X-RAY PHOTOELECTRON SPECTROSCOPY OF BSA AND ETHYL VINYL SULFONE MODIFIED BSA

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SUMMARY: X-ray photoelectron spectroscopy (XPS) can be used to detect and measure the chemical modification of bovine serum albumin (BSA) by ethyl vinyl sulfone (EVS). The analysis is based on the different binding energies (BE) of the 2p electrons from the sulfur that occurs naturally in BSA--163 electron volts (eV)--and the corresponding electrons of the introduced sulfone group--168 eV. The nitrogen: sulfur atom ratios in a series of BSA derivatives measured by XPS agree with corresponding values from elemental and amino acid analyses. The signal due to the sulfone group decreased during exposure to X-radiation in the instrument. Thus, XPS is also useful for measuring the susceptibility of protein substituent groups to X-radiation.

X-ray photoelectron spectroscopy or electron spectroscopy for chemical analysis (ESCA) is a relatively new analytical method. It is based on X-ray ejection of electrons from the core levels of atoms and measurement of the electron energies and abundance (1,2). Electrons are ejected with characteristic energies. Those that suffer no further energy losses are resolved, analyzed in a spectrometer, detected, and recorded. The binding energies of the core electrons are sensitive to atomic charge and also to the oxidation state of the element. The binding energy thus not only identifies a particular element but can sometimes permit inferences about chemical combinations. Only atoms within about 50 Å of the surface contribute to this process. The spectrometer records the number of electrons ejected vs. their energies, so that the area under each electron line is proportional to the concentration of a particular element at the sample surface. Since atomic cross-section varies for the various elements, the sensitivity of detection varies correspondingly.

Previous communications from this laboratory (1, 3-5) have described the use of XPS to detect surface properties of protein derivatives. We have also evaluated EVS as a reagent for modifying proteins (6, 7). Because electron spectra of the modified proteins show an intense line at 168 eV characteristic of sulfone sulfur, we investigated whether XPS could be a useful method for determining sulfone groups in modified proteins. Methods used and problems encountered in XPS analysis of a series of ethylsulfonylethyl derivatives of bovine serum albumin are reported in this paper.

EXPERIMENTAL

Core elemental electron lines were obtained with a Du Pont 650 electron spectrometer with a magnesium anode. The X-ray source was operated at maximum voltage and current. Freeze-dried protein samples were run as powders on tape and as pressed pellets. Spectra obtained from pellets were more satisfactory. The intensity of electron lines changed with the time of irradiation. The most accurate data were obtained by rapidly scanning a region to minimize exposure of the sample to radiation. A scan rate of 1 eV/cm was used with a recorder sensitivity of 200 counts/cm for sulfur and 1000 counts/cm for nitrogen. Elemental line widths were approximately 2.3 eV wide. Line intensities were determined by estimating a base line and measuring the peak height at half width. Atom ratios were determined by dividing the estimated line intensity by sensitivity factors taken from Wagner's data (8).

The chemically modified BSA derivatives were prepared at pH 9.5 as previously described (6, 7).

RESULTS AND DISCUSSION

Binding energies of electrons from proteins and modified proteins show discrete values characteristic of particular functional groups. Such functional groups may therefore be determined in principle by XPS without hydrolyzing the proteins. To demonstrate this possibility we analyzed BSA and a series of derivatives made by treating BSA with ethyl vinyl sulfone. The sulfur in the introduced sulfone group is readily determined in the presence of the original divalent sulfur combined in cystine and methionine because the binding energies of the respective 2p electrons are 168 and 163 eV (Tables 1 and 2 and Figures 1-3). These results were confirmed by elemental and amino acid

Table 1. ELEMENTAL NITROGEN AND SULFUR ANALYSES OF BSA AND BSA TREATED WITH ETHYL VINYL SULFONE (FIVE SAMPLES)

