THERMAL AND ELASTIC PROPERTIES OF α-KERATIN

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Received July 24, 1953

The elastic coefficient of hair fibers indicates the existence of structural changes occurring at about 5% and at about 25% extension. The volume of the hair fiber exhibits a minimum at about 5% and a maximum at about 30% stretch. There are conspicuous and sharp maxima in the energies of activation for the decay of the stress of a hair fiber at about 5% and at about 20% stretch. Typical protein denaturants have no large effects on the elastic properties of hair which indicates that hydrogen bonds are not primarily responsible for the elastic properties of hair; emphasis is placed on the disulfide bonds. It is suggested that whatever molecular folding of the peptide chains as may have been originally present in the hair follicle has been extensively deformed by the formation of inter-chain disulfide bonds.

Since the work of Astbury and Street,[1] it has been known that hair and wool are converted from what is called α-keratin into another molecular structure known as β-keratin as a fiber is progressively stretched beyond about a 20% extension.

The elastic properties of keratin fibers have been extensively studied by a number of workers and much detailed information on this subject is available. The present paper reports additional information along these lines and attempts an interpretation in terms of protein denaturation and in terms of the recent ideas of Pauling and Corey.

A simple stress–strain apparatus for the stretching of human hair immersed in water at desired temperatures was constructed; this apparatus permitted a study of the stress as a function of the stretch or of the stretch as a function of the stress applied to the hair. The human hair used was described as virgin hair by the commercial dealer. It was exhaustively extracted with alcohol, with ether and finally washed with water and electrodialyzed. It was dried and stored.

Shown in Fig. 1 is the coefficient of elasticity of the hair expressed in dynes per square centimeter as a function of the stretch at 25° and at 85°. Clearly evident at 25° are two sharp maxima in the coefficient of elasticity. The stretch up to the first maxima corresponds to the stretch in the so-called Hook's law region. The position of the first maxima is insensitive to temperature and to the previous history of the hair. On the other hand, the position and magnitude of the second maxima is very sensitive in regard to temperature and to the treatment the hair has received. As shown, the second maxima has very greatly decreased at 85° and furthermore has shifted to a position of greater extension. Not shown in Fig. 1 are the elastic coefficients at 45° and at 65°. These exhibited the expected behavior which was intermediate between 25 and 85°.

As is well known hair is a complex biological structure. It is composed of two main parts, the cuticle and the cortex. The cuticle forms a sheath around the shank or cortex of the hair fiber and makes up to 10 to 20% of the cross-sectional area of the hair fiber. We have found that when a hair fiber is extended to between 40 and 60% of its original length, the cuticle ordinarily tears in a circular direction around the hair forming sleeve-like sections of irregular lengths. If such a hair is

(1) W. B. Astbury and A. Street, *Phil. Trans. Roy. Soc. (London)*, **A230**, 75 (1930).

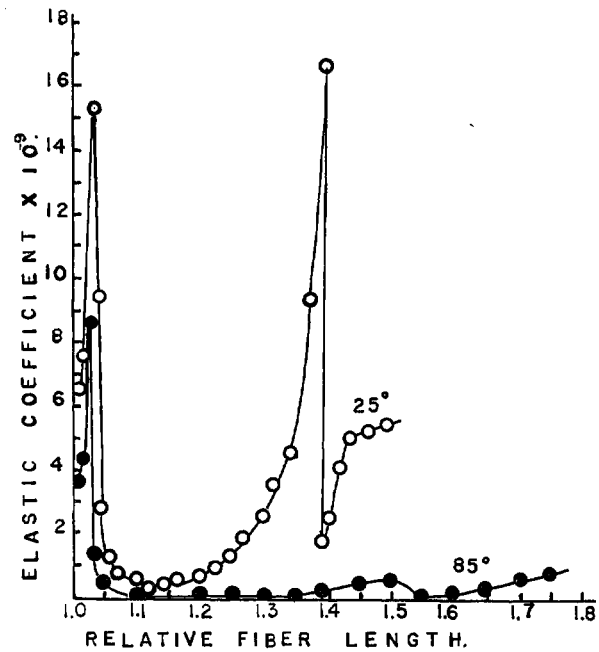

Fig. 1.—Coefficient of elasticity of human fibers at 25° and at 85° as a function of stretch, the original length of the hair being taken at unity.

