

X-ray absorption spectroscopic studies on model compounds for biological iodine and bromine

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X-ray absorption spectra of a number of organic iodine and bromine compounds of biological relevance, as well as of a series of iodine compounds with different oxidation states, have been measured. The iodine *K*-edge spectra (XANES) are found to be relatively featureless but the position of the edge is found to be sensitive to formal valence (among other factors), and the edge shape to the number of bound O atoms. EXAFS spectra of organohalogen compounds (both iodine and bromine) can be used to discriminate between aliphatic and aromatic compounds. There are differences both in the distances from the halogens to the first shell of C atoms, which are shorter for aromatic compounds, and in the patterns of shells in the Fourier transforms. This result is expected to be relevant to studies at these edges in biological systems.

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1. Introduction

In recent years, X-ray absorption spectroscopy has been applied to elucidate the chemistry of an increasing variety of elements in biological systems, initially mainly relatively abundant transition metals in metalloproteins (Cramer, 1988), more recently also trace elements like Sr (Greigor *et al.*, 1997) or Hg (Harris *et al.*, 2003), also including non-metals like S (Pickering *et al.*, 1998, 2001) and Se (Pickering *et al.*, 1999; Lee *et al.*, 2001). We have recently started studies on the halogen metabolism of brown algae, in which we expect to be able to establish both the chemical environment as well as the formal oxidation state of iodine and bromine under a number of physiologically relevant circumstances. We report here on our preparatory study on a number of model compounds relevant to the biological studies by which we explore the potential of the X-ray absorption technique to identify both chemical environments and oxidation states of iodine and bromine. The compounds were selected with the specific problem of halogen metabolism by brown algae in mind but are expected to have relevance to other future biological X-ray absorption studies at the iodine and bromine *K* edges.

The current view on the biogeochemical cycle of iodine is schematically represented in Fig. 1. The most important role of iodine in mammals is in the thyroid hormones thyroxine and T₃, which are both iodinated tyrosine derivatives that are interconverted by iodination/deiodination, and play a role in

regulating the basal metabolism rate. Iodine occurs in sea water mainly as iodate but is reduced to iodide possibly through the action of nitrate reductase in bacteria and phytoplankton (Farrenkopf *et al.*, 1997; Wong *et al.*, 2002). In this form it is actively accumulated by brown algae like *Laminaria digitata* by a factor of 10⁶ to a concentration as high as 73 mM through the action of vanadium haloperoxidase, requiring hydrogen peroxide as a cosubstrate (Küpper *et al.*, 1998). Not surprisingly, iodine was first identified as an element early in the 19th century (Courtois, 1813) in the ashes of these brown algae (kelp). Laminariales can be considered a major contributor to the biogeochemical pump of iodine from the ocean to the atmosphere (Carpenter *et al.*, 2000) as they are very active in the so-called iodovolatilization, *i.e.* the incorporation of iodine in volatile organoiodine compounds. The established role of haloperoxidase, which can both oxidize halides to the hypohalous acid level as well as incorporate halogens in organic substrates (for review, see Butler *et al.*, 2001) in the accumulation of iodine (Küpper *et al.*, 1998), raises the question as to what form it is stored in algae, possibly in specialized organelles. The question of the chemical form of accumulated iodine in brown algae had already evoked interest in the 19th century, but is still controversial despite a number of studies. Eschle (1897) reported that iodine in *Laminaria* is present 'almost exclusively' in organic form. In contrast, Kylin (1929) suggested that, in *Laminaria*, iodine was present mainly as iodide, but presumed also the existence of organically bound iodine. Roche & Yagi (1952) proposed that the iodine was present as

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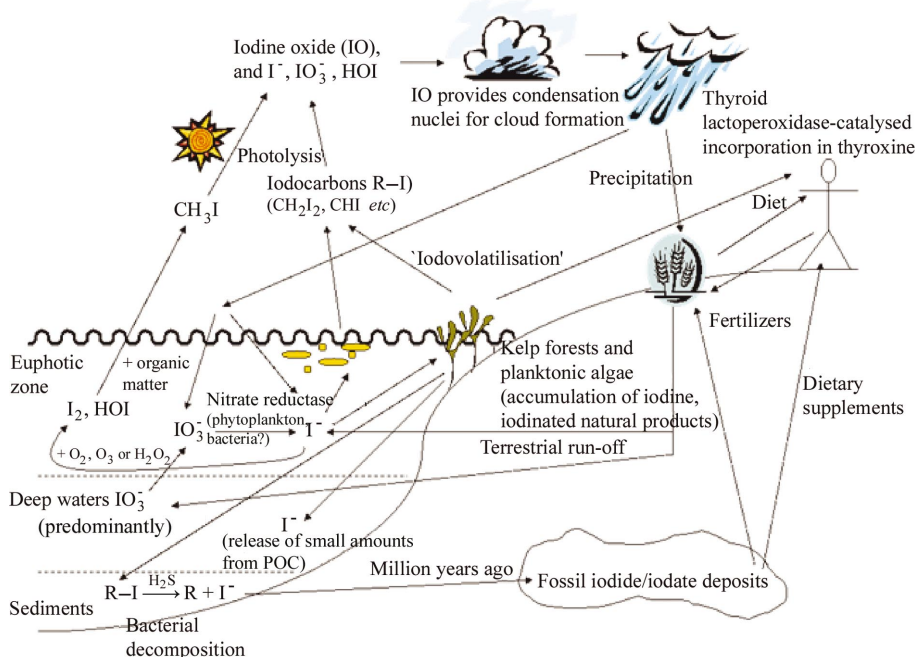


