Thermal Transitions in Reduced Wool Fibers

INTRODUCTION

Wool and other fibrous proteins are known to undergo conformational transitions at elevated temperatures.[1] These transitions can be detected by calorimetric methods,[2] by differential thermal analysis,[3] or by changes in tensile properties[4] or fiber dimensions.[5] For example, wool fibers contract rapidly in water at 120–130°C, depending upon the rate of heating. The transition temperature is lowered considerably in the presence of denaturants or reducing agents.[6] Both types of reagent are believed to destabilize the initial (ordered) state and favor the final (disordered) state.

By using a new reducing agent (tri-*n*-butylphosphine) it is possible to reduce virtually all of the disulfide bonds in wool under conditions which preserve a high degree of crystallinity in the fibers.[7] The thermal stability of the ordered regions in these reduced fibers may then be conveniently studied by measuring their lengths as a function of temperature. In the fully reduced state it may be assumed that the contraction temperatures observed will be a property of the helical microfibrils, which are no longer attached by disulfide bonds to the less-ordered components of the fiber. The low transition temperatures of such fibers (55–65°C) indicate their comparative instability. The effect of chemical modifications on the stability of the microfibrils is therefore seen more readily if thermal contraction temperatures are measured after reduction.[8] In particular, it has been found that measurements made in this way provide a useful criterion for the occurrence of crosslinking reactions.

Attempts to introduce crosslinks in polymers or proteins by treatment with bifunctional reagents are usually monitored by physical as well as chemical tests. Measurements of extension,[9] lateral compression,[10] and torsional[11] moduli as well as the swelling[12] and water sorption[12] of wool fibers have been used for this purpose. Probably the most commonly used qualitative tests for crosslinking involve measurement of the decrease in solubility of the treated wool after fission of the "native" disulfide crosslinks.[13] However, it has been shown that the interpretation of the results of such solubility tests is complicated by the fact that even monofunctional substituents, if they are sufficiently bulky, may cause a decrease in solubility of the wool, although this is generally less than that caused by bifunctional reagents.[13] Measurements of the thermal contraction behavior of wools which have been treated with mono- or bifunctional reagents seem to be free of this objection. This note describes the way in which thermal contraction measurements can be used to differentiate between the introduction of monofunctional substituents (of any size) and of crosslinks.

EXPERIMENTAL

Lincoln wools were acylated by using 0.05*M* solutions of active esters or active amides[8,14] in dimethylformamide at 60°C for 24 hr, 0.02*M* acetic acid being used as catalyst. Treatments with formaldehyde (1*M*) were carried out at 20°C for 1 hr, either in water, acetic acid (0.1*M*), or in an alkaline buffer (0.05*M* $NaHCO_3$; 0.025*M* Na_2CO_3).

Solubilities of acylated or formaldehyde-treated wools in ammonium hydroxide (0.3*M*) were determined after oxidation of the wool with performic acid.[13] The solubilities of the acylated wools have been reported previously.[13] All three formaldehyde treatments decreased the solubility of the wool from a normal value of 75% to 18–20%.

After acylation or treatment with formaldehyde the wool fibers were mounted in glass tubes so that they could be slackened for treatment or straightened for length measurement. Initial lengths were determined in 20% aqueous *n*-propanol. The fibers were slackened, tri-*n*-butylphosphine was added, and the tubes were rotated intermittently at 20°C for 48 hr. Under these conditions of reduction[7] we found that 97% of the disulfide bonds in the wool were reduced. The reduced fibers were measured, slackened,

and then heated in the reducing medium at 30°C for either 45 min or 24 hr (equilibrium was usually reached within 5 hr); the fiber lengths were then measured again. This procedure was repeated, the temperature being raised in steps of 10°C to a final temperature of 80 or 90°C. The extent of contraction at each temperature was expressed as a percentage of the length at 20°C.

