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Sorption Behavior of Mercuric Salts on Chemically Modified Wools and Polyamino Acids*

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Synopsis

Wool derivatives with sulfhydryl, thiosulfate, imidazole, pyrrolidone, or pyridine side chains were prepared and tested as potential scavengers for mercury salts in aqueous solution. More mercury is bound by these derivatives than by native wool or by certain commercial ion exchange resins under similar conditions. The presence of chloride ion, but not sulfate ion, in aqueous media decreased the extent of binding of mercuric chloride to both native and modified wools. The relative binding of mercuric chloride by various poly(amino acids) suggest that mercury is taken up by proteins by processes other than (or in addition to) specific combination with free functional groups. Two possibilities are suggested: the protein may act as a solid solvent for the mercurial, or the mercurial may form aggregated deposits within the protein after the specific binding sites have been occupied. These studies are intended to elucidate factors that govern mercury interaction with wool and other proteins and to develop improved scavengers for toxic metals.

INTRODUCTION

Sorption by wool of mercuric and methylmercuric salts, as it depends on mercurial concentration, solution pH, and time of contact, has been measured by atomic absorption spectroscopy.[1–3] The results show that under equilibrium conditions wool can efficiently adsorb inorganic mercury salts and, somewhat less efficiently, methylmercuric chloride. Sorption increased roughly in proportion to the square root of residual concentration in solution, in accordance with the Freundlich isotherm.

We wish to explore further how the mercury-binding capacity of wool can be increased by chemical modification, as would be expected if additional binding sites are introduced. We find that it is indeed feasible to increase the binding capacity of wool by such chemical modification and that modified wools appear to be useful for recovering mercurials from contaminated water.

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EXPERIMENTAL

Modified Wools

Reduced wool, S-(2-pyridylethyl) wool, and analogous S-(N-imidazolyl)-ethyl-, S-(N-pyrrolidonyl)ethyl-, and S-(*p*-nitrophenethyl) wool derivatives were made as previously described.[2,4,5] Oxidized wool was made by treating wool with $KMnO_4$ as described by McPhee.[6] Thiosulfate wool (W-S-SO_3^-) was prepared by treating wool 1 hr with 1% aqueous $NaHSO_3$ including a drop of Igepal CO-610 to enhance wetting.[7] The treated wool was rinsed three times with distilled water and air dried.

Analysis

Mercury was determined by specific atomic absorption spectroscopy with a Perkin-Elmer Model 303 spectrometer equipped with an acetylene air burner. In some instances, mercury in wool was also determined directly after digesting the Hg–wool with nitric acid and potassium permanganate.

RESULTS AND DISCUSSION

Effect of Chemical Modification

Mercury binding by wool, wool derivatives, and ion exchange resins is compared in Tables I and II. The results of this and previous studies[1–3] show that (a) chemical modification usually increased mercury binding by wool; (b) both native wool and the chemically modified wools are more effective mercury scavengers than the synthetic resins tested; and (c) the enhanced effectiveness takes place at both low and high initial mercury concentration.

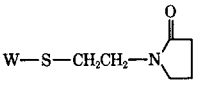
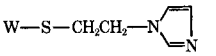
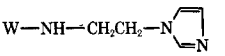
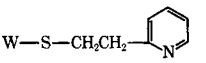
Although most derivatives bound more mercury than native wool, Table III shows that binding by S-(*p*-nitrophenethyl) wool is less than that by

TABLE I
Mercury Uptake by Native and Reduced Wool[a]

Hg Concn., mg/ml		Calcd uptake		Wt. incr. washed wool, mg/g
Initial	Final	mg/g	%	
0.1 (100 ppm)	0.002 (0.002)	4.9	98 (100)	12 (19.0)
1.0	0.2 (0.0012)	40	80 (100)	50 (48.5)
2	0.8 (0.0014)	60	60 (100)	73 (139)
4	1.7 (0.042)	115	58 (100)	116 (148)
8	3.4 (1.5)	230	57 (81)	177 (257)
16	8.6	370	46	280
24	13.0	550	46	374
32	16.5	775	49	444
40	21.5 (19.5)	925	46 (49)	510 (625)

[a] Values for reduced wool are in parenthesis; 1 gram of wool was shaken in 50 ml aqueous $HgCl_2$ solutions at 25°C for 24 hr.

