

Effect of Chemical Modification of Wool on Metal Ion Binding*

M. SID MASRI and MENDEL FRIEDMAN, *Western Regional
Research Laboratory, Agricultural Research Service,
U.S. Department of Agriculture, Berkeley, California 94710*

Synopsis

The effect of specific functional group modification of wool on the binding of a number of metal ions (individually) from aqueous solutions was studied. The metal ion uptake profiles for the different modified keratin polymers show that a degree of specificity of binding is imparted by the various modifications in terms of changes in capacities or rates of metal ion uptakes. The changes of uptake depend on both the particular ion and the particular protein modification involved. The results show the potential usefulness of the keratin derivatives for removal of toxic and industrial metal ions from water and contribute to interpretation of metal ions interactions with native wool and proteins in general. Treatments of woven wool with aqueous solutions of certain metal salts impart flame and insect resistance to the fabric.

INTRODUCTION

Binding to keratins of various metal ions including mercury(II), silver(I), copper(II), chromium(III), aluminum(III), nickel(II), iron(III), zirconium(IV), titanium(III and IV), zinc(II), cadmium(II), and lead(II) has been reported.¹⁻²¹

Because of its insolubility, content of several kinds of reactive sites, and affinity for various ions, we investigated the potential usefulness of wool as an insoluble matrix for removing mercuric and other toxic metal ions from aqueous media.²¹⁻²⁴ We have extended these studies and wish to report effects of chemical modification of wool on its binding of metal salts. Our results show that binding of various metal ions can be modified to different extents by introducing various functional groups thus giving a degree of binding specificity to the modified wool. The results contribute to understanding of wool-metal interactions through involvement of the various functional groups in the binding. Thus, they may be helpful in understanding mechanisms of metal-protein interactions in general.

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EXPERIMENTAL MATERIALS AND METHODS

Tests of Metal Ion Uptake

The tests were performed in aqueous media at room temperature as previously described.²¹ The wool used for the metal uptake tests in its native state or after modification was a fine, white top from Dubois, Idaho, 1961 clip. It was purified by extraction with benzene and methanol and air dried. The uptake measurements were made by individually contacting 1 gm wool with a solution of 10–20 mmoles salt dissolved in 50–100 ml water for 1 or 24 hr. With some salts, the solutions were acidified to dissolve the salts and minimize hydrolysis; thus, 1*N* HCl was used with BiCl₃ and SbCl₃; 0.3*N* HNO₃ with HgNO₃; and 0.5*M* acetic acid with UO₂(C₂H₃O₂)₂. With CuCl, a 1% suspension in water was used. The wool was removed after the contact time, washed thoroughly on a Buchner funnel with distilled water, then methanol, and air dried. In tests with the acidified solutions, the wool was washed with 1*N* HCl, 0.3*N* HNO₃, or 0.5*M* acetic acid prior to washing with distilled water. With cuprous chloride, the insoluble salt was shaken from the wool in a beaker filled with distilled water. This was repeated until the insoluble salt was thoroughly released. Metal content of the dried wool samples was found by x-ray fluorescence analysis.^{21,25} The treated dried wool was ground in a Wiley mill (20 mesh, Tyler), and a weighed aliquot (300 mg) was pressed in a die into a thin disc (32 mm in diameter). The disc was placed in a plastic sample holder and introduced into a Qanta Metrix energy dispersive x-ray fluorescence spectrometer with exciting energy from a rhodium x-ray source.