Sample	% N	% S
BSA	16.0	1.91
BSA-1	14.5	2.92
BSA-2	14.2	3.49
BSA-3	13.5	4.30
BSA-4	12.8	5.20
BSA-5	12.7	5.25

analyses. A plot of the N/S ratio (Y) determined by XPS against the extent of lysine modification (X) in the five BSA derivatives is linear and is described by the following equation:

$$Y = 146.3 - (9.55 \pm 0.47)X \quad (1)$$

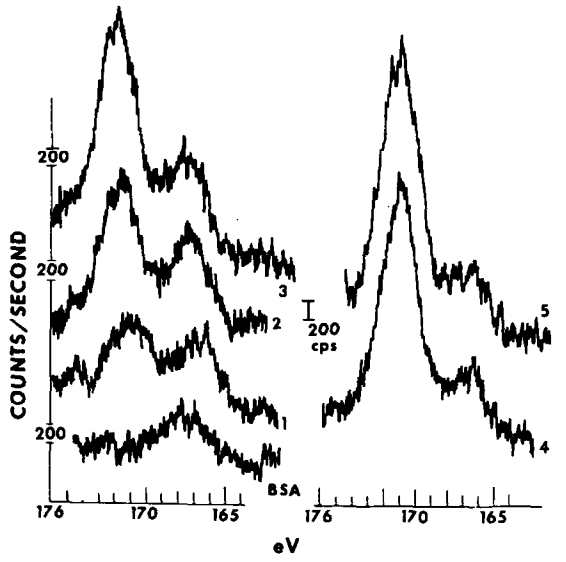
Equation (1) may be used to calculate the extent of lysine modification from N/S ratios obtained by XPS without the need of protein hydrolysis followed by amino acid analysis. Since XPS analysis requires only a few minutes compared to a much longer time for amino acid analysis, XPS measurement of chemical modification in proteins should find wide use in studies of protein structure and reactivity. The accuracy of eq. 1 ($\pm$ 5%) is less than the accuracy of amino acid analysis ($\pm$ 3%).

During this investigation we noticed that the intensity of the electron line at 168 eV due to the EVS modification decreased rapidly during exposure to the Mg Kj line at 1253.6 eV in the instrument. Decreases in the electron line intensities with increasing irradiation are shown for BSA and EVS modified BSA in Figures 2 and 3, respectively. The most striking

Table 2: ELECTRON LINE INTENSITIES AND CALCULATED NITROGEN:SULFUR ATOM RATIOS FOR BSA MODIFIED WITH ETHYL VINYL SULFONE

BSA + EVS	N/S intensity (1000 counts/sec)	I_o (corrected NI)	Total S2p intensity (counts/sec)	S(I) corrected	N/S atom ratios from XPS	N/S atom ratios from elemental analyses	Sulfur ratios[a]	% modified Lysine from amino acid analyses
No 1	20.5	49 K	2050	4420	11.1	11.3	1.00	37.5
No 2(a)	21.5	51 K	2620	5700	8.95	9.3	1.58	60.2
(b)	23.4	55.8	2960	6450	8.65			
No 3(a)	24.0	57.2	3840	8350	6.8	7.19	2.34	75.2
(b)	19.0	52.0	3140	6850	6.6			
No 4(a)	14.0	33.2	2700	5870	5.67	5.63	3.60	92.3
(b)	19.0	45.2	3760	8160	5.54			
No 5(a)	20.6	49.0	4400	9560	5.12	5.53	3.85	95.2
(b)	18.0	43.0		7270	5.9			

[a] Ratio of sulfur 2p 168 eV line (SO_2) to sulfur 2p 163 eV line (cystine and methionine sulfur).