relaxed and restretched, the cuticle disintegrates completely and the hair fiber is obtained free from cuticle. We cannot be absolutely certain that the second maxima shown in Fig. 1 is not in fact due either in part or entirely to the onset of the rupture of the cuticle before such a rupture is microscopically visible. The second maxima is greatly diminished upon restretching a relaxed hair whether the cuticle has been visibly ruptured by the first stretch or not.

The diameters of the hair fibers have been measured as a function of the extention. This has been accomplished by direct microscopic observation using a filar micrometer. The hair fibers are not perfect cylinders and have elliptical cross sections. The variation of the apparent diameters of 5 hairs have been measured as a function of stretch and these values averaged and the average volume of the hairs calculated from the averaged diameters on the assumption of cylindrical shapes. The volume of the hair fibers so calculated as a function of the extension of the hair at 25° is shown in Fig. 2. The original length and volume of hair was taken as unity. All the hairs showed the same qualitative

volume changes with stretch although there were quantitative differences between the hairs. Notable is the decrease in volume associated with stretch in the Hook's law region and the maximum in the volume at about 30% stretch.

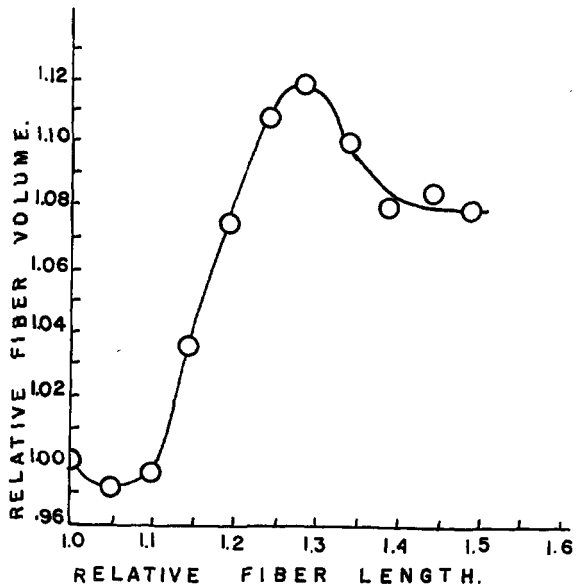

Fig. 2.—Relative volume of hair fibers as a function of extension at 25°. The original length of hair was assumed to be unity.

The $^9/_{10}$-times for the decay of the stress at selected extensions have been determined at 25° and at 45° for 5 different hairs. If we assumed that the $^9/_{10}$-times are an inverse measure of the rate of decay of the stress, we can estimate the energies of activation for the decay of the stress as a function of the extension. The hairs were first stretched to 50% extension and the loads were removed and the hairs allowed to recover. The $^9/_{10}$-times up to about 40% stretch were then measured at 25° for various intermediate extensions. The hair fibers were unloaded and allowed to relax. The temperature of the bath was raised to 45° and the $^9/_{10}$-times determined at this temperature. The $^9/_{10}$-times varied, depending on the extent of stretch, from over 2,000 seconds down to 30 seconds. Shown plotted in Fig. 3 are the estimated energies of activation expressed in kilocalories per mole as a function of the extent of stretch; the original length of the fiber being taken as unity. Clearly evident are two pronounced maxima, one at the end of the Hook's law region and another at about 20% stretch.