TABLE II
Mercury Uptake by Modified Wools and Ion Exchange Resins[a]

Material	Residual concn., mg Hg/l., (ppm)	Mercury adsorbed, %	Structures
None (original mercury concentration)	600		
Reduced wool	10	98.3	W—SH
Reduced wool plus N-vinylpyrrolidone	5.5	99.3	
Reduced wool plus N-vinylimidazole	18	97	
Wool plus $NaHSO_3$	50	91.6	W—S—SO_3^-
$KMnO_4$-oxidized wool	180	70	W—SO_3^-
Wool plus N-vinylimidazole	190	68.3	
Reduced wool plus 2-vinylpyridine	190	68.3	
Waste wool	270	55	W—S—S—W
Dowex 2 × 8 resin	320	46.6	R—CH_2—SO_3^-
Dowex 1 × 8 resin	350	41.6	R—CH_2—SO_3^-
Dowex 1-A chelating resin	470	38.3	R—CH_2—$N(CH_2COO^-)_2$

[a] Reaction conditions are as follows: 1.5-g samples of adsorbent were shaken at 21°C for 30 min in 150 ml 0.01*N* HCl containing mercuric chloride to give 600 mg mercury/liter.

native wool. Therefore, these results suggest that reduction of wool followed by alkylation with a reagent that does not introduce potential ligand sites does not necessarily improve its mercury-binding properties. In contrast, reduced wool (W–SH), oxidized wool (W–SO_3^-), and thiosulfate wool (W–S–SO_3^-) all show greater binding than with a native wool (Table I). Thus, SH, SO_3^-, and S–SO_3^- appear especially effective for mercury binding. Further improvement in the binding capacity of wool could undoubtedly be achieved by thiolation, that is, by incorporating SH groups in addition to those that can be made by reducing disulfide bonds.

Because SH groups in reduced wool tend to oxidize in air back to disulfide bonds, one of our main objectives is to develop wool derivatives as effective as reduced wool but stable in air. Results in Tables I–III suggest that we have accomplished this objective.

Mercury bound to nitrogen-containing ligands, such as amine, imidazole, pyridine, and pyrrolidone, can be removed with the aid of complexing

TABLE III
Effect of Chloride on Hg Uptake by Modified Wools[a]

Wool	NaCl, *M*	Wt. increase, mg/g
Native	0.0	510
Reduced plus vinylpyridine[b]	0.0	810
Reduced plus vinylpyridine	1.0	213
Reduced plus vinylimidazole[b]	0.0	605
Reduced plus vinylimidazole	1.0	285
Reduced plus vinylpyrrolidone[b]	0.0	680
Reduced plus vinylpyrrolidone	1.0	307
Reduced plus *p*-NO_2-styrene (HCl)	0.0	202
Reduced plus *p*-NO_2-styrene (HCl)	1.0	107
Reduced plus *p*-NO_2-styrene (H_2O)	0.0	430
Reduced plus *p*-NO_2-styrene (H_2O)	1.0	81

[a] One gram of native or modified wool was shaken for 24 hr at 25°C in 50 ml 0.2*M* $HgCl_2$ in 0.1*N* HCl without or with 1*M* NaCl as shown. The sample was washed in water and the uptake determined as the increased weight after drying.

