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The Phase Transition in α-Keratin Fibers

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This paper reviews briefly our recent results concerning the mechanism of the α-β transformation in wool keratin. The role of originally amorphous components in the α-β transformation and the process of the transformation in the α-helical components will be discussed.

KEY WORDS: α-β Transformation/α-Keratin fiber/Wool/Amorphous keratin/CD/X-ray diffraction/DTA/Microfibrillar proteins/ Matrix proteins/

INTRODUCTION

It is well known that the X-ray diffraction pattern characteristic of α-keratin fibers, such as wool and human hair, can be transformed by heating and/or stretching.[1] The stretched keratins have been termed β-keratin according to the nomenclature by Astbury and Street.[2] The structural change of α-keratin to β-keratin, since its discovery by Astbury and Street,[2] has long been of interest in investigation of the molecular structure of keratins. However, the mechanism involved in the α-β transformation has not yet been satisfactorily resolved because of the complex structure of keratin fibers.[1-10]

In the original model by Astbury and Street,[1,2] the process of the transformation was thought to be a "molecular" α-β transformation, i.e., the transformation by the same molecules from a folded α-helix to an extended β-structure. Later, Bendit[1,3] found that the changes in the X-ray diffraction pattern brought about by stretching could not be interpreted in terms of the "molecular" α-β transformation. Since Bendit's observation, the origin of the β-structure, especially the possible role of originally amorphous keratins in the transformation and the process of the transformation have been discussed.[1,3-10] Little convincing experimental evidence has yet been available for other considerations.

Recently, we have reexamined the conformation and conformational change of three main protein fractions isolated from wool, i.e., low-sulfur (Low-S), high-sulfur (High-S) and high-glycine-tyrosine (High-GT) fractions, by means of CD and X-ray diffraction.[11-16] Furthermore, the phase transition of amorphous wool fibers by heating and/or stretching has been investigated by X-ray diffraction and differential thermal analysis (DTA).[17] In this review, the experimental results concerning the mechanism of the α-β transformation in wool keratin will be discussed in terms of the role of originally amorphous components in

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the α-β transformation and the process of the transformation in the α-helical components.

EXPERIMENTAL

Materials

The wool used was Australian Merino 64's(high-crimp wool) and cleaned according to the standard method proposed by Harrap and Gillespie.[18] Deionized water was used throughout. All other chemicals were of reagent grade and used without further purification.

Preparation of Microfibrillar and Matrix Proteins

S-Carboxymethyl kerateines (SCMK) were prepared from reduced wool according to published procedures.[16] The isolation and purification of Low-S, High-S and High-GT protein fractions from SCMK have been reported in previous papers.[11,12,16] Helix-rich and non-helical fragments (SCMK-HF and SCMK-NF) were prepared from Low-S proteins of SCMK (SCMKA) by limited digestion of SCMKA with α-chymotripsin according to the method of Crewther and Dowling.[19] The purification of SCMK-HF was carried out as described before.[16] SCMK-NF, which is soluble in water at pH 4.0, was dialyzed against deionized water and recovered by lyophilization.

Preparation of Amorphous Wool Fibers

Swollen wool fibers were prepared by immersing in a 6.6M LiBr solution at 10°C for 24h and subsequently in diethylene glycol monbutyl ether (n-butyl carbitol) at room temperature for 24h.[5,10] Amorphous wool fibers were successfully prepared by washing the swollen fibers with petroleum ether and drying in vacuo.[17]

X-Ray Diffraction

The X-ray diffraction photographs were taken by a Rigakudenki model RU-3H X-ray diffractometer with a flat-plate camera, using a Ni-filtered Cuk$_\alpha$ radiation. For the films, specimens were made by mounting a number of thin films on top of one another.

CD Spectra

CD spectra were taken on a Jasco J-20 spectropolarimeter in a thermostatically controlled 1 mm jacketed cell. The mean residue weight of each derivative was calculated from its amino acid composition.[16] For CD measurements of the films, the sample solution with a different solvent was layered directly on a quartz disc used for the measurement to prepare the film. CD spectra were expressed in terms of the difference in absorbance for left and right circularly polarized light.

DTA Measurements

Specimens of about 3 mg were sealed in alminium pans. The pans were heated in a Shimadzu Differential Thermal Analyser (DT 20B) at scan rates ranging from 5 to 20°C/min.