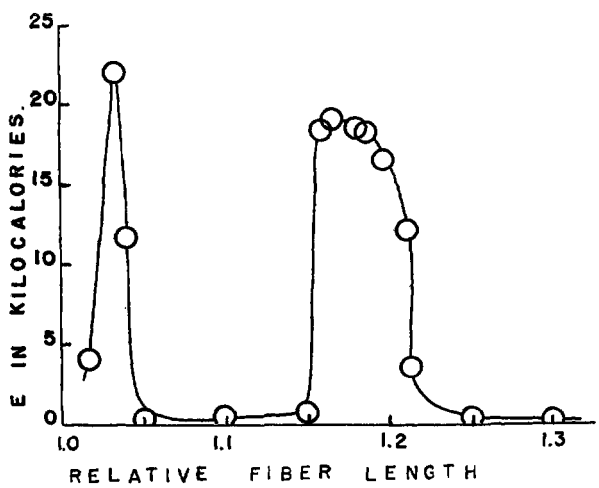

Fig. 3.—Energies of activation in kilocalories for the decay of stress for the average of 5 hair fibers.

Discussion

This research was undertaken with hopes that it might shed light on two important problems and these are: (1) protein denaturation and (2) to see if any information could be gathered which would be relevant to the polypeptide structures proposed by Pauling and Corey.[2]

The stretching of a hair fiber can be regarded in a sense as a kind of mechanical protein denaturation; the peptide chains are moved relative to each other in the stretching process and the molecular configuration in the "native" unstretched state is certainly different from what it is in the stretched state. This "denaturation" is to a large extent reversible, the unloaded hair returning to very nearly its original length after stretch. The change from the "native," unstretched state to the stretched, denatured state involves two energy humps (see Fig. 3) and these humps are not very much less than the energies of activation associated with the thermal denaturation of globular proteins in solution. The stretching of the fiber beyond the Hook's law region also shows a volume increase. Such a volume increase is characteristic of protein denaturation.[3]

The denaturing influence of concentrated urea on globular proteins is well known. Six molar urea decreases the work of extending a hair fiber about 30%. A 0.2% solution of sodium lauryl sulfate decreased the work of extension about 10%. It is known that *p*H has only a moderate effect upon the work of extension of keratin fibers except at sufficiently high *p*H to initiate the hydrolysis of disulfide bonds where the work of extension falls very drastically. The reduction of the disulfide bonds if sufficiently extensive, causes the hair to lose its elastic properties and become a plastic solid and the work of extension drops to almost zero.

It is not clear how the elastic properties of hair can be interpreted in terms of the Pauling–Corey helical structure. This structure provides for about 117% stretch in the transformation of α-keratin to β-keratin. In reality the on-set of the conversion of α- to β-keratin does not begin until the hair has been stretched about 20% and the X-ray picture is entirely of the β-keratin type at 70% extension.[1] Thus the actual stretch for the conversion of α- to β-keratin is about 45%.

Furthermore, there is no provision in the Pauling–Corey structure for two structural changes observed as hair is stretched as indicated in Fig. 3, and less certainly in Fig. 1.

It is undoubtedly true that in most proteins, the stabilizing energy arises principally from hydrogen

(2) L. Pauling and R. B. Corey, *Proc. Natl. Acad. Sci.*, **37**, 261 (1951).

(3) F. H. Johnson and D. H. Campbell, *J. Cell. Comp. Physiol.*, **26**, 43 (1945).

bond formation between the oxygen and the nitrogen atoms in the peptide chain. There are two such bonds per residue and Pauling and Corey estimate their energy of formation to be about 8 kcal. per bond. Hydrogen bonds also arise from the polar R-groups. In hair there are about 0.4 such hydrogen bonds per residue.[4] There is also van der Waals interaction leading to close packing of the R-groups. The energy contributed by the van der Waals interaction can be estimated to be about 2 kcal. per residue.[5] It therefore appears that the stabilizing energy arising from the hydrogen bond formation on the oxygen and nitrogen atoms in the peptide chain is by far the more important of the stabilizing factors. We have seen, however, that agents capable of denaturing globular proteins by hydrogen bond rupture have no pronounced influence on the work of extending a hair fiber. We feel that the reason for the failure of typical protein denaturants to influence the work of extension in any dramatic way resides in the very high cystine content of hair which provides disulfide links between adjacent peptide chains. It is principally to the inter-chain disulfide bonds that unstretched hair owes its structure and only secondarily to hydrogen bond formation.

