbon environment from 2 O atoms and a C atom in glycine, to 1 O atom, 1 N atom and 1 C atom in the amide carbonyl of glycyl-glycine. However, GSCF3 calculations [5] predict a considerably larger core level shift (−1.74 eV), but find the $\pi^*_{C=O}$ level to shift an almost similar amount (−1.79 eV), resulting in a predicted excitation energy shift in the same direction but smaller than that observed experimentally. Another measure of the reasonableness of the 0.35 eV difference between the glycyl-glycine C 1s → π^*_{CONH} and glycine C 1s → π^*_{COOH} transitions is the average 0.3 eV shift observed for a number of species in which there is a change from a (C, O, O) to a (C, O, N) environment [14,15].

The difference in the gas phase N 1s spectra is complicated because of relatively strong N 1s → 3s, 3p Rydberg transitions in both species. However, the difference spectrum (Fig. 1) is clearly dominated by a strong, narrow band at 401.7 eV which is assigned to N 1s → $\pi^*_{C=ONH}$ transitions associated with partial delocalization of the carbonyl π* orbital onto the amide N atom. The difference in the O 1s spectra of Gly and Gly-Gly is the most complicated situation since this change involves removal of an OH bond as well as

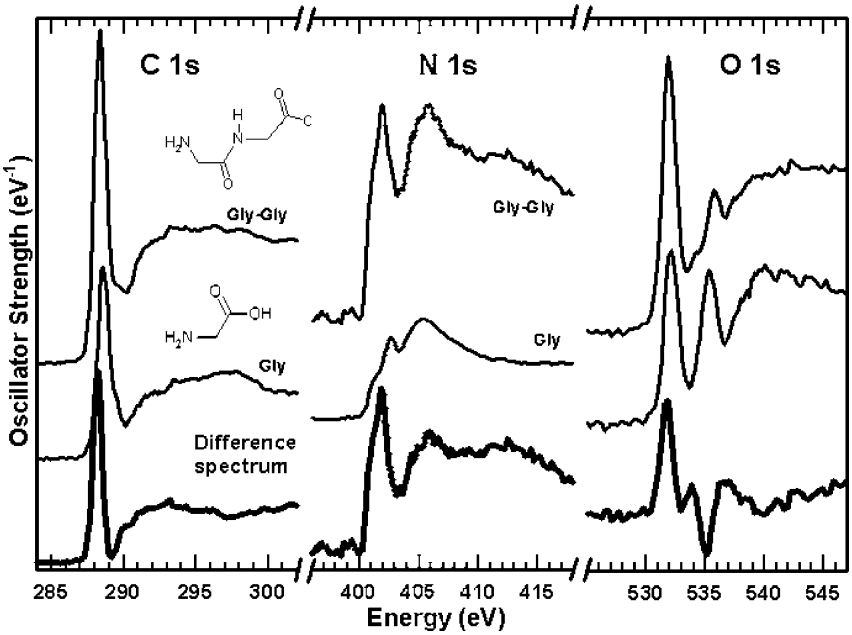

Fig. 1. Experimental C 1s, N 1s and O 1s oscillator strength spectra of gaseous glycine and glycyl-glycine measured by ISEELS, and their difference (thicker line), which is an estimate of the spectral signature of the peptide bond in the gas phase.

introduction of the peptide amide group. The most intense signal in the difference spectrum is a peak at 531.9 eV, corresponding to addition of the O 1s(CO) $\rightarrow \pi^*_{C=ONH}$ transition at the peptide bond. As for C 1s excitation, this peak is shifted to lower energy by ~0.3 eV when compared with the O 1s(CO) $\rightarrow \pi^*_{C=OOH}$ transition in glycine. Loss of the OH peak leads to the negative going peak at ~535 eV in the difference spectrum.

Fig. 2 plots the spectra of solid glycine and solid glycyl-glycine along with their differences using the same weighting scheme as applied for the gas phase spectra. Very similar trends are observed. The energy shift between the C 1s $\rightarrow \pi^*_{C=O}$ amide peak of the Gly-Gly and the C 1s $\rightarrow \pi^*_{C=O}$ carboxylic acid peak of glycine is ~0.45 eV. The difference in the size of the gas and solid state shifts may be associated with charge redistributions associated