RESULTS AND DISCUSSION

β-Structure from Microfibrillar Proteins

The Low-S proteins are considered to originate chiefly from the ordered filamentous units, termed "microfibrils". It should be of interest to compare the β-structure from the microfibrillar proteins with that of β-keratin. However, no study has been reported on the formation of β-structure from the microfibrillar proteins. We attempted to obtain the

β-pattern with relatively sharp reflections from S-carboxymethyl derivatives of these proteins, SCMKA.[13] Recently, we succeeded in obtaining the β-pattern with reflections sufficient to allow us to estimate the unit cell dimensions.[14] This section deals with the β-structure from SCMKA.

Figure 1 shows the X-ray diffraction photograph of stretched β-films of SCMKA. The oriented β-films were prepared by slowly stretching the films cast from formic acid solution in damp steam up to 300% extension over a period of 1-2h and holding them at this extension in damp steam for an additional hour. The diffraction pattern was obtained by passing the X-ray beam perpendicularly to both the plane of the thin films and the stretching direction. Astbury et al.[2] indexed the reflections in the X-ray diffraction pattern of β-keratin on an orthorhombic unit cell with a=0.93nm, b=0.664 nm and c=0.98 nm. In the unit cell, the direction of the interchain hydrogen bonding is the a axis, that of the main chain is the b axis (fiber axis) and that of the side chain is the c axis, respectively. The direction of stretching is parallel to the b axis. In the case of chemically intact keratins, the direction of the cystine crosslinks corresponds to that of the c axis. The calculated results on the stretched β-films of SCMKA showed that the reflections may be indexed on a pseudo-orthorhombic unit cell with a=0.94nm, b=0.66 nm and c=0.98 nm. This means that there is little difference in the unit cell dimesions of the β-structure from intact keratin and SCMKA.

The half-cystine (Cys/2) content of the Low-S proteins from Merino wool is about 7 residues per 100 residues of amino acids,[16] and an identical amount of S-carboxymethyl cysteines (CMCys) is introduced in the sample SCMKA. However, the similarity of the unit cell dimensions between SCMKA and β-keratin suggest that the formation of β-structure from SCMKA does not occur in the peptide sequences containing CMCys.[9]

The sequence of the Low-S proteins consists of a helix-rich and a non-helical

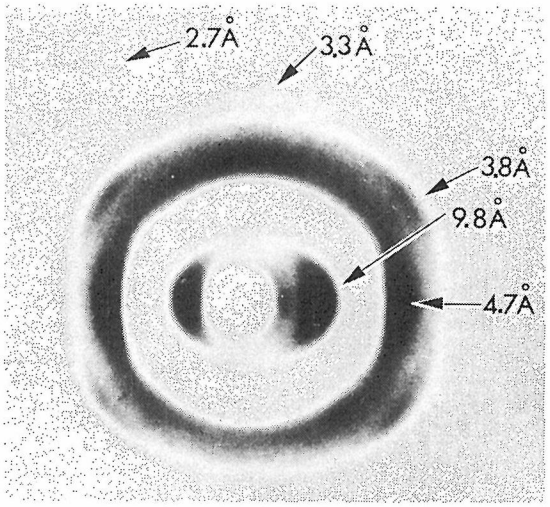

Fig. 1. X-ray diffraction photograph of SCMKA films stretched up to 300 % extension in damp steam. The X-ray beam introduced was perpendicular to both the plane of the films and the direction of the stretching.

sequence,[20] and the SCMKA from Merino wool is about 50% α-helical in water at pH 7.0.[16] The helix-rich sequences contain much fewer CMCys than the original SCMKA, whereas the non-helical sequences contain a high portion of CMCys.[20] The results obtained here may imply that the β-transformation of native wool keratin mostly occurs in the helical parts of the micorfibrillar proteins. In fact, as shown in Figure 2, the helix-rich fragments prepared from SCMKA (SCMK-HF) can be easily transformed to a β-structure by heating them in n-butanol containing a small amount of water at 100°C for 1h, whereas the X-ray diffraction patterns with only diffuse halo were obtained for the non-helical fragments (SCMK-NF) under the same heat-treatment conditions. A similar result was also obtained from DTA experiments of the samples SCMK-HF and SCMK-NF : as described later, a distinct endotherm due to the melting of the β-structure was observed in the DTA curves for the sample SCMK-HF and its heat-treated sample, but little for the sample SCMK-NF.[15]