Wool Derivatives

Reduced Wool (W-SH). This was prepared by reducing native wool (WSSW) with *n*-tributylphosphine for two days at room temperature.²⁶ The reduced wool was then washed thoroughly on a Buchner funnel with water-methanol, water, then methanol, and dried in a desiccator connected to a vacuum pump through an acetone-Dry Ice trap. When the pressure gauge indicated that the wool was dry, the vacuum was released by introducing N₂ gas into the desiccator. The wool was then equilibrated to constant weight in air of ambient temperature and humidity. We found that leaving the reduced wool in air up to three weeks at room temperature did not result in a significant change in the sulfhydryl content. The sulfhydryl content was determined by alkylation of the sulfhydryl group with 2-vinylpyridine and assaying the resulting S-β-(2-pyridylethyl)-L-cysteine by amino acid analysis as previously described.²⁷

S-β-(2-Pyridylethyl) Wool (2-PEW). This was prepared from reduced wool by S-alkylation with 2-vinylpyridine as previously described.²⁸

S-*p*-(Nitrophenethyl) Wool (NPEW). This wool derivative was prepared by S-alkylating reduced wool with *p*-nitrostyrene at pH 7.6 as previously described.²⁸

3-Nitrotyrosyl Wool (NTW). This was prepared by nitrating native wool with tetranitromethane (TNM) in a 1:1 mixture (volume) of ethanol and 0.1M Tris-HCl buffer at pH 8.0 according to the method of Riordan,²⁹ using a tenfold molar excess of TNM over tyrosyl content. The TNM was added in three portions at hourly intervals, and the reaction was then left to continue overnight. The wool (intensely orange-yellow) was washed with water, ethanol, 0.1N HCl, water, then ethanol, and air dried. Amino acid analysis of a hydrolysate showed 0.09 meq/g wool of unmodified tyrosine and the appearance of 3-nitrotyrosine (0.17 meq/g wool).

3-Aminotyrosyl Wool (ATW). This wool derivative was obtained by reducing NTW for several hours with a 20-fold molar excess of sodium dithionite in 0.1M Tris-HCl buffer, pH 7.8.³⁰

Amino Acid Analysis. Wool and modified wool hydrolysates (110°, 20 hr, 6N HCl, in sealed ampules purged with nitrogen gas) were analyzed on a Phoenix 8000 amino acid analyzer by ion exchange chromatography.^{27,31}

Tests with Wool Fabric. Some of the metal salt treatments were carried out on woven white native wool fabric to test effects on physical properties of the wool which may enhance its utilization as textile fiber. We performed preliminary tests on flame- and moth-resistance effects. Flammability tests were done in duplicates on 10 in. × 3 in. cloth swatches suspended vertically according to modified ASTM procedure D 1230. The after-flame burn time and the char length of the treated samples were compared to similar measurements with untreated control samples. Insect-resistance of treated and untreated fabrics were estimated by measuring excrement of black carpet beetle larvae exposed to the wool fabrics in a 14-day CSMA feeding tests.

RESULTS AND DISCUSSION

Uptakes of the various metal ions by native and modified wools are shown in Tables I-III. The data are presented in terms of millimoles of metal cation bound per gram of original wool. The values were calculated from weight increases of the treated wools and their metal contents as determined by x-ray fluorescence. Since the anions may affect the interaction of the keratins with the salt solutions, the particular salts used are shown in Table I. The same salts were used throughout, but for brevity only the cations are given in the rest of the tables.

Uptakes for 1-hr and 24-hr contact times are given in Tables I-III. Comparison of the data for the two times shows that much of the total uptake is accomplished in the first hour, thus indicating relatively rapid binding. This is obviously important for practical use of wool for scavenging metal ions from water, especially if column flow operation is desired. In all of these tests, the amount of bound metal was only a small fraction of that available for binding; thus, metal salt concentrations in the medium were not limiting, and we infer that the data approximate maximum uptakes under the test conditions. We summarized in Table IV the differ-

