Probing oxygen and nitrogen bonding sites in chitosan by X-ray emission

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Abstract

We present X-ray fluorescence measurements of biopolymer chitosan $[C_6H_{11}NO_4]_n$ and chitosan cross-linked with ethylene glycol diglycidyl ether (EGDE). Changes in the fine structure of resonantly excited O Kα X-ray emission spectra (XES) of unmodified chitosan are attributed to the excitation of non-equivalent oxygen sites belonging to –OH, –O– functional groups. The comparison of the nonresonant nitrogen Kα XES of unmodified chitosan cross-linked with EGDE does not show any differences, which excludes the model of chemical structure according to which EGDE is linked to chitosan via the amine group.
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1. Introduction

Chitin is a chemically stable natural biopolymer isolated from crustaceans (such as lobster, shrimp and crabs), some invertebrates and fungi [1]. It is produced in vast amounts (up to 10^{14} kg/year),

second only to cellulose on a worldwide basis [2]. Chitosan is easily prepared from chitin by deacetylating its acetoamide groups with a strong alkaline solution. Chitosan is known especially for its use as an natural adsorbent and is widely used for the prevention of water pollution by highly toxic chlorinated aromatic compounds and metal ions [3]. Chitosan is inexpensive, environmentally benign, harmless to humans, and a huge, obtainable biomass, which makes it promising and attractive for many

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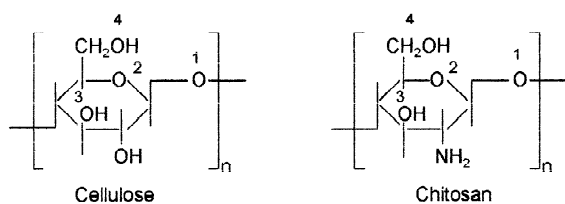E-mail address: dlederer@tulane.edu (D.L. Ederer).

Fig. 1. Chemical structure of chitosan and cellulose.

applications. The amine biomass chitosan is of particular interest; it selectively binds to virtually all group III transition metal ions at low concentrations but it does not bind to group I the (alkali) and group II (alkaline-earth) metal ions [4]. Chemical cross-linking of the linear chitosan chains with the bifunctional reagent glutaradehyde or ethylene glycol diglydyl ether (EGDE) can render chitosan insoluble in acidic media and improve resistance to chemical and biological degradation.

It is well known that the electronic structure plays an important role in the determination of the physical and chemical properties of biological and other molecules. X-ray emission spectroscopy (XES) is a probe, that is very sensitive to the local electronic structure and chemical bonding of the emitting atoms We have selected chitosan as a model to study chemical bonding by XES for several reasons. In the first place chitosan is relatively simple and has a compact geometrical structure possessing four different oxygen-based functional groups (labeled 1–4 in Fig. 1) that can be modified by cross-linking with other chemical agents. This feature makes it a suitable model object for the study of resonant X-ray scattering spectra. Secondly by chemically modifing chitosan, one can observe the XES structure that is sensitive to the different atomic sites and infer an appropriate model of the chemical structure of the chemically modified chitosan.

2. Experimental

Chitosan 10 $[C_6H_{11}NO_4]_n$ from Wako Pure Chemical Industries Ltd. was used as the sample. The chemical structure of chitosan and cellulose is shown in Fig. 1. The structure of both polymers is the same except for the substitution of one OH group in cellulose by the NH_2 group in chitosan. Four

non-equivalent oxygen sites from different functional groups are present in both compounds: C–O (*1*) which links adjacent glucose rings, C–O (*2*) which belongs to the glucose ring, OH (*3*) and CH$_2$OH (*4*).

When chitosan is cross-linked using the EGDE reagent shown in Fig. 2, the chemical structure is ambiguous because the highly strained three-membered ring in EGDE makes it much more reactive towards the nucleophilic substitution than other ethers. The hydroxyl group and the amino group in chitosan are very powerful activating groups, so they can selectively react with EGDE or produce product mixtures. Therefore the adsorption behavior of metal ions will change depending on the chemical structure of chitosan complex. Two different chemical structures of cross-linked chitosan the have been suggested according to which EGDE is linked either to the hydroxyl or to the amino group as shown in Fig. 2.