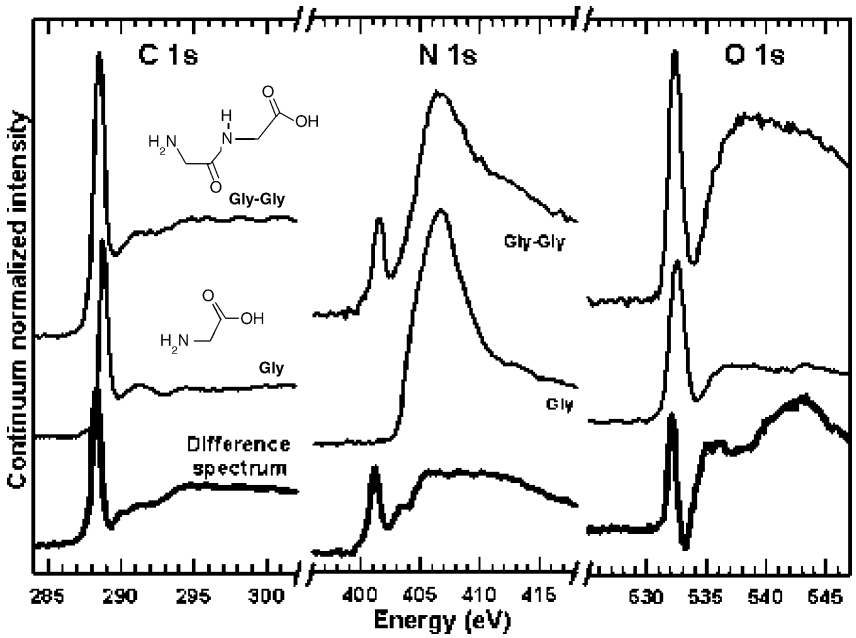

Fig. 2. Experimental C 1s, N 1s and O 1s spectra of solid glycine and glycyl-glycine measured by STXM, and their difference, which is an estimate of the spectral signature of the peptide bond in the solid phase.

with change from the neutral form in the gas phase to the di-charged zwitterion form in the solid state. The difference of the N 1s spectra of the solid species is the clearest signature of peptide bond formation. Since there are no Rydberg transitions, N 1s excitation at the amine (or, more correctly, the NH_3^+ group of the zwitterion) has no discrete structure. However, with formation of the amide peptide bond, a relatively strong N 1s $\rightarrow \pi^*_{C=ONH}$ transition occurs, giving rise to the peak at 401.1 eV in glycyl glycine. This peak position is in good agreement with that found for the corresponding peak in the N 1s spectra of proteins [5], where the N 1s $\rightarrow \pi^*_{C=ONH}$ transition totally dominates relative to the spectral contribution from the terminal NH_2 groups. The difference of the O 1s spectra exhibits a dominant peak at 532.1 eV, corresponding to the O 1s $\rightarrow \pi^*_{C=ONH}$ transition at the peptide bond. The energy shift in the solid state (~0.25 eV) is very similar to that found for the gas phase.

The differences in the C 1s, N 1s and O 1s spectra of gaseous and solid glycine and glycyl-glycine clearly contain features associated with peptide bond formation: a clear broadening and a ~0.4 eV shift of the C 1s $\rightarrow \pi^*_{C=O}$ peak from amino-acid to "peptide spectrum", a slightly smaller (~0.3 eV) shift in the O 1s $\rightarrow \pi^*_{C=O}$ peak from amino-acid to "peptide spectrum", and introduction of a new pre-edge feature in the N 1s "peptide spectrum". These features are all associated with 1s $\rightarrow \pi^*_{amide}$ transitions which are introduced with formation of the peptide bond. This shift was previously noted in previous comparisons of the C 1s NEXAFS of mono, di- and tri-amino acid systems [9]. However, that work only considered the C 1s edge and the difference spectra were not presented.

3.2. C 1s spectroscopy of alanine and phenylalanine

Fig. 3 presents the C 1s oscillator strength spectra of gaseous alanine and phenylalanine along with the previously published spectrum of benzene [16], derived from ISEELS. The C 1s spectra of alanine and phenylalanine have been previously reported in the solid phase over a rather small energy range (~284–292 eV) [9]. The present results are in good agreement with those data. If the molecular additivity concept applies, one would expect the spectrum of phenylalanine to be similar to the sum of the spectra of benzene and alanine. Small changes associated with the replacement of 2 C–H bonds with a C–C bond are to be expected, but beyond that, if the concept of molecular additivity (building block [2]) is applicable in this system, good agreement should be found. As shown in Fig. 3, this is indeed the case. The phenylalanine spectrum is dominated by the C 1s $\rightarrow 1\pi^*_{ring}$ transition at 285.20 eV. This is slightly higher in energy than the C 1s $\rightarrow 1\pi^*_{ring}$ transition in benzene, consistent with a shading of the peak to higher energy by the chemical shift of the C 1s(C – R) $\rightarrow 1\pi^*_{ring}$ component. The second strongest signal in phenylalanine is the C 1s $\rightarrow \pi^*_{C=O}$ transition at 288.7 eV. This is positioned slightly higher than the corresponding feature in alanine (288.5 eV). This shift