TABLE I
Metal Ion Uptake by Native and Reduced Wool

Aqueous salt solutions	Uptake, ^a mmoles metal/g wool			
	Native		Reduced	
	1 hr	24 hr	1 hr	24 hr
HgCl ₂	1.26	2.25	2.15	3.15
HgNO ₃	0.23	1.49	0.67	1.44
AgNO ₃	1.01	1.12	1.67	1.90
AuCl ₃ ·HCl	0.63		1.07	
CuCl	0.62	1.10	1.54	2.16
CuCl ₂		0.33		0.25
UO ₂ (C ₂ H ₃ O ₂) ₂	0.34	0.77	0.87	0.72
SbCl ₃	0.35	0.65	1.33	1.50
SnCl ₂		0.64	0.50	0.55
Pb(NO ₃) ₂	0.33	0.58	0.54	0.65
PtCl ₄ ·HCl	0.29	0.55	0.51	0.83
ZnCl ₂	0.30	0.52	1.29	1.27
BiCl ₃	0.29	0.36	0.44	0.55
CdCl ₂	0.19	0.30	0.76	0.77
PdCl ₂	0.16	0.30	1.06	2.49
FeCl ₃		0.32		0.32
Cr(NO ₃) ₃		0.11		0.18
NiCl ₂		0.04		0.17
Co(NO ₃) ₂		0.04		0.14
Ce(SO ₄) ₂		0.11		0.02
Th(NO ₃) ₄		0.01	0.23	0.43
Na ₃ AsO ₃ ^b		0.004	0.29	0.33
ZrCl ₄		0.004	0.08	0.10
TiCl ₃ ·H ₂ O ₂		0.05	0.26	0.34
TiOSO ₄ ·H ₂ SO ₄		0.03	0.05	0.05

^a When values are not given, they were not obtained.

^b Values are for As(III).

ences of uptakes for the modified wools from those for native wool; only the 24-hr data were calculated.

Native Wool

As discussed previously,²¹ native wool binds substantial amounts of many metal ions: it binds in 24 hr more than one half and up to 2.25 mmoles metal ion/g of wool from solutions of mercury(II), mercury(I), silver(I), copper(I), uranium(VI), antimony(III), tin(II), lead(II), platinum(IV), and zinc(II); about one third of a mmole/g wool for bismuth(III), cadmium(II), palladium(II), copper(II), and iron(III); about 0.1 mmole/g wool for cerium(IV) and chromium(III); and negligible amounts of nickel(II), cobalt(II), thorium(IV), arsenic(III), zirconium(IV), and thallium(I).

Native wool also binds more than 0.5 mmole gold(III) per gram wool in a short time (10–20 min) from a solution containing a limited amount (1.2 mmoles) of gold chloride. Wool appears degraded after contact with excess gold chloride (several mmoles) for a longer time (1–24 hr).²¹

TABLE II
Metal Ion Uptake by S- β -(2-Pyridylethyl) Wool (2-PEW)
and S-(*p*-Nitrophenethyl) Wool (NPEW)

Salt ^a	Uptake, ^b mmoles metal/g wool			
	2-PEW		NPEW	
	1 hr	24 hr	1 hr	24 hr
Hg(II)	1.28	2.49		2.10
Hg(I)	0.61	1.69	0.04	0.38
Ag(I)	1.02	1.34	0.89	0.89
Au(III)	1.37		0.53	0.72
Cu(I)	1.25	1.57	0.61	0.93
Cu(II)		0.13		0.18
U(VI)	0.64	0.61	0.35	0.65
Sb(III)	0.93	0.01	0.07	0.26
Sn(II)	0.62	0.63	0.60	1.41
Pb(II)	0.24	0.20	0.10	0.23
Pt(IV)	0.46	0.64	0.10	0.33
Zn(II)	0.38	0.36	0.04	0.25
Bi(III)	0.35	0.31	0.18	0.30
Cd(II)	0.72		0.06	0.25
Pd(II)	2.48	3.06	0.06	0.19
Fe(III)		0.34		0.29
Ce(IV)	0.07	0.12		
Cr(III)		0.33		
Ni(II)		0.14		
Co(II)		0.10		
Th(IV)	0.16	0.27		
As(III)	0.001	0.003		
Zr(IV)	0.25	0.52		
Tl(I)	0.0	0.0		
Ti(IV)	0.20	0.35		

^a Same as those shown in Table I.