Figure 1
Simplified biogeochemical cycle of iodine. In sea water, iodine (total concentration $0.5 \mu\text{M}$) is mainly present as iodate, yet up to 50% of this can be converted to iodide in the euphotic zone, presumably as a side reaction of nitrate reductase of planktonic algae and bacteria (e.g. Farrenkopf *et al.*, 1997; Wong *et al.*, 2002). Iodide can be taken up, possibly by the action of vanadium-containing haloperoxidase (Wever *et al.*, 1991), by a variety of algae (Küpper *et al.*, 1998), which constitute the major biogeochemical pump of iodine from the ocean to the atmosphere, in the form of iodinated halocarbons (Carpenter *et al.*, 2000). Besides emission by algae, a non-biological pathway to methyl iodide formation has been suggested, based in the reaction of organic matter in sea water with I_2 or HOI formed by oxidation of I^- in the upper water column (for review, see Luther *et al.*, 1995). Iodocarbons are rapidly photolyzed and oxidized, resulting in the formation of iodine oxide (Alicke *et al.*, 1999). IO has recently been recognized as a contributor of condensation nuclei for cloud formation in the coastal atmosphere (O'Dowd *et al.*, 2002), resulting in its deposition by precipitation. On land, iodine is accumulated by both terrestrial plants and animals, being an essential trace element for the biosynthesis of the thyroid hormone, thyroxine, by the action of lactoperoxidase. Part of the iodine entering the agricultural and human food chain nowadays originates from sea foods, including seaweeds, and from iodine supplements added to table salt from fossil iodide/iodate deposits.

iodotyrosines, which are potential products of the action of haloperoxidase on tyrosine (Almeida *et al.*, 2001). In contrast, Amat & Srivastava (1985) and Amat (1985) reported the extraction of only iodide from *Laminaria*, not detecting iodotyrosines. All these results were obtained after heating and drying or extraction of algal tissues. Owing to the chemical instability of many organic iodine compounds and the reactivity of the biological medium, they have to be taken with a certain caution, and non-invasive *in situ* measurements like X-ray absorption spectroscopy appear desirable. Furthermore, non-invasive methods are indispensable for studying functional aspects of intracellular iodine chemistry under different physiological circumstances.

Like iodine, bromine is an essential trace element for the growth of brown (Woolery & Lewin, 1973) and red (von Stosch, 1962) macroalgae. The concentration of bromine in sea water is three orders of magnitude higher (0.7 mM , virtually all bromide ions) than that of iodine, and it is incorporated by various organisms (bacteria, diatoms, algae) in a stunning variety of organic compounds (Gribble, 2000). It is imaginable

that, like that of iodine, the accumulation of bromine by algae is mediated by haloperoxidase, again raising the question in what form it is stored. In fact many of the volatile organohalogenes formed by the algae, as mentioned above for iodine, are mixed iodine/bromine compounds (Carpenter *et al.*, 2000).

In setting up the study we were particularly interested in two aspects: (i) the determination of the oxidation state from the edge, and (ii) the determination of the chemical environment from the fine structure. The X-ray absorption spectrum (XAS; Koningsberger & Prins, 1988) can be divided into two parts, *viz.* the region near the edge or X-ray absorption near-edge spectrum (XANES), and the region extending a few hundreds of eV beyond the edge, the so-called extended X-ray absorption fine structure (EXAFS). As it is expected that the edge position in the XANES part of the spectrum will shift to higher energy when the charge on the central atom is higher, this provides information on its (formal) oxidation state. The intensity of possible transitions in the edge region usually provides information on the symmetry of the environment, and the edge shape has been adequately reproduced in recent model calculations (Mijovilovich & Meyer-Klaucke, 2003; Benfatto & Della-Longa, 2001). Moreover, the white-line intensity

correlates for similar systems with the number of ligands, as demonstrated in a study on non-heme iron in phenylalanine hydroxylase (Mijovilovich & Meyer-Klaucke, 2003). The EXAFS part of the spectrum in fact measures X-ray induced electron diffraction, constituting an interference pattern of electron waves going out of the central atom and back-scattered by surrounding atoms; it can be background-subtracted and Fourier-transformed to reveal, after phase correction, a radial distribution function of atoms around the central atom.

Earlier applications of XAS to halogens in biological systems focused on the possible coordination of halogens to metal ions, *viz.* of Br to V in haloperoxidase (Dau *et al.*, 1999; Rehder *et al.*, 2000), and of Cl to Mn in photosynthetic systems (Rompel *et al.*, 1997). In an early application of XAS on iodine in a biological system, a study (Hashimoto & Ishii, 2000) on iodine in the strawberry conch *Strombus luhuanus* (Ishii, 1997), an environment of carbon neighbours, presumably in a protein, was identified, which was, however, not defined. In the case of organoiodine or organobromine compounds, it is of

interest to see if there are shells in the Fourier transforms of the spectra beyond the first shell of directly bound carbon, that allow the discrimination between the incorporation of the halogen atom in a molecule that has just a single carbon, or in a molecule where the carbon is either in an aliphatic or an aromatic environment. Besides representatives of inorganic iodine (iodide, iodate) and corresponding bromine compounds, covering the range of oxidation states of -1 to $+7$ for iodine, a selection of organohalogen compounds with the halogen in various biologically relevant environments were therefore measured, compared and simulated.

It was realised that, in the case of the presence of additional shells in the Fourier transforms, the spectra would have to be analysed and simulated with multiple-scattering calculations. In an earlier XAS study on organobromine compounds, simulations for a similar set of compounds using the simulation programme *GNXAS* were reported (Burattini *et al.*, 1993), which were in good agreement with the experimental data and described the multiple-scattering phenomena adequately. The earlier aforementioned biological Br studies used the analysis programme *FEFFIT* (Stern *et al.*, 1995). In the present study, we preferred the application of *EXCURVE* (Gurman *et al.*, 1984, 1986; Binsted *et al.*, 1992). Owing to its restrained refinement option it is better adapted to deal with one specific target of this study, the discrimination between aliphatic and aromatic compounds in an unknown biological sample.