X-ray fluorescence measurements were performed at the Photon Factory (PF, Tsukuba) and the Ad-
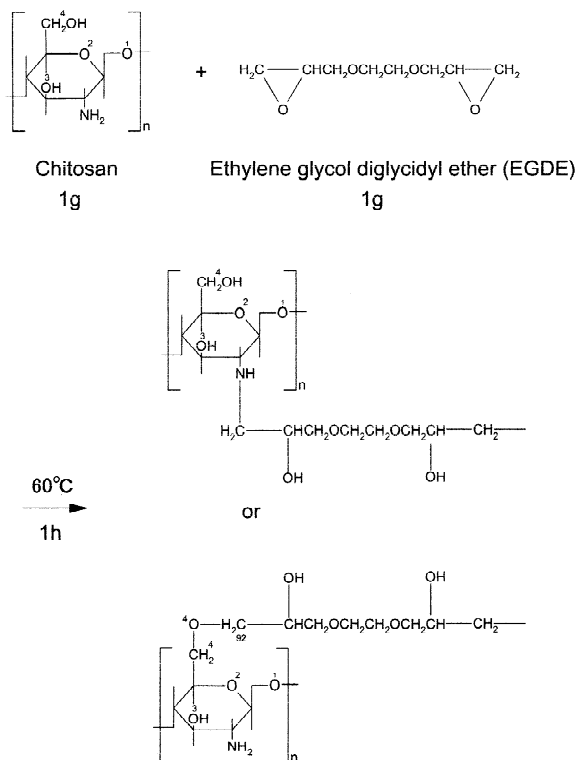

Fig. 2. Chemical structure of cross-linked chitosan with EGDE.

vanced Light Source (ALS, Berkeley) using un-
dulator beamlines that were equipped with X-ray
fluorescence endstations. The O 1s total-electron
yield spectrum and excitation energy dependence of
O Kα XES of unmodified chitosan were measured at
Beamline BL-2C of the Photon Factory consisting of
a grazing incidence soft X-ray monochromator with
a varied space plane grating [5]. The endstation is
equipped with soft X-ray emission spectrometer [6]
and a commercially available photoelectron spec-
trometer (VG-CLAM2). The oxygen spectra were
measured using laminar-type holographic spherical
grating ($R=5$ m, $N=1200$ lines/mm). A 20 μm
entrance slit width was used that provided an energy
resolution of about 0.7 eV. Spectra were obtained
between 529.7 and 615.0 eV, using exposure times of
350–1670 s. Nonresonant nitrogen Kα ($2p\rightarrow1s$
transition) emission of cross-linked chitosan was
recorded at the Advanced Light Source (Beamline
8.0) employing the soft X-ray fluorescence endsta-
tion [7]. Photons of energy 430 eV excited the
nitrogen sites above the K-edge. The nitrogen Kα
spectra were obtained with a 600 lines/mm, 10 m
radius grating and energy resolution of 0.4 eV.
Polymers may be radiation damaged under exposure
to intense X-rays. We have studied this problem [8]
in connection with X-ray fluorescence measurements
of polymer films and found that polyimide films are
rather stable to the soft X-ray excitation by mono-
chromatic synchrotron radiation.

3. Results and discussion

3.1. Resonantly excited O Kα X-ray emission spectra of unmodified chitosan

In resonantly excited X-ray emission of solids, the
core electron is promoted to unoccupied states in the
conduction band. The decay by X-ray emission is
governed by the dipole selection rule and may be
realized in two ways: via a spectator transition, when
the core hole is filled by an electron from the valence
band or via participator transitions (which includes
elastic scattering), where the excited electron itself
fills the core hole. The intensity of spectator emis-
sion is determined by the local partial density of

states in the valence band that have the appropriate
symmetry for dipole transitions.

It has been shown that the resonant X-ray emis-
sion spectra can be used for site-selective excitation
when the system consists of non-equivalent sites
occupied by chemically identical species. Such a
situation can be realized if the chemical shifts of
corresponding core levels are large enough (≥0.5
eV) and structure of the conduction band is favorable
for site-selective excitation, i.e. the electronic states
of non-equivalent sites are not strongly mixed and
well separated. It takes place for resonant excitation
of O Kα XES in high-T_c superconductors [9] and
Sr_2RuO_4 [10] and resonant excitation of S $L_{2,3}$ XES
of layered compounds $BaCo_{1-x}Ni_xS_2$ [11], where
oxygen and sulphur atoms occupy in-plane and
apical sites, but not for manganites $Eu_{1-x}Ca_xMnO_3$
[12] and $Pr_{0.5}Sr_{0.5}MnO_3$ [13] where 2p-states of
non-equivalent oxygen are strongly hybridized in the
conduction band.

One would expect that X-ray emission in mole-
cules is more site-selective because of the more
localized character of the wave functions. It has been
shown [14–16] that resonantly excited X-ray emis-
sion spectra referring to core levels shifted even
within the sub-electron-volt range will be strongly
dependent on interference effects. Thus it is possible
to assign such shifts to different sites although they
remain unresolved in the corresponding absorption
spectrum. One would therefore expect that for larger
core level shifts it is possible to use resonant site-
selective excitation of X-ray emission spectra to
obtain additional information about the electronic
structure of molecules.

