KERATIN FIBRES

VI.* MECHANISM OF THE ALLWÖRDEN REACTION

By J. H. Bradbury† and J. D. Leeder†‡

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Abstract

On treatment with chlorine water, Allwörden sacs are formed on the surface of keratin fibres and on single cuticle cells separated from fibres. Their rate of formation has been studied for wool fibres subjected to various pretreatments. The experimental results can be explained in terms of six conditions, all of which must be satisfied if Allwörden sacs are to be formed.

I. Introduction

Treatment with chlorine water causes sacs to appear on the surface of keratin fibres (Allwörden 1916). The sacs are enclosed by thin membranes, called the epicuticle, and are filled with soluble protein. The membranes (about 35 Å thick) were isolated and analysed by King and Bradbury (1968) and consist almost entirely of a keratinous protein; this has been confirmed by Lofts and Truter (1969). There has been some discussion over whether the epicuticle membrane covers the whole fibre in a continuous sheath (Mercer 1953) or surrounds each cuticle cell individually, but our recent work on the formation of sacs on single cuticle cells separated from the fibre shows that the latter explanation is correct (Leeder and Bradbury 1968, 1971*b*; Bradbury and Leeder 1970).

Müller (1939), Hock, Ramsay, and Harris (1941) and Schuringa, Konings, and Ultée (1953) consider that sacs are produced by the action of chlorine water in dissolving protein material underlying the epicuticle which is then unable to escape since the epicuticle acts as a semipermeable membrane. The chemical potential of the water beneath the epicuticle is thus reduced to a value less than that in the bulk solution and, as a result, water flows through the epicuticle into the fibre, producing sacs which cover each cuticle cell. The soluble proteins which fill the Allwörden sacs have an amino acid analysis which is similar to that of cuticle (King and Bradbury 1968). In this paper we report on experiments which assist in ascertaining a set of conditions which are necessary for the formation of Allwörden sacs.

II. Experimental

(*a*) *Treatments of Wool*

Virgin Merino 64's root wool was extracted in a Soxhlet apparatus with light petroleum (b.p. 60–80°C) for 8 hr. This was followed by washing six times in distilled water at 50°C and two 24-hr washes in deionized distilled water. Chemical treatments of wool were as follows.

(i) *Peptide Hydrolysis.*—Wool was heated at 100°C for 20 hr in 0·01M hydrochloric acid at 200:1 liquor–wool ratio. Weight loss was 12·5%.

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† Chemistry Department, Australian National University, P.O. Box 4, Canberra, A.C.T. 2600.

‡ Present address: Division of Textile Industry, CSIRO, Belmont, Vic. 3216.

(ii) *Ammonium Hydroxide Treatment.*—Wool was immersed in concentrated ammonia solution at 20°C for 24 hr (200:1 liquor–wool ratio). Weight loss was 1·3%.

(iii) *Treatment with Potassium Cyanide* (Crewther *et al.* 1967).—Wool was treated at 60°C for 20 hr with 1% potassium cyanide in water at 100:1 liquor–wool ratio. Weight loss was 10·2%.

(iv) *Potassium Hydroxide Treatment.*—Wool was treated at 50:1 liquor–wool ratio with 1M potassium hydroxide in water at 20°C for 5 min, then neutralized in 0·005M sulphuric acid. Weight loss was 1·5%.

(v) *Potassium Bromate–Salt Treatment* (McPhee 1960).—Wool was treated with 2% potassium bromate (on weight of wool) in 0·05M sulphuric acid saturated with potassium chloride, for 1 hr at 20°C with a 25:1 liquor–wool ratio, then neutralized with dilute sodium bicarbonate solution. Weight loss was 0·7%.

(vi) *Sulphuryl Chloride Treatment* (Leeder and Bradbury 1971*a*).—Wool was dried *in vacuo* and sealed in an evacuated glass tube containing 100% dry sulphuryl chloride. After heating at 40°C for 70 hr, the wool was washed four times in carbon tetrachloride and four times in ethanol. Weight loss was less than 0·2%.

(vii) *Ammonium Thioglycollate* (Farnworth 1961).—Wool was treated at 40°C for 30 min in 1% (v/v) thioglycollic acid + 6% (v/v) concentrated ammonia in ethanol at 20:1 liquor–wool ratio, then washed in ethanol.

Following all chemical treatments, the modified wools were washed for 1 week in twice-daily changes of deionized distilled water. Weight losses were measured after heating for 1 hr at 100°C *in vacuo* (10^{-4} torr).