^b Values were not obtained when not shown.

TABLE III
Metal Ion Uptake (24 hr) by 3-Nitrotyrosyl Wool (NTW)
and 3-Aminotyrosyl Wool (ATW) (in mmoles Metal/g Wool)

Metal ion ^a	Uptake by		Metal ion ^a	Uptake by	
	NTW	ATW		NTW	ATW
Hg(II)	1.92	1.94	Bi(III)	0.32	0.11
Hg(I)	1.42	2.07	Pb(II)	0.32	0.14
Sn(II)	1.61	0.19	Cd(II)	0.31	0.05
Cu(I)	1.35	0.94	Sb(III)	0.27	0.0
Cu(II)	0.30	0.14	Fe(III)	0.23	0.41
Ag(I)	1.25	1.49	As(III)	0.22	0.0
Pd(II)	0.85	1.65	Ni(II)	0.19	0.07
U(VI)	0.64	0.40	Tl(I)	0.15	0.23
Au(III) ^b	0.62	0.62	Zr(IV)	0.12	0.0
Pt(IV)	0.46	1.36	Co(II)	0.12	0.08
Zn(II)	0.42	0.61			

^a From aqueous solutions of salts shown in Table I.

^b Values for Au(III) are the uptakes in 10–20 min of contact time.

TABLE IV
Differences of Metal Ion Uptake (24 hr) of Modified Wools^a from Native Wool
(in mmole Metal/g Wool)

Metal ion	WSH	2-PEW	NPEW	NTW	ATW
Hg(II)	0.90	0.24	-0.15	-0.33	-0.31
Hg(I)	-0.05	0.20	-1.11	-0.07	0.58
Ag(I)	0.78	0.22	-0.23	0.13	0.37
Au(III) ^b	0.44	0.74	-0.10	0.0	0.0
Cu(I)	1.06	0.47	-0.17	0.25	-0.16
Cu(II)	-0.08	-0.20	-0.15	-0.03	-0.19
U(VI)	-0.05 ^c	-0.16 ^d	-0.13	-0.13	-0.37
Sb(III)	0.85	-0.64 ^e	-0.38	-0.38	-0.65
Sn(II)	0.07	0.0	0.78	0.98	-0.44
Pb(II)	0.07	-0.38	-0.35	-0.26	-0.44
Pt(IV)	0.28	0.09	-0.22	-0.09	0.81
Zn(II)	0.75	-0.16	-0.27	-0.10	0.09
Bi(III)	0.20	-0.04	-0.05	-0.03	-0.24
Cd(II)	0.47	0.55 ^f	-0.05	0.01	-0.25
Pd(II)	2.19	1.59	-0.11	0.55	1.35
Fe(III)	0.0	0.02	-0.03	-0.09	0.09
Ce(IV)	-0.09	0.01	—	—	—
Cr(III)	0.08	0.23	—	0.06	—
Ni(II)	0.13	0.10	—	0.15	0.03
Co(II)	0.10	0.06	—	0.08	0.04
Th(IV)	0.42	0.26	—	—	—
As(III)	0.33	0.0	0.0	0.22	0.0
Zr(IV)	0.10	0.52	—	0.12	0.0
Tl(I)	0.28	-0.05	—	0.10	0.17
Ti(IV)	0.02	0.32	—	—	—

^a Modified wools are: reduced (WSH), S- β -(2-Pyridylethyl) wool (2-PEW), S-(*p*-nitrophenylethyl) wool (NPEW), 3-nitrotyrosyl wool (NTW), and 3-aminotyrosyl wool (ATW).

^b Uptake in 10-20 min.

^c Value for 1 hr uptake = +0.53.

^d Value for 1 hr uptake = +0.30.

^e Value for 1 hr uptake = +0.58.

^f 1-Hr value.