2. Material and methods

2.1. Compounds

3-Iodotyrosine, 3,5-diiodotyrosine, 4-bromophenylalanine and 3,5-dibromotyrosine were obtained from Sigma; iodoform was from Acros; all other compounds were from Aldrich.

2.2. EXAFS measurements

EXAFS measurements were carried out in the European Molecular Biology Laboratory (EMBL) Outstation in the Hamburg Synchrotron Laboratory (HASYLAB) at the Deutsches Elektronensynchrotron (DESY) in Hamburg, Germany. For I *K*-edge measurements, a Si 311 monochromator was used and the station was set up without a focusing mirror. For Br *K*-edge measurements the set-up comprised a Si 111 monochromator and a focusing mirror with a cut-off energy of 21.5 keV. In both cases monochromators were order-sorting and set at 50% of peak intensity to suppress harmonics (Hermes *et al.*, 1984). During data collection, the storage ring DORIS III was operated at 4.5 GeV with ring currents between 150 and 90 mA. The EXAFS station features a CANBERRA 13-element solid-state fluorescence detector which was used for the solution measurements. Solid samples were diluted in BN to a concentration which would approximately give an edge step of the X-ray absorption coefficient μ of 1. HESAR glass cells with Kapton (or polyimide) windows were used for the solid samples and the aqueous solutions; for organic solvents we

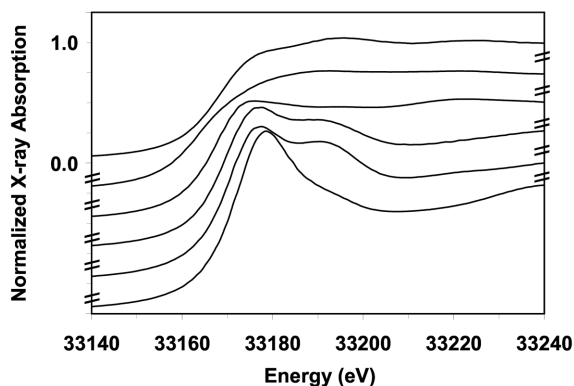
used a glueless cell with Kapton windows (Sprakel *et al.*, 2002). For the Br *K*-edge measurements, the energy-calibration device (Pettifer & Hermes, 1985) was applied. Scans at the I *K*-edge were calibrated by reference to the absorption spectrum of a NaI sample between the signal ion chamber and the calibrator detector. Typically three to five scans per sample were taken. The sample was kept at 20 K during the measurements and moved in between scans so that the part of the sample that was exposed to the beam was varied as much as possible. No spectroscopic differences between scans were observed.

2.3. EXAFS data reduction and simulations

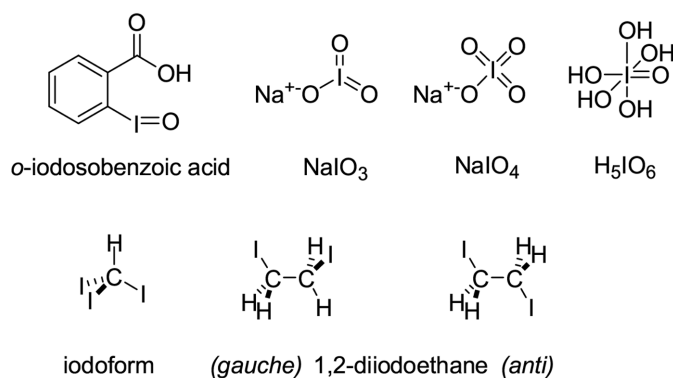
Data reduction was carried out at the EMBL and in Nijmegen with the EMBL Outstation data-reduction package (Nolting & Hermes, 1992), including the energy calibration programmes *CALIB* and *ROTAX*, the averaging programme *AVERAGE* and the background-subtraction programme *REMOVE*. Simulations of the calibrated, averaged and background-subtracted EXAFS were carried out on the CLRC Daresbury Laboratory (Warrington, UK) dedicated EXAFS computer, *xrserve1*, and at the EMBL computer, *butthead*, using the 1998 release of the EXAFS simulation programme *EXCURVE* (Gurman *et al.*, 1984, 1986; Binsted *et al.*, 1991). The *ab initio* calculation of phase shift and back-scattering factors in *EXCURVE* was run with its default options, *i.e.* Hedin–Lundqvist ground state (Hedin & Lundqvist, 1969) and the von Barth exchange potential (von Barth & Hedin, 1972). Multiple-scattering simulations were run in the default setting of small-atom approximation (Rehr & Albers, 1990) with the maximum path length (*plmax*) extended from the default value of 10 to 15 Å where appropriate. *EXCURVE* was selected for the EXAFS simulations because of the possibility of simulating and iteratively refining the multiple scattering of rigid ring systems with restraints (Binsted *et al.*, 1992). The difference between constrained refinement, in which an idealized geometry is imposed, and restrained refinement is that in the latter more parameters are refined but that deviations from idealized values for the interatomic distances are weighted and incorporated in the fit index during the iteration. In the present work, restrained refinements of the EXAFS of aromatic iodine and bromine compounds were run by analogy to our previous work on Cu pyridine complexes (Feiters *et al.*, 1999).