The insert in Fig. 3 shows the measured O 1s total
electron yield (TEY) absorption spectrum, probing
the unoccupied O 2p-states. Based on the fine
structure of the O 1s TEY spectrum of chitosan, we
have selected photon excitation energies at 529.7 eV
(below the threshold), 531.7, 533.2 and 536.5 eV
(corresponding to the absorption peaks labeled *a, b*
and *c*) and far from the threshold (at 547.9 and 615.0
eV). The excitation energy dependence of O Kα XES
at the selected photon excitation energies for
chitosan is given in Fig. 3. The spectra are normal-
ized to the number of incoming photons represented
by a mesh current. The intensity of O Kα XES
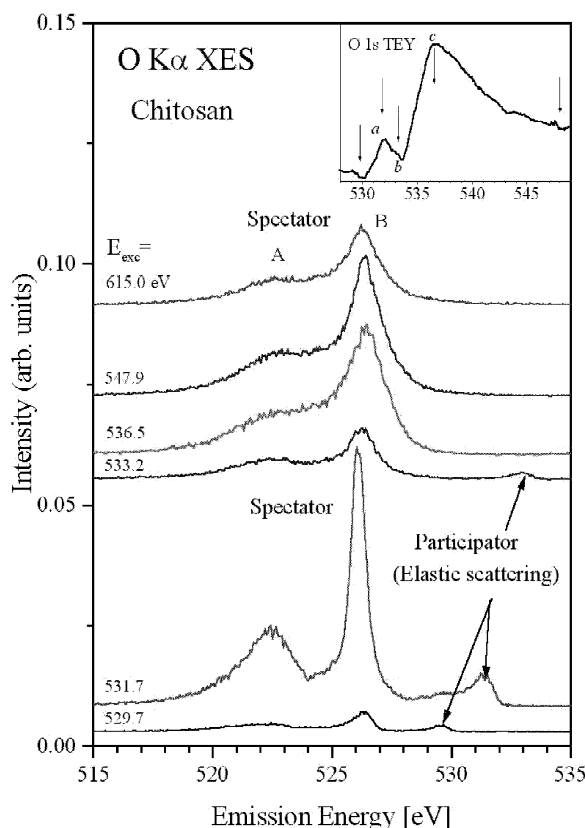shows resonant behavior at 531.7 eV (peak *a*) that is

Fig. 3. Excitation energy dependence of O Kα emission of chitosan near O1s threshold. The O1s total electron yield spectrum (TEY) of chitosan is shown in the insert.

Fig. 4. Resonantly excited O Kα emission of chitosan normalized to the intensity maximum. Note the oxygen bonding sites from Fig. 1, are shown as the numbers in perenthesis after the excitation energy.

associated with the –OH site, but, in general, the O Kα intensity follows the structure of the absorption spectrum. The spectator part of the spectrum consists of two main peaks, *A*, and, *B*, centered at 522.5 and 526.0 eV, respectively. The energy position of peak *A* is essentially unchanged as a function of excitation energy, while peak *B* shows a 0.2 eV energy shift. We note that the FWHM (full width at the half of maximum) of peak *B* is narrow when exciting at 531.7 eV and becomes broader at excitation energies of 533.2 and 536.5 eV. Further increase of the excitation energy does not change the width of this peak. This behavior is more clearly seen in Fig. 4 where the same spectra, excited at different excitation energies, are normalized to the same spectral intensity and each spectrum, measured at higher excitation energy, is compared with the previous one.
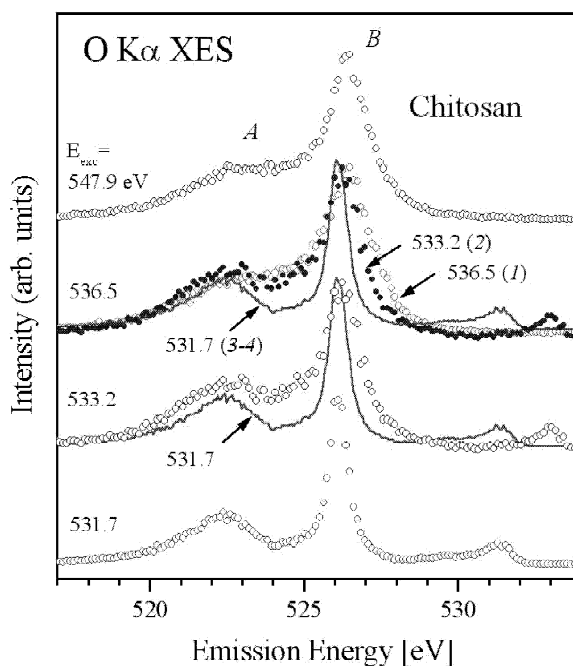
Cellulose and chitosan have similar chemical structures differing by the replacement of an OH group in celluose with an amine group to form chitosan, as shown in Fig. 1. According to Ref. [17], the O1s XPS of cellulose consists of two peaks centered at $E=532.9$ and 533.5 eV, that can be attributed to non-equivalent oxygen sites. Considering these XPS results and measurements of O 1s X-ray absorption spectra of ethanol and diethyl ether reference samples ([18]; Hitchcock, private communication), we can attribute the origin of the absorption peaks *a* and *b* of O 1s TEY of chitosan (see Fig. 3) to the excitation of an oxygen 1s electron to unoccupied 2p-states belonging to –O– and –OH functional groups, labeled (1, 2) and (3, 4) in Fig. 1, respectively. We suggest that the observed peak of O 1s TEY of chitosan at $E=536.5$ eV (*c*) may be due to excitation of an O 1s electron to unoccupied 2p-orbitals belonging to –O– groups between the unit rings, or this structure may be associated with a shape resonance.