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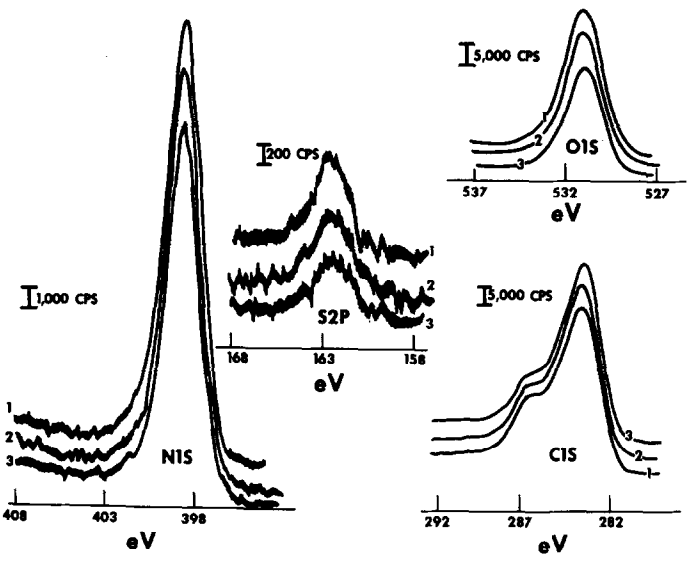

2.

Fig. 1. Sulfur 2p electron spectra of native BSA and of BSA modified to different degrees with ethyl vinyl sulfone. Extent of modification is shown in Table 1. The bottom left-hand curve is for native BSA. The other curves correspond to the BSA-EVS derivatives listed in Table 1.

Fig. 2. Carbon (C1S), oxygen (O1S), sulfur (S2P), and nitrogen (N1S) electron lines for BSA as a function of time of irradiation: 1, initial line; 2, after 10 minutes; 3, after 30 minutes.

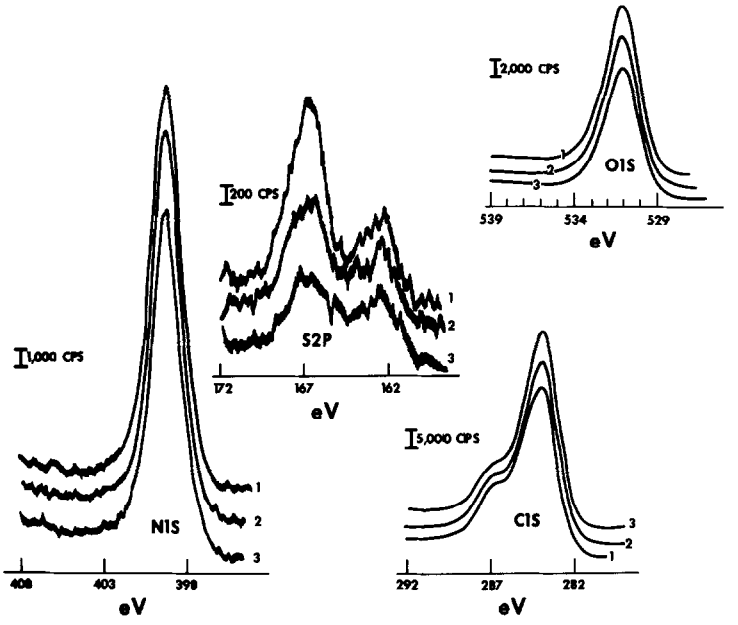

Fig. 3. Carbon (C1S), oxygen (O1S), sulfur (S2P), and nitrogen (N1S) electron lines for BSA treated with ethyl vinyl sulfone as a function of time of irradiation in the instrument: 1, initial electron line; 2, after 10 minutes; 3, after 30 minutes.

difference in these two series of spectra is the rapid decrease in the intensity of the sulfur 2p line at 168 eV. Evidently, the sulfone group is much more labile to radiation than other functional groups in the protein.

Radiation chemistry of polyaliphatic and polyaromatic sulfone polymers has been studied by Brown and O'Donnell (9, 10) in the solids under vacuum. The principle step in radiolysis was deduced to be carbon sulfur bond cleavage followed by liberation of SO_2. The rapid decrease in the electron line at 168 eV for the BSA derivatives correlates with specific carbon sulfur bond rupture and elimination of SO_2.

Other authors have noted radiation damage evidenced by changes in XPS spectra with time (1). Wurtzbach (11) recently observed changes in the XPS spectra of amino acids that he interpreted in terms of known

radiation decomposition processes such as decarboxylation and deamination. It appears that XPS is a uniquely informative technique for observing radiation effects on proteins. With this in mind, we are currently examining other protein derivatives.

Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others which may also be suitable.

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