3. Results and discussion

A comparison of edges of compounds with different formal oxidation states for iodine is made in Fig. 2 (see Fig. 3 for structures). The energy at half-height of such edges has been found to be a reasonably reliable measure of the edge position (Dau *et al.*, 2003). For a series of iodine compounds, we observe that the edge position shifts to higher energy with valence, going from iodine in BN (formal valence 0, 33164.1 eV) to *o*-iodosobenzoic acid (+1, 33165.4 eV), iodate (+5, 33167.8 eV) and periodate (+7, 33168.5 eV). However, it

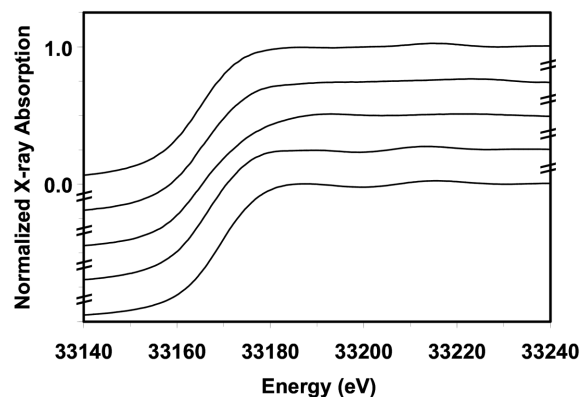

Figure 2

Iodine X-ray absorption K edges of compounds (see Fig. 3) of different valency of iodine. From top to bottom: NaI (20 mM in water, valency -1); I_2 (with BN, 0); *o*-IO-benzoic acid (+1); $NaIO_3$ (10 mM in water, +5); $NaIO_4$ (with BN, +7); H_5IO_6 (with BN, +7).

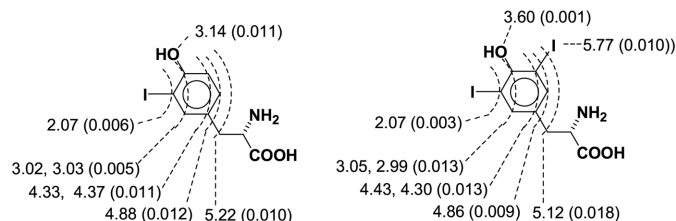

Figure 3

Structures of iodine compounds.

should be noted that iodide (formal valence -1 , 33167.7 eV) does not follow the trend, having approximately the same energy at half-height as iodate (+5), and there is still a considerable energy step from periodate to H_5IO_6 (33170.3 eV) although there is no change in formal valence. The iodine and iodide spectra do not exhibit special features, but for the other compounds the intensity of the white line between 33170 and 33180 eV is found to increase roughly with the number of O atoms bound to iodine, going from *o*-iodosobenzoic acid *via* iodate to periodate and its fully hydrated form, H_5IO_6 , whereas the shoulder at 33190 eV is particularly strong in iodine compounds with a large number of doubly bound O atoms. Considering that the doubly bound O atoms are on average at a shorter distance from the iodine than the other O atoms, the shift to higher energy for the edge position going from periodate to H_5IO_6 is in line with the observed shift to higher energy with longer average ligand distance for Mn compounds (Dau *et al.*, 2003). Compared with Fig. 2, the edges of the organic iodine compounds (Fig. 4, see Figs. 3 and 5 for structures) do not show characteristic features, and therefore do not allow distinction of the chemical speciation of iodine in these compounds, unlike the situation for *e.g.* sulfur compounds when measured at the sulfur K edge (George & Gorbaty, 1989; Pickering *et al.*, 2001). The position of the edge


Figure 4

Iodine X-ray absorption K edges of organoiodine compounds (see Figs. 3 and 5) compared with elementary iodine. From top to bottom: 1,2-diiodoethane in BN; CHI_3 in BN; I_2 in tetrahydrofuran/acetonitrile 1/1; 3-iodotyrosine; 3,5-diiodotyrosine.


Figure 5

Structures of 3-iodotyrosine (left) and 3,5-diiodotyrosine (right) with parameters of the refined simulations of Figs. 7(d) and 7(e), respectively; all atoms were included in multiple-scattering units whose occupancies refined to 1.2 and 1.0, respectively. Distances in Å, Debye–Waller-type factors quoted as $2\sigma^2$ in Å² in parentheses. 3-Iodotyrosine: energy range, 18–600 eV; ΔEF , -4.137 eV; fit index (k^3 -weighting), 0.1785. 3,5-Diiodotyrosine: energy range, 18–600 eV; ΔEF , -0.367 eV; fit index (k^3 -weighting), 0.1718.

for the organic compounds could have been expected to depend, for example, on the number of other iodine atoms bound to a carbon, or the hybridization of the carbon, but the energy resolution (± 2 eV) is too low to allow these trends, if at all present, to be distinguished. Compared with the Br K -edge spectra, which will be discussed below, the I K edges are relatively featureless. The difference is due to the core-hole lifetime effect for iodine, *i.e.* the broadening of the spectral features because of the reduced lifetime of the excited state. The iodine compounds might show more characteristic features at the L edge but that was not included in this study because (i) the mean free path of photons in biological material at the energy of the I L edge (L_3 edge at 4560 eV) is relatively short, resulting in a large absorption and a low fluorescence yield compared with the I K edge, and (ii) the EXAFS energy range for the I L edge is limited to 300 eV, owing to the partial overlap of the three L edges.

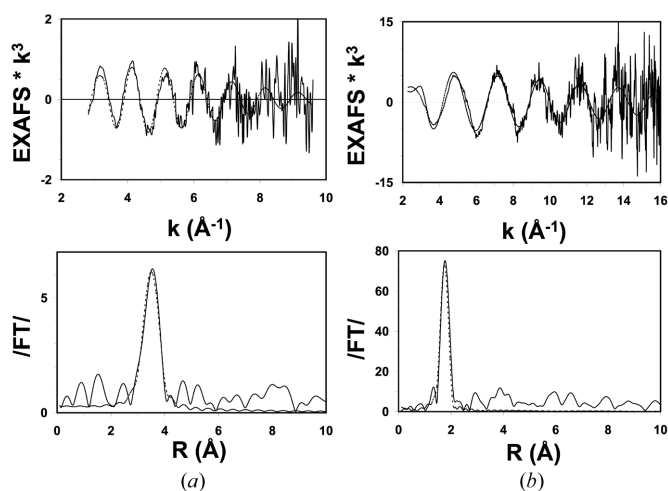
The EXAFS and Fourier transform of sodium iodate in aqueous solution (Fig. 6b) show very strong signals, which can be simulated with a shell of close O atoms at 1.79 Å (Table 1) in line with recent literature results (Bonhoure *et al.*, 2002). On