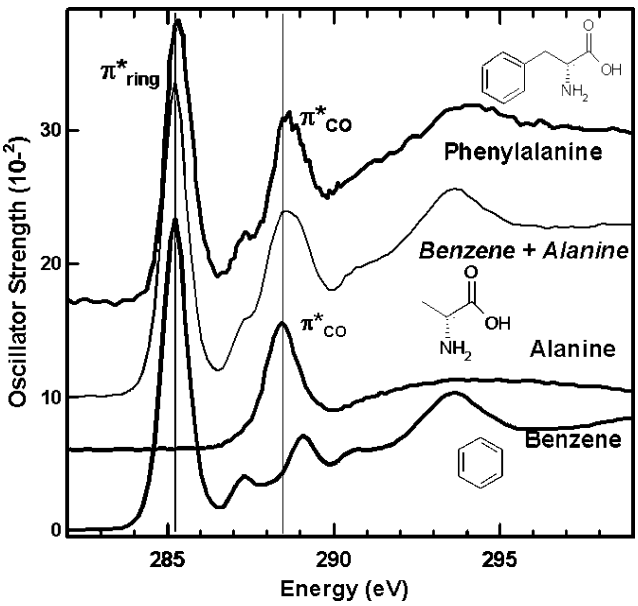

Fig. 3. Experimental C 1s oscillator strength spectra of gaseous benzene [16], alanine and phenylalanine derived from ISEELS measurements. The sum of the alanine and benzene spectra is also shown (thinner line).

is clearly associated with the contribution of the relatively strong C 1s $\rightarrow 2\pi^*_{ring}$ peak at 289.0 eV in benzene. The agreement of the additivity estimate with the experimental spectrum of phenylalanine is remarkably good. At least in this case, it can be seen that the so-called "building block principle" [2] for inner shell excitation spectra of complex molecules works very well. This is valuable information for NEXAFS and X-ray microscopy studies of amino-acids and peptides, since it indicates that the phenyl moiety of phenylalanine, and by extension possibly the aromatic parts of other aromatic amino acids (e.g. tyrosine), may be modelled to a good first approximation as the sum of an aromatic and a non-aromatic part.

3.3. NEXAFS spectroscopy of crystalline and gel forms of glycine

Fig. 4 compares NEXAFS spectra of two orientations of crystalline glycine with the NEXAFS spectra of a randomly oriented glycine gel. STXM micrographs recorded at 288.6 eV photon energy ($\pi^*_{C=O}$) are presented on the right side of this figure. The measurements were made using linescan spectral mode. In the case of the crystalline sample, in one part of the line the sample was oriented such that the π* out-of-plane transitions are excited, while the sample in a different part was oriented such that the σ* in-plane transitions are excited. For the gel sample, the spectrum was uniform in shape along the line. Because the gel contained a lot of water, the O 1s spectrum plotted for the randomly oriented sample is actually an average of the two oriented spectra. These results indicate that one must be sensitive to possible orientation effects when dealing with NEXAFS