We conclude from these results that by selectively exciting at photon energies of 531.7, 533.2 and 536.5 eV, electrons from energetically separated O 1s core levels are promoted to unoccupied molecular orbitals. The primary contribution to the spectra comes from the functional groups, –OH, –O– in the ring and possibly –O– between the rings. The corresponding X-ray emission O Kα XES selectively probes O 2p-states in occupied molecular orbitals formed by the same functional groups as suggested in Fig. 4.

3.2. Non-resonant X-ray emission spectra of cross-linked chitosan

Non-resonant X-ray emission spectra are measured by exciting above the core ionization threshold. In this case the intensity distribution of the emission spectrum, that occurs when the electron undergoes a transition from an occupied valence level to fill the core hole created by the incident photon absorption, is decoupled from the excitation. According to the dipole selection rule, $\Delta l = \pm 1$, the 1s core level holes in carbon and nitrogen atoms can only be filled by p-valence electrons. Therefore, the intensity of non-resonant X-ray emission spectra of light elements maps the p-density of states at each particular atomic site, and in a molecular orbital picture, the contribution of the local p-type atomic orbital. X-ray transitions are localized within the first coordination sphere of emitting atoms which makes X-ray emission spectra sensitive to the local order and can be used as a tool for study of the local structure of particular atoms.

To test the models used for the chemical structure of cross-linked chitosan, shown in Fig. 2, we use nitrogen Kα emission because carbon and oxygen atoms are present in both chitosan and EGDE. On the other hand, nitrogen is present only at one site in unmodified chitosan or in the chemically modified chitosan (as shown in Fig. 2). Therefore, if EGDE is linked with chitosan via the amine group, the nitrogen Kα XES must show some changes with respect to spectrum of unmodified chitosan.

Fig. 5 shows the comparison of the nitrogen Kα XES of cross-linked and unmodified chitosan. As seen, the nitrogen Kα XES of both materials are found to be identical. The complete correspondence

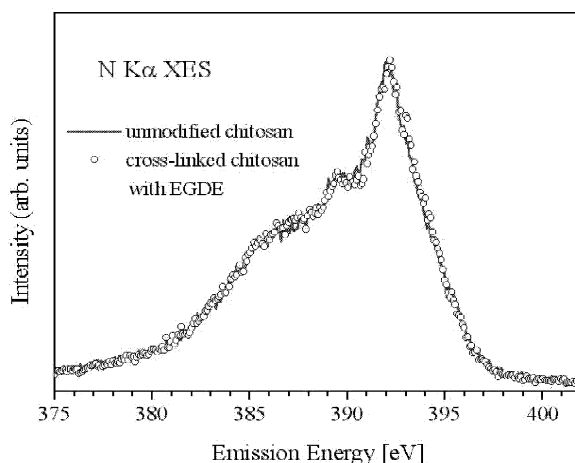Fig. 5. N Kα emission of unmodified (solid line) and cross-linked (open circles) chitosan.

of N Kα XES of unmodified and cross-linked chitosan completely excludes the model of chemical structure according to which EGDE is linked via the amine group. Therefore the alternative model with EGDE linked via hydroxyl group is preferable.

4. Conclusion

We have performed X-ray fluorescence studies of biopolymer chitosan and cross-linked chitosan including the measurements of excitation energy dependence of O Kα XES near O 1s threshold and O 1s total electron yield spectra. Based on these measurements we conclude that the changes in the width of resonantly excited O Kα XES are due to site-selective excitation of oxygen atoms belonging to different functional groups (OH and –O–). The comparison of nitrogen Kα spectra of unmodified and cross-linked chitosan shows that the structural model is preferable according to which EGDE is linked via the hydroxyl group.

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