Formation of β-Structure from Matrix Proteins

It has long been considered that the matrix proteins, i.e., High-S and High-GT proteins can not assume any ordered structure both in solution and in solid state. During the course of the conformational studies on High-S and High-GT protein derivatives,[11,12] we have found that these matrix proteins can assume a distinct β-structure in films cast from formic acid solution. In this section, the formation of the β-structure from High-S and High-GT proteins is demonstrated.

Figure 3 shows the CD spectra of the two types of SCMKB films cast from formic acid and aqueous solution. For the sake of comparison, Figure 3 also shows the CD spectrum of poly (L-lysine) film in the random conformation. It can be seen that the spectra of SCMKB films are definitely different from that of poly (L-lysine) film in the random conformation, suggesting that these SCMKB films contain partly ordered structures. It should be particularly noted that the spectrum of the film cast from formic acid solution was very similar to that of poly (L-lysine) film in the β-conformation. Figure 4 compares the CD spectrum of the SCMKB film cast from formic acid solution with that of the poly (L-lysine) film in the β-conformation.

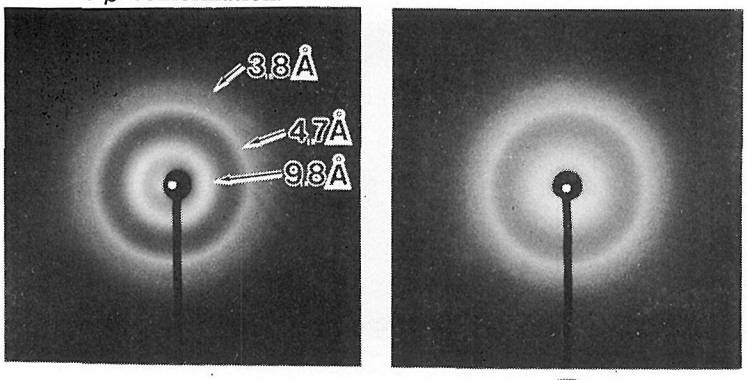

Fig. 2. X-ray diffraction photographs of samples SCMK-HF (A) and SCMK-NF (B). The samples were heated in n-butanol containing a small amount of water at 100°C for 1h.

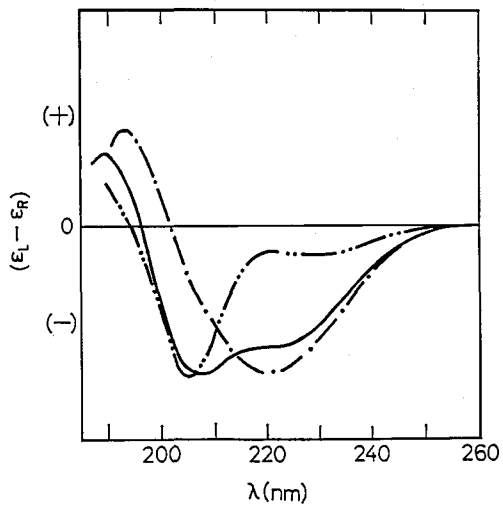

Fig. 3. CD spectra of SCMKB films cast from formic acid (—·—·) and aqueous solution (———) and of poly (L-lysine)film in random conformation (—··—··).

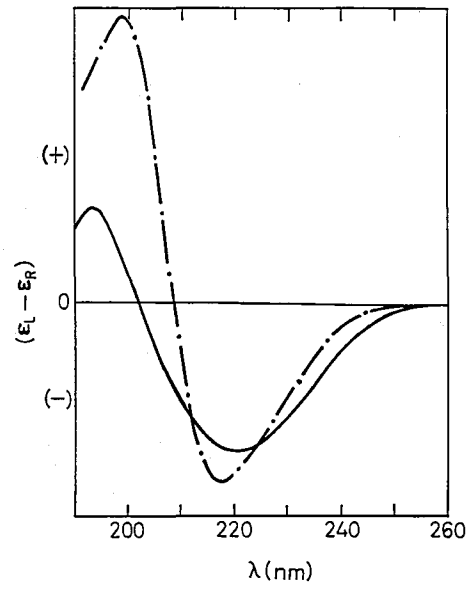

Fig. 4. CD spectra of SCMKB film cast from formic acid solution (———) and of poly (L-lysine) film in β-conformation (—·—·).