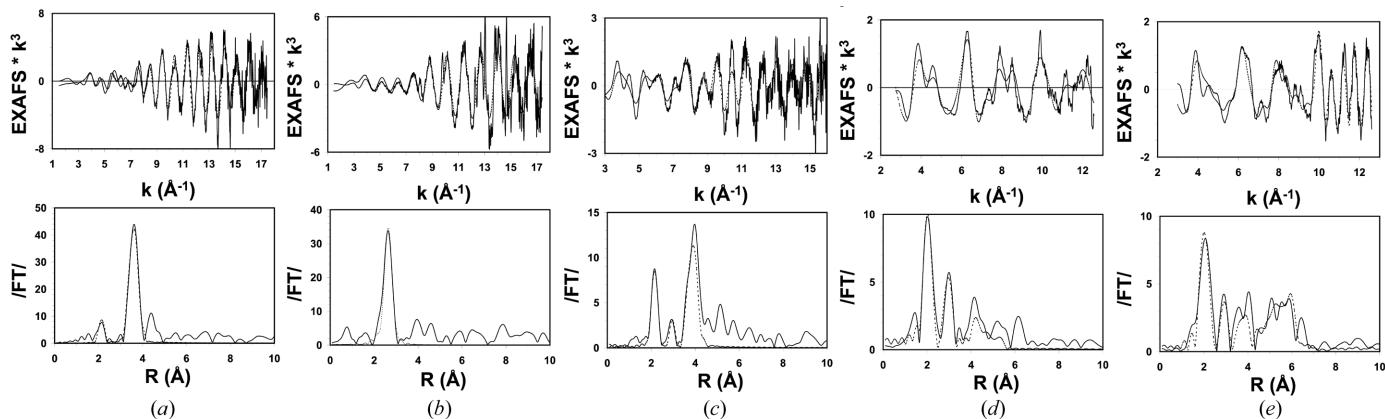
Table 1

Parameters for the simulations of the EXAFS of iodine compounds.

 Energies in eV; distances in Å; Debye–Waller-type factors quoted as $2\sigma^2$ in Å² in parentheses. The parameters for the simulations of the aromatic iodine compounds are given in Fig. 5.

	NaI (aq.) (Fig. 6a)	NaIO ₃ (aq.) (Fig. 6b)	CHI ₃ /BN (Fig. 7a)	I ₂ /ethanol (Fig. 7b)	EtI ₂ /BN (Fig. 7c)
ΔEF (eV)	-5.16	-7.55	-10.66	-11.24	-3.711
Energy range (eV)	20–350	13–976	5–1166	5–1160	5–647
O	10.0 at 3.56 (0.034)	3.4 at 1.79 (0.004)	–	–	–
C	–	–	0.7 at 2.16 (0.001)	–	1.0 at 2.18 (0.005)
C	–	–	–	–	0.7 at 2.99 (0.006)
I	–	–	2.6 at 3.57 (0.005)	0.8 at 2.71 (0.004)	1.0 at 3.85 (0.006)
Fit index (k^3 -weighting)	2.6948	1.3019	0.6964	1.2472	1.1285


Figure 6

 Experimental (solid) and simulated (dashed) k^3 -weighted EXAFS (top) and phase-corrected Fourier transform (bottom) of (a) 20 mM NaI in water and (b) 10 mM NaIO₃ in water. The parameters of the simulations are listed in Table 1.

Figure 7

 Experimental (solid) and simulated (dashed) k^3 -weighted EXAFS (top) and phase-corrected Fourier transform (bottom) of (a) CHI₃/BN, (b) I₂ in tetrahydrofuran/acetonitrile 1/1, (c) 1,2-diiodoethane/BN, (d) 3-iodotyrosine and (e) 3,5-diiodotyrosine/BN. The parameters of the simulations in (a), (b) and (c) are listed in Table 1, those for (d) and (e) in Fig. 5.

the contrary, the weak signal observed for the sodium iodide solution (Fig. 6a) reflects the absence of any atoms covalently bound to the atom of the iodide ion, the only detectable contribution being the O atoms of the first shell of solvation by water molecules. The distance of 3.56 Å is in good agreement with the values of 3.60 Å found in an X-ray diffraction study on a concentrated aqueous NaI solution (Maeda & Ohtaki, 1975) and the 3.50 Å from earlier EXAFS studies (Tanida & Watanabe, 2000). The occupancy of 10 appears to be somewhat overestimated compared with the value of 6 that was assumed in those studies, but agrees well with the 10–11 close O atoms identified in the environment of the iodide ion in an X-ray crystallographic study (Thiele & Putzas, 1984).

The EXAFS and Fourier transforms of iodine (Fig. 7b) show the effect of iodine backscattering, which can be distinguished from other backscattering contributions like that of carbon by its characteristic higher amplitude at higher k , as also shown by the spectra of various organic compounds containing multiple iodine atoms, like iodoform (CHI₃, Fig. 7a) and 1,2-diiodoethane (EtI₂, Fig. 7c). The spectra of the organoiodine compounds indicate that there are contributions of other atoms than just the carbon bound to the central iodine atom; this means that, unlike the I K -edge spectra, the I K EXAFS would offer the possibility of distinguishing different chemical environments for iodine even within the class of organoiodine compounds. It is therefore of interest to compare the spectra of aliphatic iodine compounds like that of 1,2-diiodoethane (Fig. 7c) with those of aromatic compounds, for example 3-iodotyrosine (Fig. 7d). Interestingly, the latter spectrum shows in its Fourier transform the pattern that is known from studies of metal complexes to be characteristic for coordination of a metal by a (hetero-)aromatic ring, such as observed for Cu pyridine complexes (Karlin *et al.*, 1988; Feiters *et al.*, 1999); even the atoms that would appear to be

very remote from the central atom contribute very strongly to the spectrum, owing to the rigidity of the ring and the presence of reinforcing multiple scattering effects in it. Clearly, aliphatic and aromatic organoiodine compounds can be distinguished on the basis of the EXAFS spectra, in particular their Fourier transforms. This is a very important insight which is expected to have its impact on biological iodine EXAFS studies.