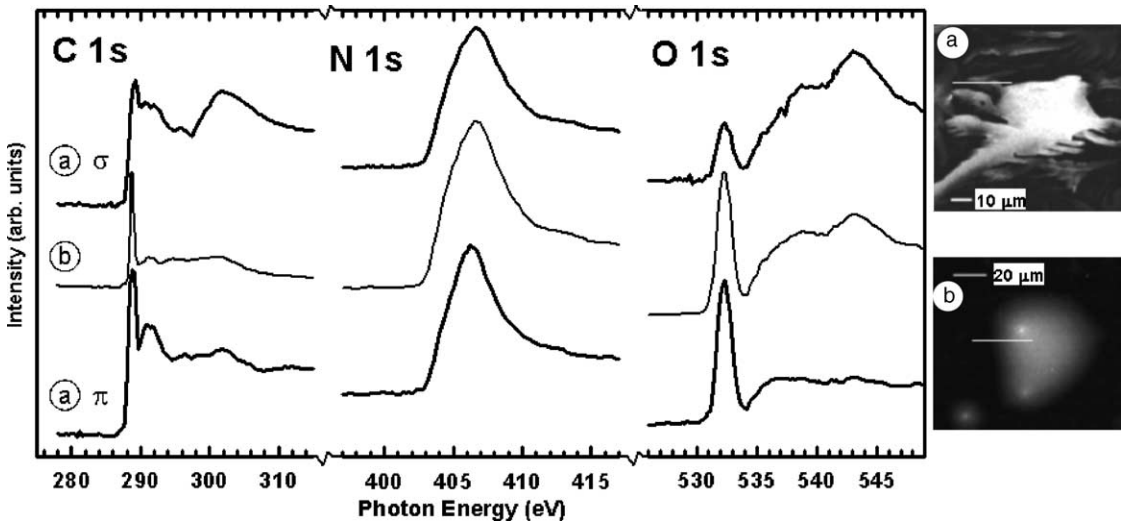

Fig. 4. NEXAFS spectra of solid glycine in the C 1s, N 1s and O 1s regions recorded with STXM from a crystalline sample from which samples in two orthogonal orientations relative to the *E*-vector of the light were sampled (upper and lower traces); and in the C 1s and N 1s region from a randomly oriented gel sample (central traces). The O 1s spectrum for randomly oriented glycine is the average of the two limiting crystalline spectra since the O 1s spectrum of the gel was dominated by the spectrum of water. STXM images at 288.6 eV for the crystalline and gel samples in the regions measured are displayed on the right.

spectra of molecular solids. It also illustrates the power of STXM microscopy to obtain meaningful reference spectra even for a species with a strong tendency to form oriented crystals. Advantages of using STXM microscopy as opposed to non-spatially resolved spectroscopy include: adaptability to both wet and dry samples, requirement for only microscopic amounts of sample, and an ability to tolerate wide variations in sample thickness.

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References

[1] A.P. Hitchcock, J. Electron. Spectrosc. Rel. Phenom. 112 (2000) 9.
[2] J. Stöhr, NEXAFS Spectroscopy, Springer Tracts in Surface Science, 1992, p. 25.
[3] N. Kosugi, in: T.K. Sham (Ed.), Chemical Applications of Synchrotron Radiation, World Scientific, 2002, p. 228.
[4] K. Kaznacheyev, A. Osanna, C. Jacobsen, O. Plashkevych, O. Vahtras, H. Ågren, V. Carravetta, A.P. Hitchcock, J. Phys. Chem. A 106 (2002) 3153.
[5] M.L. Gordon, G. Cooper, T. Araki, C. Morin, C.C. Turci, K. Kaznatcheev, A.P. Hitchcock, J. Phys. Chem. A 107 (2003) 6144.
[6] J. Hasselström, O. Karis, M. Weinelt, N. Wassdahl, A. Nilsson, M. Nyberg, L.G.M. Pettersson, M.G. Samant, J. Stöhr, Surf. Sci. 407 (1998) 221.
[7] M. Nyberg, J. Hasselström, O. Karis, N. Wassdahl, M. Weinelt, A. Nilsson, L.G.M. Pettersson, J. Chem. Phys. 112 (2000) 5420.
[8] M. Tanaka, K. Nakagawa, T. Koketsu, A. Agui, A. Yokoya, J. Synchrotron Rad. 8 (2001) 1009.
[9] J. Boese, A. Osanna, C. Jacobsen, J. Kirz, J. Electron. Spectrosc. Rel. Phenom. 85 (1997) 9.
[10] V. Carravetta, O. Plashkevych, H. Ågren, J. Chem. Phys. 109 (1998) 1456.
[11] L. Yang, O. Plashkevych, O. Vahtras, V. Carravetta, H. Ågren, J. Synchrotron Rad. 6 (1999) 708.
[12] T. Warwick, H. Ade, A.L.D. Kilcoyne, M. Kritscher, T. Tylisczcak, S. Fakra, A.P. Hitchcock, P. Hitchcock, H.A. Padmore, J. Synchrotron Rad. 9 (2002) 254.
[13] A.L.D. Kilcoyne, T. Tylisczak, W.F. Steele, S. Fakra, P. Hitchcock, K. Franck, E. Anderson, B. Harteneck, E.G. Rightor, G.E. Mitchell, A.P. Hitchcock, L. Yang, T. Warwick, H. Ade, J. Synchrotron Rad. 10 (2003) 125.
[14] S.G. Urquhart, H. Ade, J. Phys. Chem. B 106 (2002) 8531.
[15] R.J. Lessard, E. Al-Hassan, D. Tulumello, G.C. Cooper, A.P. Hitchcock, unpublished.
[16] J.A. Horsley, J. Stöhr, A.P. Hitchcock, D.C. Newbury, A.L. Johnson, F. Sette, J. Chem. Phys. 83 (1985) 6099.