Reduced Wool

Comparison of the 24 hr uptakes by WSH to corresponding values for WSSW shows increased uptakes per gram of wool of 0.9, 0.78, 1.06, 0.85, 0.75, and 0.47 mmoles of mercury(II), silver(I), copper(I), antimony(III), zinc(II), and cadmium(II), respectively. These increases indicate that the sulfhydryl polymer has great affinity for these metal cations. That the observed increases are not due to conformational changes of the protein following reduction of the disulfide crosslinks (which may uncover new sites for interaction) is supported by comparison to the results with NPEW. This conclusion is also supported by the observation that in most instances the increases in metal uptake for reduced wool compared to the native form were roughly equivalent to the sulfhydryl content of WSH (about 0.9

meq/g). Furthermore, these increases appear to have been achieved within the first hour, as can be seen by comparing the nearly equal differences of uptake between WSSW and WSH for both the 1-hr and 24-hr periods with these cations. This result suggests rapid binding to sulfhydryl groups of wool to form covalent metal mercaptides. Firm binding of these cations to WSH is expected owing to the general stability and low solubility product of various metal mercaptides. The significance of the natural (though small) sulfhydryl content of WSSW in the highly effective binding by native wool of mercuric ions, at low concentrations, has been pointed out.²⁴

Since uptakes of these metal ions was increased by amounts roughly equivalent to the sulfhydryl content of WSH, we suggest that these ions do not interact appreciably with the disulfide function (certainly no more than the difference between the observed uptakes and the potential sulfhydryl content of 0.9 meq/g, which is small).

In view of the relative stability of WSH to air oxidation in the dry state (no significant decrease of sulfhydryl content was detected after three weeks at room temperature; see experimental section), reduced wool appears to be a practical and highly effective insoluble substrate for removing these cations from water solutions. Comparison of metal ion uptakes by WSH and WSSW also shows the following:

1. While the 24-hr uptake of mercury(II) by WSH was 0.9 meq/g more than by WSSW, no change was obtained with Hg(I). This may be interpreted as indicating that uptake of Hg(I) by WSSW (about 1.5 meq/g) is to a large extent due to the interaction with the disulfide bond. Further evidence for this conclusion comes from the observed negligible uptake of Hg(I) by NPEW (0.38 meq/g) in which the disulfide bond function has been abolished. Also, if it is assumed that reaction of Hg(I) with sulfhydryl groups is rapid while that with disulfide bonds is much slower (which is reasonable), then the observed difference between 1-hr uptakes of 0.44 mmoles Hg(I) by WSH and WSSW is readily explicable. It should also be mentioned that the color of WSSW (originally white) that had been treated with mercurous nitrate was dark purple, while that of NPEW similarly treated was unchanged (white). Due to the tendency of mercury to undergo disproportionation, part of the uptake of 1.5 meq cation/g WSSW from HgNO_3 may represent uptake of the cation as Hg(II) rather than Hg(I).

2. While copper(I) uptake by WSH was increased by 1 mmole/g wool, the uptake of copper(II) was essentially unchanged from that by WSSW. We suggest that this difference may be related to the known ability of Cu(II) to catalyze oxidation of sulfhydryl groups in proteins.^{5,6} The uptake by WSH of Fe(III), which similarly catalyzes oxidation of sulfhydryl groups,¹⁰ also was not increased over the uptake by WSSW. We suggest that the lack of difference in uptakes by WSH and WSSW of Ce(IV), Cr(III), and perhaps Ni(II), Co(II), and As(III) may reflect a tendency of these cations also to catalyze oxidation of sulfhydryl groups in proteins.

3. The uptake of Pd(II) by WSH was increased over that by WSSW by about 2.2 mmoles/g wool (24 hr). This increase is more than twice the stoichiometric amount of sulfhydryl groups generated in WSH. This observation was reproducible. It is interpreted as due to increased accessibility of the wool substance due to the absence of disulfide crosslinks (thus making sites that are not available in the crosslinked WSSW available for interaction). The result with 2-PEW, in which the disulfide function was also abolished, also showed more than stoichiometric increase in Pd(II) uptake over the 0.9 meq of pyridylethyl groups/g wool, and can be explained similarly. Results of Pd(II) uptake by NPEW (no disulfide), however, showed no increase from that of WSSW, perhaps owing to steric or electrostatic conditions or the potential of the nitro group of NPEW to oxidize Pd(II) (see results of Pd(II) uptake by NTW and ATW).

