THALLIUM IN HUMAN ERYTHROCYTE MEMBRANES: AN X-RAY PHOTOELECTRON SPECTROSCOPY STUDY

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Summary

X-ray photoelectron spectra were made of the surface of vacuum dried human red cells in an unetched state and after argon ion etching into the surface for various times. A thallium signal appeared after 30 minutes (at a depth of ∿100 Å) and then disappeared with further etching. The region of the membrane containing thallium may be involved in ion transport, i.e., site of Na+ - K+ ion activated adenosine triphosphate. A iron signal was observed after reaching a depth >500A.

Introduction

X-ray photoelectron spectroscopy (XPS) (1) is being employed to study the elemental composition of the human red cell surface. XPS offers the opportunity to determine the distribution of elements with sampling depth of the order of 20A in the intact cell surface before and after argon ion etching. The ion etching procedure removes a small amount of membrane surface, then the elemental analysis is determined by XPS. In this manner the elemental composition at various depths in the cell membrane is determined. This procedure was repeated until the iron line was observed.

We report in this communication a new finding; the presence and apparent levels of thallium observed in normal human red cells using the XPS method.

Method

Six normal erythrocyte samples were collected in heparinized polypropylene tubes and centrifuged at 3000 g for 30 minutes at 4°C to separate plasma and white cells. The interstitial fluid remaining after this treatment is about one percent. After centrifuging, the plasma and one-third of the upper layer of packed red cells were removed to insure adequate removal of white cells.

Another series of samples were collected in glass tubes and washed three times with isotonic saline. These samples showed a large increase in the amplitude of the Na and Cl lines, and a very prominent Si line appeared. All of the reported data were, therefore, obtained from samples collected with plastic syringes and tubes.

The X-ray photoelectron spectra were obtained using a Hewlett Packard 5950 A ESCA spectrometer. In this study the red blood cells were deposited upon a gold plate and vacuum dried. The cells were deposited over the entire area sampled by the X-ray beam, a 1 x 5 mm rectangle. The etching was performed with a 950 volt Ar+ beam defocused to a FWHM of 1 cm. Using sputter yields based upon data from Carter and Colligan (2), a calculated etch rate of 100A per 30 minutes was obtained.

Scanning electron micrographs of the red cell surfaces unetched and etched 6 ½ hours disclosed no preferential etching; the etched surfaces were smooth within the resolution of the microscope (<100Å). The atom fractions of the elements making up the sample volume were calculated from peak heights according to Wagner (3).

Results

The XPS spectra of the vacuum dried red cells in the unetched state and after argon ion etching to various depths into the red cell surface are shown in Figure 1, over a range of electron binding energies from 0 to 250 eV. In the spectrum of the unetched sample seven lines, due to the following electrons, are apparent: phosphorus 2_s and 2_p, sulfur 2_s and 2_p, chlorine 2_p, potassium 3_s and oxygen 2_s. In addition to these, there are very prominent lines from C_{1s}, O_{1s} and N_{1s} at binding energies of 284 eV, 532 eV and 399 eV, respectively. (The gold 4f lines are from the sample holder).

After 30 minutes etching the S, Cl and K line intensities have increased while the P line intensities have markedly decreased. In addition, two very prominent lines have appeared at binding energies of 118 and 122 eV. We have assigned these lines to thallium $4f_{7/2}$ and $4f_{5/2}$ electrons, respectively.

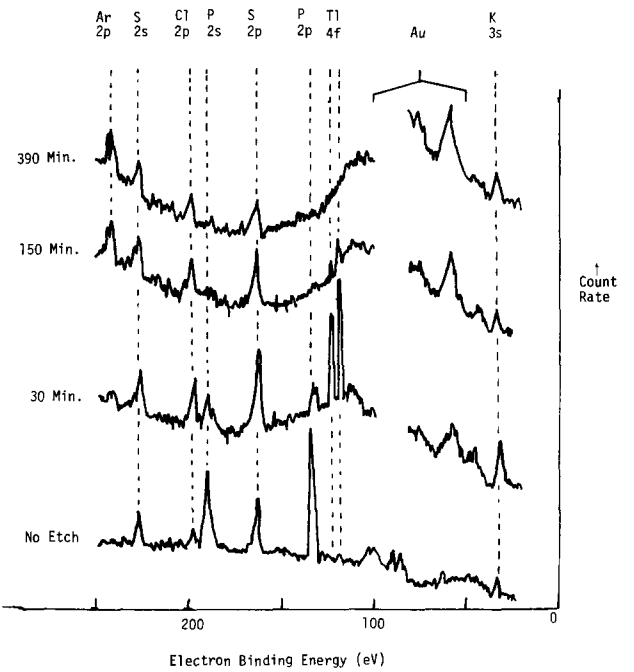

Figure 1. X-ray Photoelectron Spectra of Erythrocytes as a Function of Argon Ion Etching Time.