[b] See Table II for structures.

reagents such as chloride and thiocyanate ions, mercaptoacetic acid, citric acid, or EDTA[1,2] For example, 1*M* NaCl removes 50% of the mercury from high-mercury wool (0.5 g Hg per g wool) under equilibrium conditions (24 hr, room temperature, 1:100 wool:liquid ratio). In contrast, some of the mercury adsorbed to wool appears hard to remove by extraction with complexing reagents. The amount of such firmly bound mercury is greater in reduced wool. We infer that it is covalently bound to mercaptan sulfur. Thus, the different wool derivatives may find useful application for different purposes, depending on whether reversible or irreversible binding is needed.

Salt Effects

Mercuric chloride tends to associate with chloride ions to form negatively charged complexes,[3] as illustrated:

(a) Mercury Binding:

$$\underset{\text{wool}}{\text{W—NH}_2} + \text{HgCl}_2 \rightleftharpoons \underset{\text{mercury-wool}}{\text{W—N(H)—Hg—Cl}} + \text{HCl}$$

(b) Interference by Cl^-:

$$\text{HgCl}_2 + 2\text{Cl}^- \rightleftharpoons \text{HgCl}_3^- + \text{Cl}^- \rightleftharpoons \text{HgCl}_4^{2-}$$

Cl^- competes with $HgCl_3^-$ and $HgCl_4^{2-}$ for binding sites in wool.

(c) Desorption by Cl^-:

$$\text{W—N(H)—Hg—Cl} + 3\text{Cl}^- + \text{H}^+ \rightleftharpoons \text{W—NH}_2 + \text{HgCl}_4^{2-}$$

For this reason, it was expected that chloride ion (e.g., in lakes, sea water, and industrial effluents) would interfere with mercuric chloride bind-

TABLE IV
Effect of NaCl on Mercury Uptake by Wool

Concentration of added NaCl, *M*	Solution pH 2		Solution pH 7	
	Residual concn., mg Hg/l. (ppm)	Binding, mg Hg/g	Residual concn., mg Hg/l.	Binding, mg Hg/g
0	170	43	320	28
0.15	270	33	450	15
0.5	380	22	520	8
1	460	14	520	8
2	480	12	540	6
Initial concn.	600		600	

[a] One gram of wool was shaken for 1 hr at room temperature with 100 ml of 0.01*N* HCl (pH 2) or water (pH 7) with added sodium chloride as indicated. The initial mercury content was 600 mg/l. Mercury binding was calculated from residual concentration of Hg in solution.

ing to wool, an effect already demonstrated by Speakman and Coke[9] and Leach.[10,11] More systematic studies, summarized in Tables III and IV, show that this phenomenon operates for both native and various chemically modified wool. Chloride interference due to complexing with $HgCl_2$ should decrease with increasing pH leading to hydrolysis as Cl^- is replaced by OH^-. This is indeed the case (Table IV).

It is also noteworthy that (a) sulfate ion does not inhibit mercury binding since $MgSO_4$, $ZnSO_4$, and Na_2SO_4 did not affect mercury uptake by wool; (b) the chloride ion effect appears to be at a maximum limiting value at about one molar chloride concentration; (c) the chloride effect is independent of the particular cation used since our studies show that NaCl, KCl, $CaCl_2$, $FeCl_2$, or $CuCl_2$ all inhibit mercury binding to wool; and (d) we observe a similar halide ion effect with methyl mercuric chloride and with sodium iodide.

Mechanism of Binding

Reactions of mercury compounds with amino acids containing sulfhydryl,[12-14] sulfide,[15] disulfide,[16] amino,[3,17] and imidazole[18] groups lead to the expectation that these in wool will bind mercury under suitable conditions. Much study has been devoted to developing conditions under which reactions of mercuric salts or organic mercury derivatives can be used to determine sulfhydryl contents of simple mercaptans and proteins.[12] Thus, reaction of various organic mercurials, but especially methylmercuric iodide at pH 9 is judged to measure free sulfhydryl reliably. At this pH, sulfhydryl is ionized (therefore reactive) and amino groups protonated (therefore relatively unreactive).