The simulations for the compounds containing multiple iodine atoms in Figs. 7(a)–7(c) and Fig. 7(e) show that the characteristic amplitude envelope of iodine is well reproduced at various distances. The EXAFS in Figs. 7(a)–7(c) is dominated by the backscattering of the other iodine atoms in the molecule, which has a high amplitude at high k ; this tends to suppress the significance in the refinement of weaker contributions that dominate the spectrum at lower k . This effect results in deviations between experimental and simulated spectra but according to the Fourier transforms these are due only to contributions at relatively large distances from the central atom; hence there is no reason to question the result of the simulation for the contributions at low R in the Fourier transform. Refining in k - instead of k^3 -weighting did not result in a better agreement for the low- k region of the EXAFS, as judged by visual inspection. The result for the simulation of the spectrum of elementary iodine for the iodine–iodine distance (Table 1, 2.71 Å) is in reasonable agreement with the literature value of 2.67 Å (Cotton & Wilkinson, 1988). In the simulation of 1,2-diiodoethane, the I–I distance (3.85 Å) is relatively short, indicating that the preferential relative conformation for the iodine substituents on the C–C bond is gauche; an antiperiplanar conformation would have resulted in a much longer I–I distance (~ 5 Å). In the simulations of iodoform (Fig. 7a) and 1,2-diiodoethane (Fig. 7c), the distances between the I and the sp^3 -hybridized C of 2.16 and 2.18 Å (Table 1) are longer than those between the I and the sp^2 -hybridized C of the aromatic rings in the I-substituted tyrosines (Figs. 7d and 7e) which are only 2.07 Å (Fig. 5); this is in line with the shorter atomic radius for sp^2 -hybridized carbon compared with sp^3 -hybridized carbon. In addition to the aforementioned characteristic pattern of aromatic rings, the accurate distance information derived from the EXAFS offers another approach to distinguishing between incorporation of iodine in an aliphatic or an aromatic compound. The multiple-scattering simulations for the aromatic compounds in Figs. 7(d) and 7(e) were calculated on the basis of the geometries given in Fig. 5, and refined in EXCURV98 using so-called restrained refinement (Binsted *et al.*, 1992), with the optimum values of the distances between adjacent C atoms in the phenyl ring set to 1.39 Å and those between C atoms with one carbon in between to 2.41 Å.

The edges of the organic bromine compounds (Fig. 8, see Figs. 9 and 12 for structures) exhibit much more fine structure than those of the iodine compounds, because of the shorter core-hole lifetime effect for heavier elements, as mentioned above. Although the traces for the two aromatic compounds, 4-bromophenylalanine and 3,5-dibromotyrosine, resemble each other most, it is not possible to use the edge structures to classify compounds as aliphatic or aromatic on the basis of the

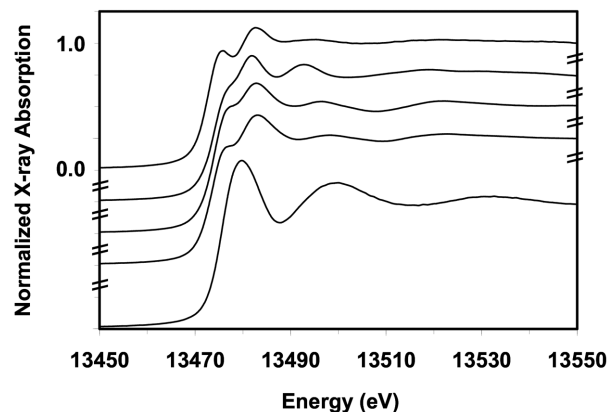


Figure 8

X-ray absorption K edges of bromine (see Figs. 9 and 12 for structures) in (top to bottom) bromoform (CHBr_3); 11-Br-undecanoic acid; 4-Br-phenylalanine; 3,5-dibromotyrosine; NaBr (10 mM in water).

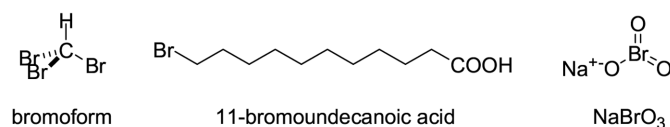


Figure 9

Structures of bromine compounds.

Br edge. The edge of a 10 mM aqueous solution of sodium bromate (not shown) was found to have a very intensive (intensity approximately doubled) white line compared with that of 10 mM sodium bromide (Fig. 8, bottom trace), but in spite of the higher oxidation state it was only at a marginally (<0.5 eV) higher energy.

The EXAFS spectra of aqueous sodium bromide (Fig. 10a) and sea water (Fig. 10b) could be simulated by O atoms (of solvating water molecules) at 3.33 and 3.29 Å (Table 2), respectively. Owing to the large distance of these O atoms to the bromine and in spite of their large number, the EXAFS signal is relatively weak compared with that of other compounds as well as to that of aqueous sodium bromate (Fig. 10c) which has a strong oxygen contribution at 1.65 Å (Table 2). The bromine–oxygen distance of 3.29–3.33 Å in our simulations is significantly longer than that found in a room-temperature EXAFS study (3.19 Å; Tanida *et al.*, 1994) but in good agreement with a combined room-temperature EXAFS/molecular dynamics study (3.34 Å; d'Angelo *et al.*, 1994). The occupancy found in our refined simulations (10.7–11.1) is much higher than that found in the EXAFS/molecular dynamics study (6.7; d'Angelo *et al.*, 1994); the value in the literature is probably more accurate in view of the adequate modelling of the asymmetry in the radial distribution of the shell of solvating O atoms derived from the molecular dynamics.