To check our assignment of thallium 4f for these lines $TlCl_3$ was added to an unetched red blood cell sample and examined. It was observed that the position and intensity ratio of the resulting thallium lines agreed with the observed lines in the etched sample.

After 150 minutes etching the P lines are missing, and the Tl lines are markedly reduced. After 390 minutes of etching the Tl 4f lines are also totally missing. The atomic fractions of the elements detected in the sampling volumes in the red cell surface as a function of etching are given in Table I. The lines for Fe $2p_{3/2}$ and $2p_{1/2}$ at 708 and 721 eV were observed after etching to a depth of ∿1000Å.

(Note: That the iron line was not observed until we had reached a depth of ∿1000Å into the erythrocyte surface suggests membrane thickness of ∿1000Å. Various values have been reported for the erythrocyte membrane thickness. The electron microscopy technique gives a value of ∿70Å for the dense trilamellar structure (4). X-ray diffraction investigation on dried membranes suggested a value in the range of 80 - 85Å (5). Energy

TABLE 1. ATOM FRACTION OF ELEMENTS COMPRISING SAMPLED VOLUME AS A FUNCTION OF ETCHING TIME

Etching Time (minutes)	Approximate (3) Depth in Membranes in Angstroms	Element							
		C	N	O	P	S	Cl	K	Tl
0	20	.84	.11	.039	.006	.002	.0006	.001	ND
30	100	.92	.036	.035	.001	.003	.0008	.003	.0006
150	500	.92	.036	.041	ND	.002	.001	.002	.0001
390	1,300	.91	.034	.048	ND	.002	.001	.001	ND

ND: not detected

transfer experiments reported a value of ∿65Å for dried and 100Å for rehydrated membranes (6). These results indicate that the electron dense region measured by the above procedures indicate a membrane thickness of 65 to 100Å. However, other measurements on membrane thickness using analytical leptoscope gave values of 150 - 250Å (7). Quantitative birefringence measurements of human red cell ghosts gave a value of 5000Å (8). Also, see Ponder (9) review of erythrocyte membrane thickness in relationship to hydration.

A recent report of erythrocyte membrane thickness determined by measuring membrane excluding volume, gave a value of 600 ± 70Å (10). A new model for the erythrocyte membrane applying polymeric domain concepts is consistent with an erythrocyte membrane thickness greater than 200Å (11)).

Discussion

It appears that Tl+ is concentrated in and just below the phospholipid region of the red cell membrane. The presence of both nitrogen and sulfur in this region suggests the presence of protein in the lipid bilayer region. The human red cell membrane contains at least two major proteins which have been shown to transverse the lipid bilayer of the cell membrane (12-17). A complete discussion of experimental data as well as current models proposed for the red cell membrane are found in the above references.

Investigations of Tl+ binding to the Na+ - K+ ion-activated adenosine

triphosphate (Na+ - K+, ATPase) obtained from various sources all demonstrate that Tl+ can act as an activator of Na+ - K+ ATPase and that Tl+ binding affinity for this enzyme is much greater than for K+ (18-23). Kyte (24) has recently shown that Na+ - K+ ATPase spans the red cell membrane.

Another enzyme system known to be activated by Tl+ is pyruvate kinase (E.C. 2·7·1·40) (25-27). Thallium has been shown to be an activator of this enzyme, and it has a binding affinity much greater than K+ (25-27). Pyruvate kinase has been shown to be bound to the red cell membrane (28).

The fact that Tl binds strongly to both Na+ - K+ ATPase and pyruvate kinase suggests that the metal binding of these enzymes may be located in and below the phospholipid bilayer. The Tl+ binding site for Na+ - K+ ATPase is consistent with the proposal of Grisham and Mildvan (29) that the transport site for monovalent cations is very near the catalytic site of Na+ - K+ ATPase.

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