4. Although the uptakes by WSH compared to WSSW of nickel(II), cobalt(II), thorium(IV), arsenic(III), zirconium(IV), and thallium(I) increased much less than the potential of 0.9 meq/g wool, these increases are significant in view of the fact that the uptakes of these ions by WSSW are essentially nil. The relationship of limited uptake of some of these ions and their probable tendency to reoxidize WSH was mentioned above. The limited uptake of Zr(IV) may be related to the polynuclear (polymeric) nature of Zr(IV), to its tendency to hydrolyze in water even at low pH and low Zr(IV) concentrations,¹⁸ and to salt links between free amino and free carboxyl groups of wool.

5. Uptake of gold(III) by WSH, with a solution of about 1.2 mmoles gold chloride per gram of wool, was almost quantitative within 10–20 min of contact. WSH appears to undergo degradation as with WSSW with excess gold chloride and longer treatment times.

6. The 1-hr uptake of uranium(VI) by WSH was higher than the corresponding 1-hr uptake by WSSW, but the 24-hr uptakes were the same. WSW treated with uranium(VI) for 24 hr lost weight and appeared degraded.

S- β -(2-Pyridylethyl) Wool

The behavior of 2-PEW, already noted in part, can be summarized as follows. In general, the introduced pyridethyl groups are usually not so effective in the modified wools as the sulfhydryl for binding the various cations. However, increased binding (compared to either WSSW or WSH) was observed with chromium(III), zirconium(IV), titanium(IV), and with gold(III). Furthermore, treating 2-PEW with excess gold chloride for periods even as long as 24 hr appeared not to degrade the wool (no weight loss). The 1-hr uptake of Sb(III) by 2-PEW was about 0.6 mmoles higher than the 1-hr uptake by WSSW, but the 24-hr uptake by 2-PEW was nil. This unusual observation was reproducible. It may indicate hydrolysis or degradation of 2-PEW by prolonged treatment with the Sb(III) solution.

S-(p-Nitrophenethyl) Wool

The slightly lower uptakes usually observed with NPEW compared to WSSW may reflect minor participation of the disulfide of WSSW in the

binding of the various metal ions. The decreased uptake of Hg(I) was mentioned earlier. Another outstanding result is the increased uptake of tin(II) by NPEW compared to WSSW. Similar increases were also seen with other keratins modified to contain nitro groups, for example, NTW (Table III) and 3-nitrotyrosyl silk (not shown in the tables).

Thus, native silk treated with SnCl_2 for 24 hr contained only about 0.5% Sn(II), while 3-nitrotyrosyl silk took up about 10% Sn(II). A possible interpretation is that the nitro groups of the modified keratins oxidize the stannous ion, Sn(II), to the stannic ion, Sn(IV), which in turn may be the ionic species that interacts with potential binding sites in the keratin (in which the nitro groups would be reduced to amino groups).

3-Nitrotyrosyl- and 3-Aminotyrosyl Wools

In contrast to the high uptake of Sn(II) by NTW and NPEW, uptake of Sn(II) by 3-aminotyrosyl wool (ATW) was less than by WSSW, again suggesting that oxidation to stannic ion rather than the generation of amino groups in keratin is the important circumstance responsible for the increased Sn(II) uptake by the nitro derivatives. Similarly, uptake of Sn(II) by 3-aminotyrosyl silk (not shown) was decreased to about one eighth the uptake by 3-nitrotyrosyl silk. Uptake of Pd(II) was increased with both NTW and ATW, especially with the later. In contrast, Pd(II) uptake by NPEW was not increased. The substituted tyrosyl phenolic residue may therefore be an active binding site. Oxidation of Pd(II) by the nitro-keratins may occur.