Results and Discussion

The thermal contraction of reduced wool does not occur over as narrow a temperature range (see Figs. 1 and 2, curves c) as more homogeneous and crystalline protein fibers such as collagen. However, it is sufficiently sharp and reproducible (under standard conditions of heating) to provide a useful index of the stability of the crystalline regions of wool, presumably microfibrils.[15] Figure 1 shows the effects of acylation on the thermal contraction behavior of wool when the wool is heated under nonequilibrium conditions, viz., for 45 min at each temperature. The active esters and active amides used for acylation have previously been shown to react with most of the lysyl residues in wool (200 μmole/g),[8,16] although under the conditions used some hydroxylic side chains are also acylated.[14] Most of the lysyl residues in wool occur in the low-sulfur proteins derived from the microfibrils and are concentrated in the helical portions of these proteins.[17] It is not surprising, therefore, that acylation has a considerable influence on the transition temperature (which we measure at the point of maximum slope of the contraction vs. temperature curve). The introduction of either small (acetyl) or large (*n*-decanoyl) groups lowers the transition temperature, i.e., it decreases the stability of the microfibrils to thermal denaturation. On the other hand, acylation with bifunctional reagents raises the transition temperature by at least 30°C. We regard this stabilization of the microfibrils as evidence for the introduction of crosslinks.

The time allowed (45 min) for thermal contraction at each temperature step in the above study was insufficient for the contraction to reach equilibrium. However, Figure 2 shows that, even after allowing 24 hr at each temperature,* the results are qualitatively the same, although the actual transition temperatures have been displaced to lower values. Treatment with the bulky monofunctional reagent still lowers the transition temperature, whereas treatments with bifunctional reagents raise it. In view of the dependence of the observed transition temperature on the heating rate, it is important to use standard conditions when comparing the thermal contraction behavior of treated fibers.

In general, we have found (see Fig. 1) that the introduction of long acyl groups (e.g., stearyl) displaces the transition to lower temperatures than small acyl groups (e.g., acetyl). This is analogous to the denaturation of proteins by the binding of detergent anions at basic sites and is explicable in terms of enhanced opportunities for nonpolar interactions in the disordered state. A comparison of Figures 1 and 2 shows that fibers treated with bifunctional active esters and amides not only contract at higher temperatures than the control fibers but also contract more slowly. The slower contraction of crosslinked fibers is probably due to an increase in the internal viscosity. The size of the crosslinks introduced, —CO— or —$OC(CH_2)_8CO$—, does not appear to influence greatly either of these effects, although we do not know the relative numbers of the two types of crosslinks introduced.

Treatment of collagen with formaldehyde raises the shrinkage temperature, indicating the introduction of crosslinks.[18] Formaldehyde treatment also raises the transition temperature of wool (see Fig. 2), which supports other evidence[19,20] that some crosslinks are introduced into microfibrillar regions. Solubility measurements confirm that the formaldehyde treatment used in this work introduces crosslinks.

The microfibrils in normal wool are intrinsically unstable (after reduction), since they have a preponderance of acidic side chains.[21] Thus any reaction which destroys the

* A separate study showed that contraction ceased after 5–6 hr, even with crosslinked wools.

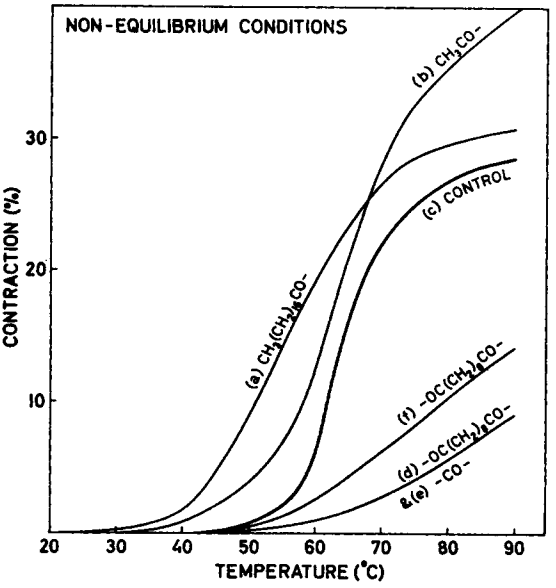

Fig. 1. Effect of substituents on the thermal contraction of wool. The fibers were initially treated with (*a*) *N*-stearylimidazole, (*b*) *N*-acetylimidazole, (*d*) *N,N'*-sebacoxydisuccinimide, (*e*) di-*p*-nitrophenyl carbonate, or (*f*) *N,N'*-sebacyldiimidazole. They were then reduced and measured after heating for 45 min at each temperature shown on the abscissa.

