CHEMICAL STATE ANALYSIS OF SULFUR ATOMS IN HUMAN KERATINIZED TISSUES

ZHAO LIANGZHONG (赵良仲), LIU SHIHONG (刘世宏) AND WANG DANGHAN (王当憨)
(Institute of Chemistry, Academia Sinica, Beijing)

Received June 12, 1981.

Stratum corneum epidermidis, callosity, nail, and hair are chief parts that compose human keratinized tissues. Biologically, they are all made up of keratin. Disulfide bonds between peptide links in keratin macromolecules make it firm and stable. Different sorts of keratin have been known, for example, hair and nail belong to hard keratin which are rich in sulfur, whereas epidermis belongs to soft keratin with less sulfur content. Sulfur contents in human keratinized tissues have been reported earlier[1,2], but changes of chemical states of sulfur atoms under natural conditions (wthout chemical pretreatment) have not been reported yet. Understanding of these changes is of consequence for keratin macromolecular and keratinized process studies. In this paper, changes of chemical states of sulfur atoms in human keratinized tissues under natural conditions are revealed by X-ray photoelectron spectroscopy (XPS).

I. MATERIALS AND METHODS

(1) *Preparation of samples.* Human epidermis was obtained by means of a surgical operation. Callosity, nail and hair were all obtained from healthy persons. These were all washed with acetone and dried at 50°C in a vacuum drier and then determined by XPS.

(2) *Instrument and methods.* A KRATOS XPS instrument, model ES-300, was used to record the spectra. Samples were adhered on double sticky tapes and excited with MgKα radiation source. S2p electron peaks were recorded. All binding energy values were determined with a reference line of C1s at 285.0 eV as calibration. The pressure in the analyzer chamber was maintained at $1—2\times10^{-7}$ torr.

II. RESULTS AND DISCUSSION

(1) *Epidermis.* A typical sulfur 2p electron spectrum from human epidermis is shown in Fig. 1. Obviously, there are at least two different chemical states of sulfur atoms. The S2p binding energy of sulfur atom in lower valence state is about 164.0± 0.2 eV and sulfur atom in higher valence state is about 168.2±0.2 eV. According to the studies of keratin of human hair[3] and the correlation table between S2p binding energy and oxidation states of sulfur atom[4], the former corresponds to sulfur in cystine (or cysteine), the latter corresponds to sulfur in sulfonate. Sulfur atoms in intermediate valence states may exist, since the S2p peaks are a little broader than the S2p peak of cystine.

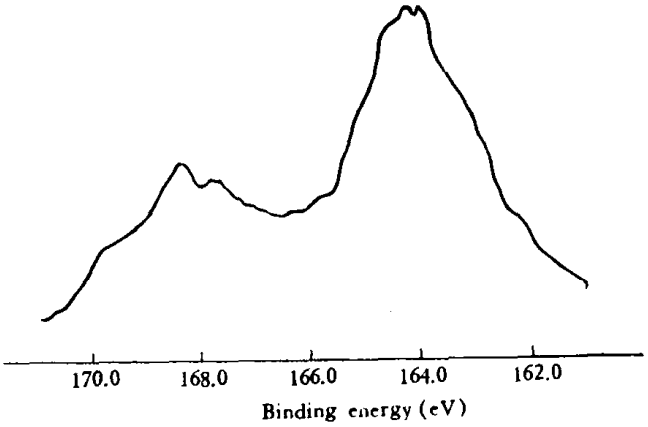

Fig. 1. S2p electron spectrum of human epidermis

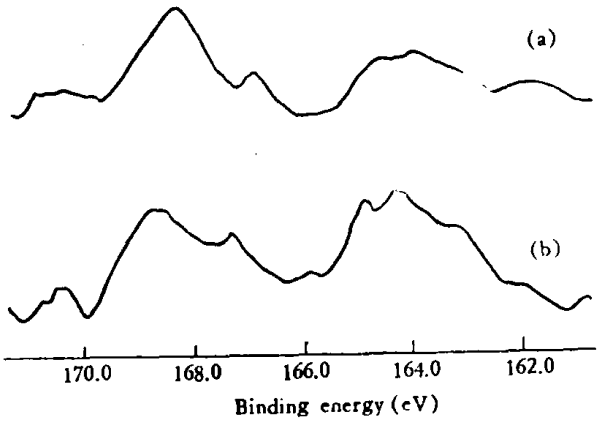

Fig. 2. S2p electron spectra of human callosity.
(a) surface of callosity; (b) inner part of callosity.

It has not been expected that a lot of sulfur atoms in higher valence state appear in human epidermis. This may be because the keratin in human epidermis can be oxidized naturally by weathering during the time of growing.

(2) *Callosity.* The S2p electron spectra from callosity is shown in Fig. 2, in which (a) was obtained from the surface of callosity, (b) was obtained from inner part of callosity. In both of them, the sulfur atoms can be seen existing at least in two valence states, higher and lower. Why a lot of sulfur atoms in higher valence state are presented in the inner part of callosity remain unknown. Maybe along with the process of keratinization, higher valence state sulfur atoms occur simultaneously.