Results with ATW also showed improved uptakes with Hg(I), Ag(I), and Pt(IV). Therefore, the 3-aminotyrosyl residue appears to be an effective binding site for these cations.

The uptakes by ATW of Sb(III), Pb(II), and U(VI) were less than those by WSSW; perhaps the phenolic tyrosyl residues in native wool are binding sites for these cations, and substitution of these residues interferes with their suitability as binding sites.

Wool Fabric

Flame resistance and insect resistance were imparted to the wool fabric by treatment with some of the metal salts. Results shown in Table V are illustrative. We confirm improved flame resistance due to treatment with zirconium and titanium salts as shown previously by Benisek.²⁰ We also obtained improved flame- and insect-resistance properties of wool with antimony and bismuth salts. We hope to report on this aspect of the work in more detail in future publications.

SUMMARY

Wool and wool derivatives bind a substantial amount of many of the metal ions tested. Furthermore, a degree of specificity of metal binding can be imparted to wool by certain chemical modifications, in that the relative binding capacities and rates for various ions can be differently affected.

TABLE V
Effect of Metal Salt Treatment on Flame and Moth Resistance of Woven Wool Fabric

Treatment (salt) ^a	Weight increase, % of original wool	Flammability ^b		Moth-resistance excrement per larvae, mg
		After-flame, sec	Char length, in.	
SbCl ₃	1.9	8.9	5.8	0.35 ^c
BiCl ₃	5.6	3.0	4.9	0.88
ZrOCl ₂	d	7.8	5.9	1.9
TiCl ₄	d	3.3	4.5	2.2
CuCl	9.0	14.0	9.0	0.25
AgNO ₃	12.3	23.5	total (10 in.)	0.19
Pb(NO ₃) ₂	3.1	27	total (10 in.)	1.38
Untreated controls	—	21.5	total (10 in.)	3.2

^a SbCl₃ and BiCl₃ were dissolved in 1N HCl. With CuCl treatment, HCl was added to a 1% suspension of CuCl in H₂O to bring solution to 0.01N HCl. AgNO₃ and Pb(NO₃)₂ were dissolved in H₂O. All treatments were carried out at 25°C for 16 hr, except with ZrOCl₂ and TiCl₄. With ZrOCl₂ and TiCl₄, citric acid was added to the reaction mixture and the treatment was carried out by boiling for 45 min as described by Benisek.²⁰ All fabrics were washed thoroughly and air dried before flame- and insect-resistance tests.

^b Treatments are considered effective when the after-flame burn time is less than 12 sec and the char length is less than 6 in.

^c Also about 3% mortality was observed.

^d Not determined.

The results also include information about the modes of binding of the various ions. This information is very likely pertinent to binding of these cations to other proteins. The keratin modifications tested were reduction of the disulfide bonds to give a polysulfhydryl polymer, and S-alkylation of the generated sulfhydryl groups with reagents which introduce into the keratin molecule functional groups which are potentially either binding sites, such as S-pyridylethyl groups, or nonbinding sites, such as S-*p*-nitrophenethyl groups. Tyrosyl residues were also modified to 3-nitrotyrosyl or 3-aminotyrosyl residues.

We suggest that further specificity of binding may be imparted to wool by other chemical modifications such as esterification of glutamyl and aspartyl free carboxyl residues which appear to be binding sites for many cations,^{3,4,7,11-13,32} and by appropriate choice of modifying reagents. Also, modification of more than one functional group in the same keratin molecule may be considered.

Finally, the interaction of wool with metal salts may impart useful properties to the wool as a textile fiber as shown in many instances in the literature.^{1,6,11-13,16-20} We also find that in some cases reduced flammability and increased insect resistance are imparted to wool as a result of treatment with some of the metal salts.

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 that may be suitable.

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