charge of cationic side chains will further destabilize the reduced fiber. This undoubtedly accounts for the effect of monofunctional acylating agents (which react with amino groups) in lowering the transition temperature. Bifunctional acylating agents and formaldehyde must also have this effect, but this must be more than offset by the stabilizing effect of the crosslinks which are introduced, since a net increase in the transition temperature is actually observed.

Although the transition temperature is determined by the thermodynamic stability of the contractile material, the extent of contraction of the denatured fiber is determined by the proportion of contractile material which has melted. In normal wool the α-helical segments of the microfibrils vary in their thermal stability,[22] so that it is not surprising that a broad thermal transition is observed. Since the final extent of contraction falls short of the value (ca. 40%) which is usually reported for completely denatured fibers, it appears that some α-helical segments fail to melt below 90°C under our conditions. However, treatment of wool with small and/or highly reactive reagents which can react with and destabilize all helical segments in the microfibrils might be expected to cause the contraction to approach the upper limit. This is the case with *p*-nitrophenyl acetate, which not only decreases the transition temperature but also increases the total extent of contraction above that of normal wool (Fig. 1). Formaldehyde, which reacts readily with many protein side chains, would also be expected to react with all of the microfibrillar segments indiscriminately. Although the introduction of some crosslinks by formaldehyde will stabilize the fiber to thermal denaturation, raising the transition temperature, heating the reduced fiber beyond this point will result in the contraction of all of the helical segments and lead to maximal contraction of the fiber (Fig. 2). The sharpness of the transition suggests that the stability of the various contractile segments is less variable after formaldehyde treatment than before. Bulkier or otherwise less reactive reagents do not react with all of the helical segments, some being inaccessible.

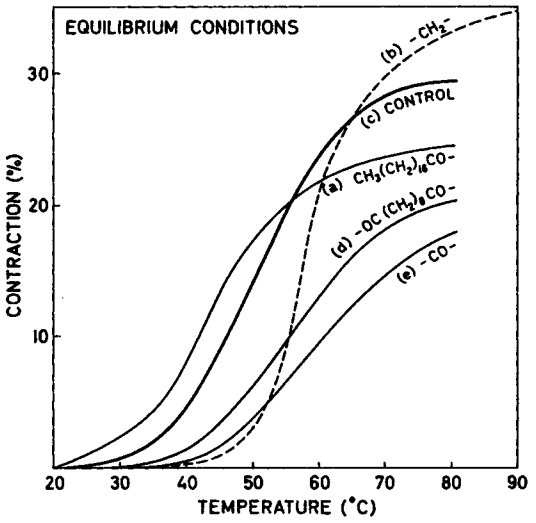

Fig. 2. Effect of substituents on the thermal contraction of wool. The fibers were initially treated with (*a*) *N*-stearylimidazole, (*b*) formaldehyde, (*d*) *N,N'*-sebacoxydisuccinimide, or (*e*) di-*p*-nitrophenyl carbonate. They were then reduced and measured after heating for 24 hr at each temperature shown on the abscissa.

Thus, fibers treated with bulky acylating agents still show as broad a transition as the control fibers and also contract incompletely when heated above the transition temperature.

Many qualitative tests for crosslinking in wool, e.g., solubility and diametric swelling measurements, fail to differentiate between the introduction of a small number of crosslinks and a large number of bulky groups. However, the thermal contraction behavior of wool provides an unambiguous test for crosslinking, in that it clearly differentiates between the introduction of bulky groups and of crosslinks.

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