It should be noted that the sodium bromide and sea-water spectra contain low-frequency contributions, visible in the EXAFS as a significant depression around $k = 4\text{--}5 \text{ \AA}^{-1}$, and in the Fourier transform as an apparent shell of atoms at a distance lower than the first shell of simulated O atoms, which

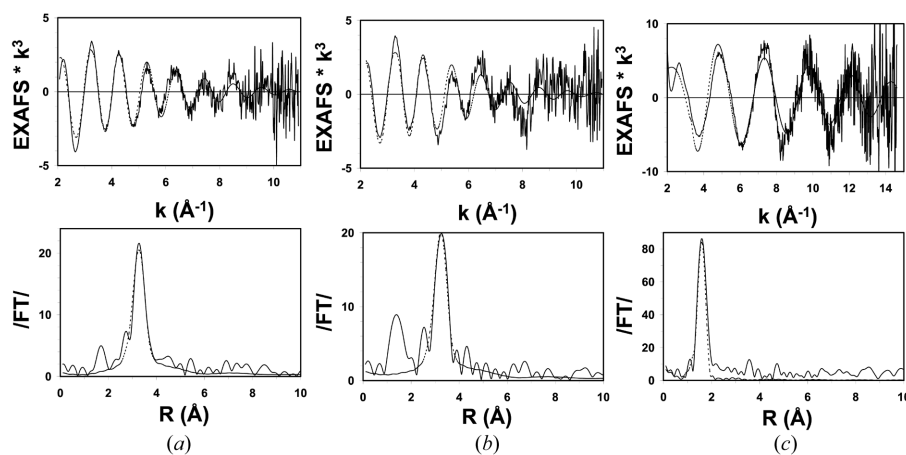
Table 2

Parameters for the simulations of EXAFS of bromine compounds.

 Energies in eV; distances in Å; Debye–Waller-type factors quoted as $2\sigma^2$ in Å² in parentheses. The parameters for the simulations of the aromatic bromine compounds are given in Fig. 12.

	NaBr (aq.) (Fig. 10a)	Sea water (Fig. 10b)	NaBrO ₃ (aq.) (Fig. 10c)	CHBr ₃ (aq.) (Fig. 11a)	11-Br-undecanoic acid/BN (Fig. 11b)†
ΔEF (eV)	−3.25	−0.82	−9.29	−3.24	−6.37
Energy range (eV)	3–450	3–450	4–817	4–815	4–620
O	10.7 at 3.33 (0.032)	11.1 at 3.29 (0.034)	2.7 at 1.65 (0.006)	–	–
C	–	–	–	at 1.92 (0.005)	1.3 at 1.96 (0.003)
C	–	–	–	–	2.86 (0.007)
Br	–	–	–	2.9 at 3.17 (0.009)	–
C	–	–	–	–	4.27 (0.007)
Fit index (k^3 -weighting)	0.3790	0.4769	0.2266	0.9013	0.1900

† All the atoms included in the simulation of 11-bromoundecanoic acid are part of a single multiple-scattering unit; the occupancies for the remote shells are the same as given for the carbon attached to the central bromine.


Figure 10

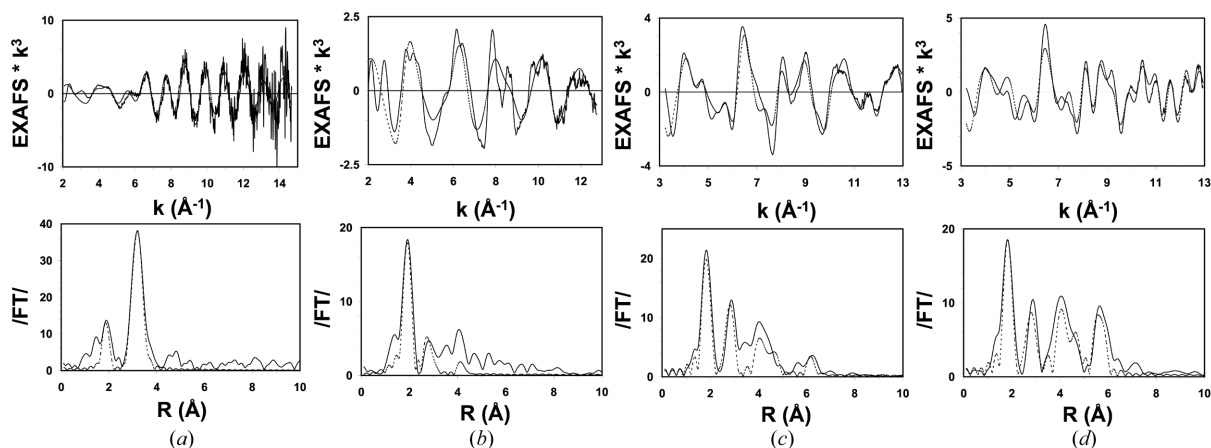
 Experimental (solid) and simulated (dashed) k^3 -weighted EXAFS (top) and phase-corrected Fourier transform (bottom) of (a) 10 mM NaBr in water, (b) Br in sea water and (c) 10 mM NaBrO₃ in water. The parameters of the simulations are listed in Table 2.