The binding stoichimetry of inorganic mercuric salts is less certain because of the possibilities of one mercury atom combining with two sulfhydryl groups or only one. When mercury is not in large excess and the

TABLE V
Mercury Sorption by Model Substances[a]

Material	Monomer unit weight	Mercury absorbed		Residual concn., milligrams Hg/l.
		Milligrams/g	Micromoles per monomer unit	
None				600
Polyamide-6,6	125.2	8	0.24	520
Poly(methyl glutamate)	143.1	6	0.14	540
Poly(glutamic acid)	129.1	7	0.19	530
Polytyrosine	163.2	9	0.21	510
Polyglycine	57.1	15	1.18	445
Poly(arginine hydrobromide)	237.1	22	0.42	380
Poly(lysine hydrochloride)	164.6	56.7	1.66	33
Polyhistidine	137.1	56.2	2.01	28
Egg albumin		38		220
Bovine serum albumin		33		290
Horse γ-globulin		22		380

[a] Fifty-mg samples of polymer in 2 ml 0.01*M* HCl in dialysis bags were equilibrated for 24 hr against 3 ml 0.01*M* HCl without the polymer. The initial $HgCl_2$ concentration in both compartments was 600 ppm. Calculations of bound Hg are based on the residual concentrations in the total volume (5 ml) as measured for the solutions outside of the bags.

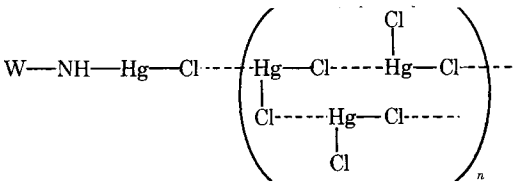
substrate is mobile, approximate 1:2 combining ratios may be observed. Under other conditions, binding can greatly exceed the small amount corresponding to the free sulfhydryl content of wool. Leach[10] has shown that the binding from mercuric chloride in acid is scarcely affected by previously blocking the free sulfhydryl groups. Speakman and Coke[9] assumed that (besides sulfhydryl), the basic groups of lysine and arginine residues in wool would bind mercury because of the recognized formation of mercury–amino complexes. For supporting evidence, they measured mercury uptake of wool that had been treated with nitrous acid to destroy primary amino groups. Binding was, indeed, decreased by this treatment, but because the drop in mercury binding was less than proportional to the drop in acid binding, existence of additional binding sites was inferred. Combination with peptide bonds was therefore proposed.

These studies do not separate effects on rates of adsorption versus total uptake at equilibrium. A satisfactory theory should consider electrical charge of the protein, the ionic states of the mercurial and of the reactive sites in wool, and the influence of complex-forming sites with higher or lower binding constants at any particular pH. Clear understanding is important in designing chemical treatments for improving the adsorption characteristics of wool and in selecting conditions for adsorption and recovery.

Data on relative affinities of model poly(amino acids) for mercuric chloride (Table V) show that although all protein functional groups may be

involved in mercury binding, amino groups of lysine side chains and imidazole groups of histidine residues appear most effective after SH groups. (We have already demonstrated the importance of both primary and secondary amino groups in polyamine–carbohydrates in mercury binding[3]). Arginine residues show an intermediate effectiveness, but, surprisingly, polyglycine appears to bind a moderate amount of mercuric chloride. Evidently either peptide bonds or aliphatic (hydrophobic) parts of the polymer may participate in mercury binding. However, the situation is not straightforward since the other poly(amino acids) as well as nylon, a polyamide, exhibit a lesser tendency to bind mercury. Possibly conformational and proximity factors are important here. Furthermore, since poly(glutamic acid), poly(methyl glutamate), and polytyrosine show a low affinity for mercuric chloride, evidently carboxyl, ester, and phenolic groups are not significant in mercury binding by proteins.