(*b*) *Allwörden Reaction*

The time taken for the appearance of Allwörden sacs on fibres treated with freshly prepared chlorine water (Bradbury and Leeder 1970) was determined using a Leitz Dialux microscope as described previously (Leeder and Bradbury 1971*a*). Fibres treated with saturated chlorine water could be observed over periods of several weeks by sealing the wool and the reagent between a microscope slide and a coverslip with paraffin wax.

III. Results

The times required for the appearance of Allwörden sacs on treated wool fibres are given in Table 1. The normal reaction obtained with an untreated Merino fibre

Table 1

Effect of treatments on the Allwörden reaction

Treatment	Time for appearance of sacs (sec)	Observations
Untreated	15	Normal reaction (see Fig. 1)
Potassium bromate–salt	5	Sacs slightly smaller than normal
Peptide hydrolysis	10	Sacs slightly larger than normal
Thioglycollate	15	Normal reaction
Potassium hydroxide	21	Normal reaction
Ammonium hydroxide	120	Small sacs slowly develop
Sulphuryl chloride	1–2 hr	Very slow sac development; nearly complete coverage after 16 hr
Potassium cyanide	No reaction	No reaction after 6 weeks contact with chlorine

is shown in Figure 1. When a fibre is treated with chlorine water at room temperature in a sealed system for a prolonged period the Allwörden sacs retain their shape but gradually become thinner, and after 1 week they cannot be seen clearly. On the

other hand, fibres which had been pretreated with potassium cyanide showed no sac formation in chlorine water after 6 weeks immersion in a sealed system, and there was no visible evidence of attack of the fibres by the chlorine water.

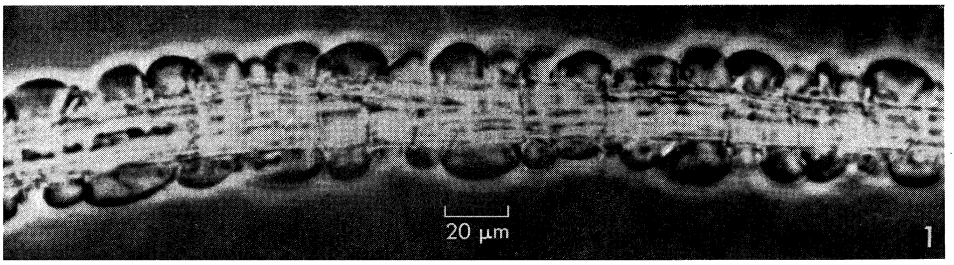

Fig. 1.—Merino wool fibre immersed in chlorine water.

Samples of wool, in which varying amounts of cystine had been reduced with tri-n-butyl phosphine and the thioi groups coupled with iodoacetic acid or ethyleneimine, were kindly supplied by Dr. J. A. Maclaren (Sweetman and Maclaren 1966; Maclaren and Sweetman 1966). The time for appearance of Allwörden sacs on these fibres is shown as a function of disulphide content in Figure 2.

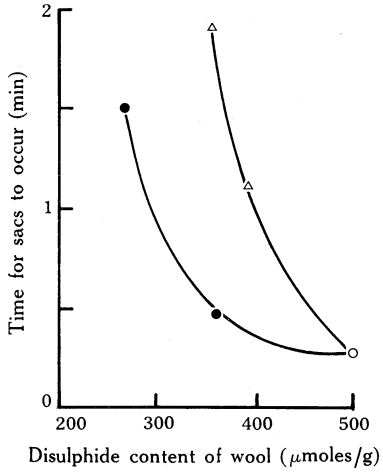

Fig. 2.—Time of appearance of sacs in relation to disulphide content of wool for untreated wool (○), and for wool in which some disulphide links are replaced by $-SCH_2COOH$ (●) or $-SCH_2CH_2NH_2$ (△) groups.

IV. Discussion

In order to interpret the experimental results, we will consider first of all the basic conditions which must be satisfied if Allwörden sacs are to form on keratin fibres or on single cuticle cells separated from fibres. No claim is made for originality, except for points (3) and (6), but a series of simple statements are necessary, as follows:

(1) The reagent diffuses into the cuticle and dissolves protein underlying the epicuticle.

(2) Sufficient protein is dissolved for the epicuticle no longer to adhere to the underlying cuticle.

(3) The solubility of the proteins in solution are sufficient to allow an appreciable osmotic pressure to be generated, which results in the formation of the sacs. This requires the presence of a large number of cysteic acid residues (from the oxidation of disulphide bonds) in the soluble protein.