The existence of β-structure for the SCMKB film cast from formic acid solution was also confirmed from the X-ray diffraction measurements. Figure 5 shows the X-ray diffraction photograph from the SCMKB film cast from formic acid solution. The diffraction pattern was obtained with the X-ray beam passing parallel to the planes of the thin film. The values of spacings observed from three prominent reflections were found to be 0.753 nm (strong reflection), 0.467 nm (strong) and 0.366 nm (weak). The 0.467 nm reflection is characteristic of a β-structure, which is consistent with the result of the CD spectrum. Although attempts to obtain good fiber diagrams were unsuccessful, the β-films from SCMKB were also obtained by heating the films cast from aquenus solution in alcohol solution containing a small amount of water, suggesting that the formation of β-structure is one of the structural characteristics for High-S proteins. The details of the β-structure have been published elsewhere.[11]

Similar results were obtained for the High-GT proteins from CD and X-ray diffraction measurements of their cast films.[12] These results on High-S and High-GT proteins clearly demonstrate that the originally non-helical matrix proteins may be transformed to an extended β-structure under some specific conditions.

Conformational Change α-Helical Proteins by Heating

The conformational change of α-helical microfibrillar proteins (Low-S proteins) by heating and its reversibility have been studied in detail using the Moffitt-Yang parameter b_o given by ORD measurement.[21] S-carboxymethylated Low-S proteins (SCMKA) have been known to undergo a conformational change in the temperature range 25-80°C. However, the nature of the conformational change, namely, whether the conformational change of

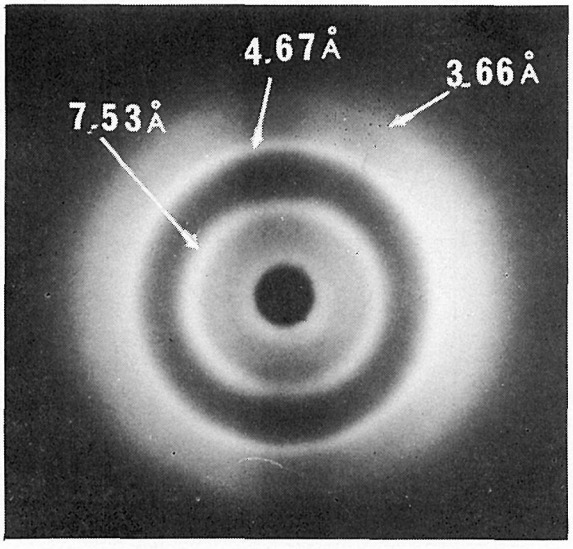

Fig. 5. X-ray diffraction photograph of SCMKB film cast from formic acid solution. The X-ray beam was introduced parallel to the plane of the films.

SCMKA is a helix-coil or helix-β transition, has not been elucidated. We have examined the conformational change of SCMKA by heating through CD.[16]

Figure 6 shows the variation of CD spectra for SCMKA in aqueous solution (pH 6.7) with increasing temperature. It should be noted that all the spectra intersect at 204 nm. From our previous study on CD analysis of SCMKA,[16] it was found that (i) the secondary structure can be satisfactorily estimated using a set of reference spectra proposed by Chang

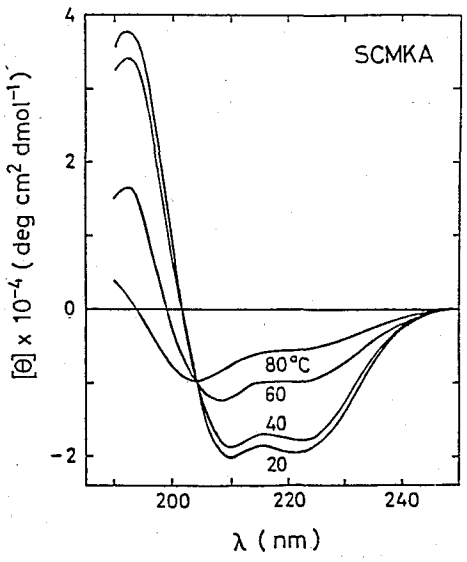

Fig. 6. Variation in CD spectra of SCMKA with increase in temperature.