are not included in the simulation. Such contributions have been observed before for bromine (d'Angelo *et al.*, 1993; Burattini *et al.*, 1993) and for other elements, like Sr (d'Angelo *et al.*, 1997), where they have been ascribed to the presence of multi-electron excitations, like the KN_1 and $KM_{4,5}$ edge (= double electron excitation involving one $1s$ and $3d$ electron). An alternative explanation is to assume that, in addition to the fine structure caused by electron backscattering off surrounding atoms, the EXAFS, an additional fine structure exists, which is caused by electron backscattering off the interatomic potential (atomic EXAFS, or AXAFS) (Rehr *et al.*, 1995; O'Grady & Ramaker, 1998; Wende & Baberschke, 1999). In both cases the question arises as to why we observe the phenomenon only in the specific case of the samples with aqueous bromide. We propose that this is due to incomplete removal of the low-frequency contributions in our background-subtraction procedure in the case of weak EXAFS. In other studies at the Br K edge (Dau *et al.*, 1999), contributions

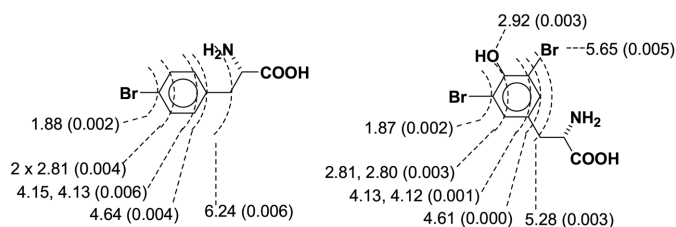
at lower distance, *e.g.* 2 Å in the case of aqueous sodium bromide, were also observed; in this case an explanation specific to the hydrated bromide ion, *viz.* an assignment to H atoms (from the water molecules) was proposed (in addition to other explanations). This is of course reasonable as the H atoms are the closest atoms in the environment of the hydrated bromide and, recently, hydrogen contributions have even been discerned in the EXAFS spectra of hydrated metal ions, where O atoms, not H atoms, are the atoms closest to the central atom (d'Angelo *et al.*, 2002). In our spectra, however, we were unable to simulate the low-frequency contribution with H atoms, as they did not match these shells when allowed to float in a refinement. Therefore, we prefer an explanation of the low-frequency oscillation as due to a general physical atomic absorption effect and assume that it is observed only in the hydrated bromide spectra because it could not be removed in the background subtraction owing to the weakness of the EXAFS in these spectra.

The comparison of the Fourier transforms of the organobromine compounds (Fig. 11, bottom panels) reveals that a distinction between aromatic and aliphatic compounds can be made for bromine, as concluded before for iodine; the patterns of shells in the Fourier

transforms in Figs. 11(c) and 11(d) resemble those in Figs. 7(d) and 7(e), although they are shifted to lower R (distance) because bonds with bromine are shorter than bonds with iodine. The spectrum of CHBr₃ (Fig. 11a) can be simulated with carbon and bromine contributions at 1.92 and 3.17 Å, respectively; that of 11-bromoundecanoic acid (Fig. 11b) by C atoms of the alkyl tail at 1.96, 2.86 and 4.27 Å. As noted before in a study of aqueous solutions of small organobromine compounds, intermolecular effects such as interaction with solvent (d'Angelo *et al.*, 1994) or, in our case, BN and other organobromine molecules can affect the spectra; simulations of these effects fall outside the scope of the present study but this neglect does result in significant differences between the experimental spectra and fits based only on considerations of intramolecular effects. The spectrum of 4-bromophenylalanine (Fig. 11c) is simulated by the contribution of a phenyl ring and an additional carbon (Fig. 12, left, including multiple scattering), and that of dibromotyrosine (Fig. 11d) by a phenyl


Figure 11

Experimental (solid) and simulated (dashed) k^3 -weighted EXAFS (top) and phase-corrected Fourier transform (bottom) of (a) CHBr_3 (aq.), (b) 11-bromoundecanoic acid/BN, (c) 4-bromophenylalanine/BN and (d) 3,5-dibromotyrosine/BN. The parameters of the simulations in (a) and (b) are listed in Table 2, those for (c) and (d) in Fig. 12.


Figure 12

Structures of 4-bromophenylalanine (left) and 3,5-dibromotyrosine (right) with parameters of the refined simulations of Figs. 10(c) and 10(d), respectively; all atoms were included in multiple-scattering units whose occupancies refined to 1.0 for both spectra. Distances in Å, Debye-Waller-type factors quoted as $2\sigma^2$ in Å² in parentheses. 4-Bromophenylalanine: energy range, 25–635 eV; ΔEF , -2.162 eV; fit index (k^3 -weighting), 0.1324. 3,5-Dibromotyrosine: energy range, 25–635 eV; ΔEF , -3.186 eV; fit index (k^3 -weighting), 0.1981.

ring with the second bromine at 5.65 Å as well as contributions from the oxygen of the hydroxyl group in the ring and the first carbon of the side chain (Fig. 12, right, including multiple scattering). Although the difference in bond length between bonds of bromine with sp^3 - or sp^2 -hybridized carbon is not as large as that found for iodine (1.92–1.96 versus 1.87–1.88 Å), it is large enough to give significant differences in the EXAFS, thus providing an additional feature to distinguish bromine in aliphatic and aromatic environments.

4. Conclusions

We conclude that the iodine K -edge positions and structures are sensitive to formal valence, showing an edge shift to higher energy except for iodide. The multiple-scattering effects in the aromatic rings of aromatic organohalogen compounds are adequately reproduced with the restrained refinement simulations in *EXCURVE*. EXAFS spectra of organohalogen compounds (both iodine and bromine) can be used to discriminate between aliphatic and aromatic compounds. There are differences both in the distances from the halogens to the first

shell of C atoms, which are shorter for aromatic compounds, and in the patterns of shells in the Fourier transforms. This result is expected to be relevant to studies at these edges in biological systems.

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