(4) The dissolved protein is of such a molecular weight that it is unable to diffuse through the semipermeable epicuticle membrane or escape from the cuticle by any other route.

(5) The epicuticle membrane must remain intact.

(6) With keratin fibres a sufficient area of each cuticle cell must be exposed on the surface of the fibre to allow the normal sacs to be raised.

Pretreatment with potassium bromate or dilute hydrochloric acid causes some bond fission which facilitates the dissolution of protein underlying the epicuticle [points (1) and (2)] and hence increases the rate of formation of Allwörden sacs as shown in Table 1. Thioglycollate pretreatment has no effect since the thiol groups produced by the treatment will readily be oxidized to sulphonic acid groups by the chlorine water. The reduced rate of formation due to alkaline pretreatments (Table 1) agrees with previous work by Schuringa, Isings, and Ultée (1952), Leveau and Cebe (1953), and Lindberg (1953). It was suggested that disulphide were converted to monosulphide (lanthionine) cross-links, which are more stable towards oxidation by chlorine water. An extreme case of this type of stabilization results from treatment with potassium cyanide, which is known to convert cystine to lanthionine (Cuthbertson and Phillips 1945; Crewther *et al.* 1967) and in this case seems to protect the whole fibre from oxidation by chlorine water.

Inhibition of sac formation by pretreatment with sulphuryl chloride may result from conversion of $-CH_2S-S-CH_2-$ groups to $-CH_2Cl$ and $-CH_2SO_2Cl$ groups (Hall 1939; Farnworth and Speakman 1949), which would not produce as many sulphonic acid groups on oxidation with chlorine water as would be produced by oxidation of virgin wool. The sulphonic acid anions are excellent solubilizing groups for proteins and lack of them may produce a protein which is not soluble enough to form sacs [point (3)]. This is also the explanation of the results shown in Figure 2, where the effective replacement of some of the $-CH_2SO_3^-$ groups by the less solubilizing (at low pH) $-SCH_2COOH$ or $-SCH_2CH_2NH_3^+$ groups (Gillespie 1963; Crewther *et al.* 1965) causes an increase in the time needed for the development of sufficient concentration of soluble protein to raise the sacs [point (3)].

The stability of Allwörden sacs for periods of more than 1 week in chlorine water, together with the gradual disappearance of the membranes, can be attributed to a continuing process of dissolution of keratin and chain scission, producing peptides which diffuse through the semipermeable membrane. Preliminary results on this diffusible protein, obtained by Dr. J. M. O'Shea using chromotography on Sephadex G200 in 8M urea, indicates a maximum molecular weight of the order of 14,000.

Allwörden sacs form on single cuticle cells separated from keratin fibres, thus the soluble protein material cannot escape through the exocuticle and endocuticle into the solution [point (4)]. It is unlikely that either of these cuticle layers would prevent the passage of soluble proteins, since fibres from which the epicuticle is

removed by chlorine water treatment fail to produce the usual thick sacs on subsequent treatment with bromine water (King and Bradbury 1968). Leeder and Bradbury (1968, 1971*b*) therefore proposed that the epicuticle membrane surrounds each cuticle cell completely and that this effectively prevents the loss of soluble protein from inside the sac. However, if this is true why does the sac form on one side only of the separated cuticle cell? The reason for this may be that the endocuticle is not readily dissolved by chlorine water [points (1), (2), and (3)], or that the dissolved endocuticle protein is not soluble enough, because of its low cysteic acid content (Ley 1971), to raise Allwörden sacs [point (3)] or for both reasons together. By contrast, the soluble protein from the "a" layer of the exocuticle contains a large amount of cysteic acid (Ley 1971), is hence very soluble, and readily raises the sacs.

Furthermore, because of the similar amino acid analyses of epicuticle and of resistant membranes from whole wool, we have suggested than an epicuticle-type membrane surrounds cortical cells as well as cuticle cells (Bradbury, Leeder, and Watt 1971), yet treatment of cortical cells with chlorine water does not lead to the formation of Allwörden sacs (Leeder 1969). This could be explained on the basis of either point (2) or point (3) above.

Finally, it has been shown by Bradbury and Leeder (1970) that the normal type of Allwörden sacs are not readily produced on keratin fibres such as human hair, where there is a multiple cuticle layer and only about one-fifth to one-sixth of the cuticle is exposed on the surface [point (6) above].

V. Acknowledgments

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VI. References

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