(3) *Nail.* Fig. 3 shows the S2p electron spectra of human nail, in which lines (a), (b) and (c) were obtained from the surface of nail, a little beneath the surface of nail and the inner part of nail respectively. In (a) and (b), at least two different chemical states of sulfur atoms correspond to disulfide bonds and sulfonate in chemical environment respectively, however in line (a), sulfur atoms in higher valence state predominate, whereas, in line (b) sulfur atoms in lower valence state predominate. These results show that these sulfur atoms in higher valence state were formed by natural oxidation of sulfur atoms in disulfide bonds. In line (c), fewer sulfur atoms in higher valence state are present and this gives a further support to the fact that the higher

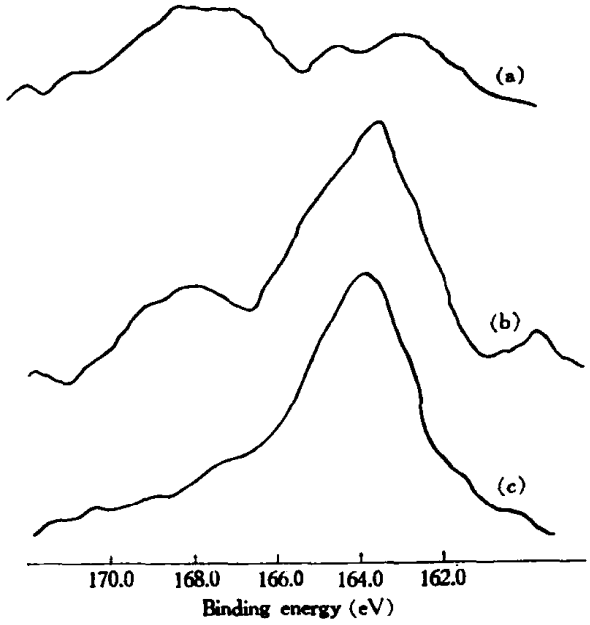

Fig. 3. S2p electron spectra of human nail.
(a) surface of nail; (b) a little beneath the surface of nail; (c) inner part of nail.

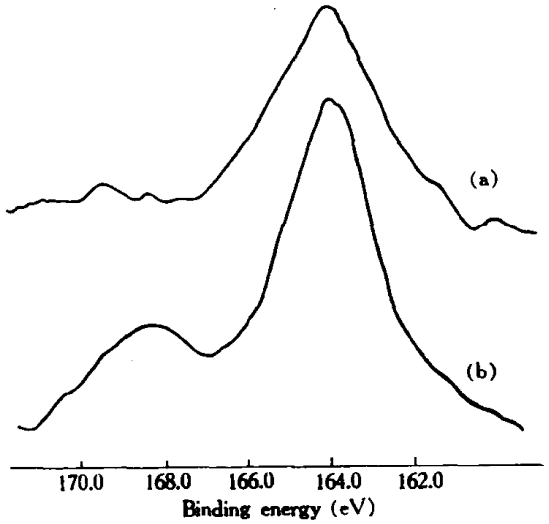

Fig. 4. S2p electron spectra of human canities.
(a) about 0—2 cm from hair root; (b) about 8 cm from hair root.

valence state sulfur comes from natural oxidation.

(4) *Canities.* The oxidative extents of disulfide bonds at different positions on surface of black hair are quite different. Difference between black hair and canities lies in the fact that the latter lacks melanin. Are the chemical states of sulfur atoms in keratin on surface of both the same? Fig. 4 gives an answer to this qestion. Line (a) in Fig. 4 shows the S2p electron spectra from short canities (about 0—2 cm from hair root) of an adult man, (b) is the S2p electron spectra from an end cut 8 cm from the hair root of a moderate long canities. Obviously, sulfur atoms in higher valence state are 20—30 percent richer in (a) than those in (b). This is quite the same as that obtained from black hairs.

III. Conclusion

Sulfur atoms in lower valence state which correspond to disulfide bonds in cystine in their chemical environment are similar in all kinds of human keratinized tissues. However, sulfur atoms in higher valence state, corresponding to sulfonate in their chemical environment, are presented in these keratinized tissues as well. An explanation is that all these sulfur atoms in higher valence state come from the disulfide bond sulfur by natural oxidation. Whether in epidermis and callosity, the sulfur atoms in higher valence state formed by keratinization exist remains to be Studied.

References

[1] Clay, R. C. et al., *J. Amer. Chem. Soc.*, **62**(1940), 2709.
[2] Baden H. P. et al., *Biochim. Biophys. Acta*, **322** (1973), 269.
[3] 赵良仲等，生物化学与生物物理学报，**13** (1981)，503.
[4] Lindberg, B. J. et al., *Physica Scripta*, **1**(1970), 286.
[5] Siegbahn, K. et al., *Nova Acto Reqiae Soc. Sci. Upsoliensis*, Ser. IV, 1967. 20.