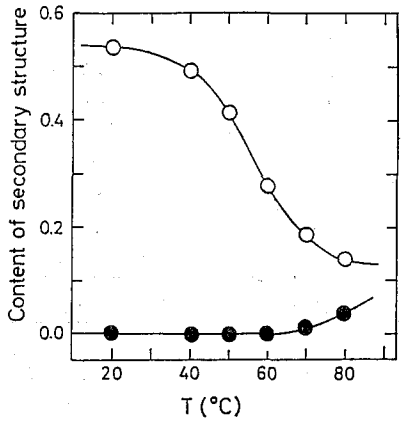

Fig. 7. Variation in SCMKA secondary structure with increase in temperature. (○)α-helix; (●) β-form.

et al.[22] and (ii) SCMKA is 52-54% α-helical in aqueous solution at 20°C and has little β-structure. Therefore, if the content of β-structure became appreciable in SCMKA at higher temperatures, the CD spectra would not intersect at a common wavelength. The results of CD analysis showed no occurrence of β-structure on heating, as can be seen in Figure 7. The CD analysis was made with the method of Chang et al.[16,22] It may be concluded that the conformational change in SCMKA, which occurs around 50°C, is a so -called helix-coil transition. This also suggests that the α-helical proteins may be transformed to β-structure via an intermediate disorder phase.

Last to be mentioned here is that most of the α-helical proteins undergo a conformational change on heating, but about 15% of the helical chains are very resistant to heat. Furthermore, the phase transition temperature of soluble proteins isolated from wool fibers is much lower than that of keratin proteins in the native state.

Phase Transition of Amorphous Wool Fibers

When amorphous wool fibers are immersed in pure water, the axially oriented fiber pattern of the native α-keratin structure is known to be completely regenerated.[5,10] No study has been reported on the phase transition of amorphous wool fibers by heating and/ or stretching. A detailed study on the phase transition in amorphous wool fibers may be expected to provide information about the mechanism of the α-β transformation in wool keratin. This section deals with the phase transition of amorphous wool fibers.

The effect of relative humidities on the coil-helix transition of the amorphous wool fibers was at first examined by using X-ray diffraction. To this end, the amorphous wool fibers in the dry state were exposed to atmosphere of different relative humidities at room temperature. The results are shown in Table 1. Different relative humidities were generated in a desiccator over saturated salt solutions. No significant change in the structure was observed for the samples at low humidities (<35 % r.h.) even after 60 days, indicating that water plays an important role in the transformation to α-keratin structure. A similar coil -helix transition was observed by stretching the amorphous wool fibers at 65 % r.h. and 20°C. On the other hand, the coil-β transition was observed by heating the amorphous sample at 150°C for 3h. It was found that such a transformation occurred even in the absence of water, for example, in vacuo. Figure 8 compares the X-ray diffraction pattern of the sample fibers in the β-structure with that of the original amorphous wool fibers. The 0.47nm reflection

Table 1 Structural changes of amorphous wool fibers exposed to atmospheres of different humidities at 20°C

Relative humidity (%)	Treatment time (day)[a]							
	0	0.5	1	3	7	14	30	60
100	am	α						
95	am	– am	α					
75	am	———	am	α				
65	am	———	———	———	am	α		
35	am	———	———	———	———	———	———	am
0	am	———	———	———	———	———	———	am

a) am, amorphous keratin; α, α-keratin.