Since, at high initial mercury concentration, more mercury is bound to wool than can be accounted for in terms of free functional groups, additional mechanisms must operate in the binding of mercurials to proteins. One possibility is that the protein could act as a solid solvent for mercurials. This possibility is supported by the previous observations[1,2] that the extent of binding can be quantitatively accounted for in terms of a partitioning of the mercurial between the liquid phase and the solid (wool) phase. Another possibility is that mercuric chloride forms polymeric lattices at some places within the wool structure.[19] The nature of the aggregated deposits is presently unknown. In the case of $HgCl_2$, polymer formation could result from interactions between mercury and chloride atoms, as illustrated:



mercuric chloride "polymer" bound to wool

Evidence in support of these possibilities could come from future x-ray diffraction, Raman spectroscopy,[20] and chlorine nuclear magnetic resonance studies.[21,22]

In conclusion, from a study of factors that govern mercury binding to model compounds, native, and modified wools, we believe that the binding sites for mercury in proteins are sulfhydryl, amino, imidazole, and guanidino groups but that other sides significantly contribute to binding. Finally, by suitable chemical modification it is possible to enhance significantly the binding properties of wool for mercury salts.

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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.

References

1. M. Friedman, C. S. Harrison, W. H. Ward, and H. P. Lundgren, *J. Appl. Polym. Sci.*, **17**, 377 (1973).
2. M. Friedman and A. C. Waiss, Jr., *Environ. Sci. Technol.*, **6**, 457 (1972).
3. M. S. Masri and M. Friedman, *Environ. Sci. Technol.*, **6**, 745 (1972).
4. M. Friedman and A. T. Noma, *Text. Res. J.*, **30**, 349 (1970).
5. M. S. Masri, J. J. Windle, and M. Friedman, *Biochem. Biophys. Res. Commun.*, **47**, 1408 (1972).
6. J. R. McPhee, *Text. Res. J.*, **30**, 349 (1960).
7. J. A. Maclaren, W. E. Savige, and B. J. Sweetman, *Austr. J. Chem.*, **18**, 1655 (1965).
8. J. L. Webb, *Enzyme and Metabolic Inhibitors*, Vol. 2, Academic Press, New York, 1966, p. 729.
9. J. B. Speakman and C. E. Coke, *Trans. Faraday Soc.*, **35**, 246 (1939).
10. S. J. Leach, *Austr. J. Chem.*, **13**, 547 (1960).
11. S. J. Leach, in *A Laboratory Manual of Analytical Methods of Protein Chemistry*, Vol. 4, P. Alexander and H. P. Lundgren, Eds., Pergamon Press, New York, 1966, Chap. 1.
12. M. Friedman, *Chemistry and Biochemistry of the Sulfhydryl Group in Amino Acids, Peptides, and Proteins*, Pergamon Press, New York, in press, Chap. 11.
13. T. Norseth, *Acta Pharmacol. Toxicol.*, **31**, 138 (1972).
14. R. Arnon and E. Shapira, *J. Biol. Chem.*, **244**, 1033 (1969).
15. D. F. S. Natusch and L. J. Porter, *J. Chem. Soc.*, **A**, 2527 (1971).
16. P. R. Brown and J. O. Edwards, *Biochemistry*, **8**, 1200 (1969).
17. R. H. Fish and M. Friedman, *J. Chem. Soc. Chem. Commun.*, **812** (1972).
18. P. Brooks and N. Davidson, *J. Amer. Chem. Soc.*, **82**, 2118 (1966).
19. J. Sikorski, W. S. Simpson, and H. J. Woods, *Proceedings of the 4th International Conference on Electron Microscopy*, Vol. 1, G. Möllenstadt, Ed., Springer, Berlin, 1960, p. 707.
20. P. R. Carey, H. Schneider, and H. J. Bernstein, *Biochem. Biophys. Res. Commun.*, **47**, 588 (1972).
21. T. S. Stengle and J. D. Baldeschwieler, *J. Amer. Chem. Soc.*, **89**, 3045 (1967).
22. R. G. Bryant, *J. Amer. Chem. Soc.*, **91**, 3045 (1969).

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