It is probable that a large fraction of the α-helical forms of hair have suffered extensive deformations due to the formation of disulfide bonds as the hair fiber leaves the hair follicle and the sulfhydryl groups are oxidized to disulfide.

The structural change initiated at the end of the Hook's law region probably arises from a straightening of the partly folded peptide chains and the second structural change occurring in the 20 to 30% stretch region probably involves the start of the conversion of the partly folded chains to the α-keratin structure.

(4) H. B. Bull, *J. Am. Chem. Soc.*, **66**, 1499 (1944).

(5) W. D. Harkins, "Surface Chemistry," Pub. No. 21, Am. Assoc. Adv. Sci., 1943.

DENATURATION OF HEMOGLOBINS BY ALKALI[1]

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Received July 24, 1953

The decomposition of hemoglobin and oxyhemoglobin from man, rabbit and beef blood, at *p*H 11.9 and at temperatures between 2 and 30° proceeds according to first-order kinetics. The first-order velocity constants for human, rabbit and beef oxyhemoglobin at 2° are 2.3×10^{-2}, 2.8×10^{-4}, and 9.3×10^{-6}, respectively. The presence of two or more hemoglobins in rat blood is indicated by a break in the denaturation velocity. The alkali denaturation of hemoglobins differs from that of oxyhemoglobins by the lower values for $\Delta H^{\ddagger}$, and by more negative values for $\Delta S^{\ddagger}$. This is ascribed to configurational changes accompanying oxygenation. The denaturation velocity of human, rabbit and beef globin at *p*H 11.9 and 11.0 is much higher than that of the corresponding hemoglobins and is similar for all three types of globin. The differences in the resistance of various hemoglobins against alkali are attributed to different degrees of complementariness of a non-polar "anti-heme" pattern in the globin surface, high resistance against alkali indicating a high degree of spatial adjustment of globin to the rigid planar porphin structure of the heme molecule.

In contrast to most proteins the hemoglobins of different species of animals differ from each other considerably in their resistance to the action of alkali. Thus, human oxyhemoglobin is changed by 0.05 *N* NaOH within less than one minute to a brown product, whereas bovine oxyhemoglobin remains bright red for many hours under the same conditions.[3,4] The change in color and absorption spectrum is due to denaturation as shown by the parallel loss of solubility at *p*H 7–8.[4] The extent of denaturation can, therefore, be measured spectrophotometrically. When the per cent. of denatured oxyhemoglobin is plotted against reaction time, continuous curves are obtained in most cases. Breaks in the curves reveal the presence of two or more hemoglobins in the investigated blood. In this manner multiple hemoglobins were detected in the blood of new-born children[5] and adult persons.[6,7] In spite of the frequent use of the alkali denaturation method in biochemical and clinical work,[8–13] not much is known about the mechanism of the reaction. We investigated, therefore, the rate of alkali denaturation at different temperatures and also compared the denaturation rate of oxyhemoglobin with that of hemoglobin and globin.

Methods.—In order to prevent the denaturation of oxyhemoglobin during its preparation, organic solvents and lengthy manipulations were avoided. The red cells from oxalated blood were washed rapidly three times with 0.9% NaCl solution, hemolyzed by water, and centrifuged to obtain a clear solution. The hemolysate was then diluted to give an absorbancy of 0.5–0.8 on the Beckman spectrophotometer. The freshly prepared solution was placed in a 10-mm. absorption cell, mixed with one-fifth of its volume of 0.25 *N* NaOH, and the reaction followed photometrically at 576 mμ. The denaturation of reduced hemoglobin was

(1) Support of this work by research grants from the U. S. Public Health Service, National Institutes of Health, and from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council, is gratefully acknowledged. The authors are indebted to Mrs. Margaret Kennedy for assistance in some of the experiments.

(2) U. S. Public Health Service predoctorate fellow, 1951–1952.

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