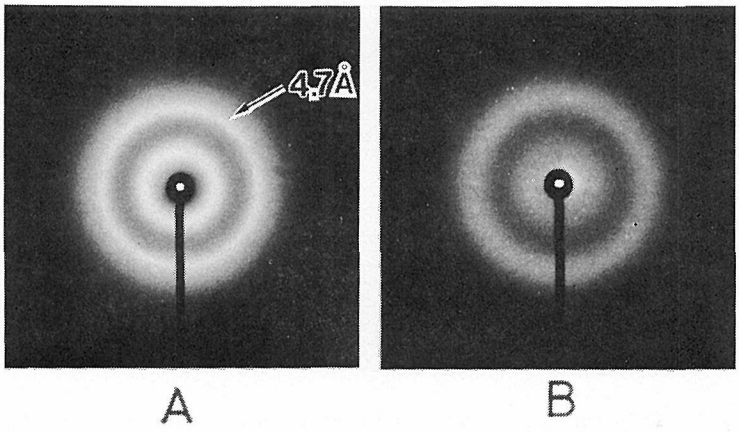

Fig. 8. X-ray diffraction photographs of wool fibers in the β structure (A) and amorphous wool fibers (B).

characteristic of the β-structure appeared in the former sample fibers, whereas only a diffraction halo characteristic of the amorphous wool fibers in the latter. The 0.47 nm reflection was a continuous ring. This indicates that the amorphous keratin was transformed to randomized β-keratin. It is of interest to note that the amorphous keratin can be converted to β-keratin by heat-treatment even in the absence of water. On the other hand, no structural change was observed in the native α-keratin fibers under the same heat -treatment conditions as mentioned above.

The β-transformation of amorphous wool fibers by heating in the absence of water was also confirmed from DTA measurements. Figure 9 shows the DTA curves of Merino wool fibers in vacuo at a heating rate of 20°C/min. Three endotherms in a range of 200-300°C are observed in the DTA curve of the native wool fibers (Fig. 9A). The peaks a, b and c have been assigned to the melting of the α-helix structure, the melting of the β-structure and the pyrolysis of keratin proteins, respectively.[15] On the other hand, the DTA curve of the wool fibers in the β-structure (β-keratins), which were prepared by stretching in steam to about 100 %,[14] exhibits only a broad endotherm in a range of 270-300°C (Fig. 9B). It can be seen that the DTA curve (Fig. 9D) of the sample fibers, which were prepared by treating the amorphous wool fibers at 150°C for 3h in vacuo, is almost the same as that of β-keratin fibers. For the sake of comparison, the DTA curve of amorphous wool fibers is shown in Fig. 9C.

Furthermore, it was found that the amorphous wool fibers can be converted not to α-keratin but to β-keratin fibers by immersing in boiling water for 1h. No structural change was detected in the native α-keratin fibers from the X-ray diffraction measurements under the same treatment conditions.

From these results it may be concluded that the coil-β transition in wool keratin occurs much more easily than the disruption of the α-keratin stucture to amorphous structure, namely, the helix-coil transition. These results also support the consideration by Mitchell

and Feugelman that the phase transition process in wool keratin is highly co-operative.[8)]

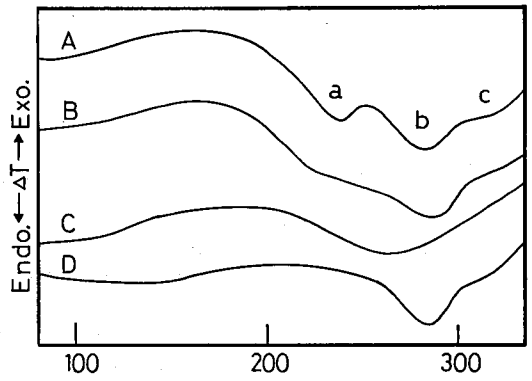

Fig. 9. DTA curves of Merino wool fibers in vacuo at a heating rate of 20°C/min.
(A) native wool fibers; (B) wool fibers in the β-structure; (C) amorphous wool fibers; (D) heat-treated amorphous wool fibers.

CONCLUDING REMARKS

The present investigation has confirmed the basic features of the phase transition in wool keratin previously described by a number of workers. However, some new informations have been obtained, particularly on the role of originally amorphous keratin proteins in the α-β transformation and the process of the transformation of α-helical keratin to β-keratin. The main results obtained are as follows.

(i) The major component which is transformed to β-keratin structure by heating and/or stretching is α-helical keratin proteins.

(ii) Originally non-helical keratin proteins, especially the matrix proteins may be also involved in the β transformation in wool keratin.

(iii) The process of the β transformation in α-helical keratin proteins is crystal (α-helix) $\longrightarrow$ amorphous $\longrightarrow$ crystal (β-structure).

(iv) The α-β transformation in wool keratin is highly co-operative.

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