[CONTRIBUTION FROM THE WESTERN REGIONAL RESEARCH LABORATORY, BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE]

The Molecular Structure of Fibers Made from Native Egg Albumin*

BY K. J. PALMER AND JAMES A. GALVIN

The formation of fibers from typical corpuscular proteins is of great interest because of the practical implications and also because of the additional information obtained on the structure of these proteins. From the extensive X-ray diffraction studies of Astbury and co-workers it is now generally recognized that all proteins are structurally related in the sense that the denatured state is a fibrous state arising from the liberation of polypeptide chains. As a result of his earlier investigations Astbury suggested that when corpuscular proteins are denatured the peptide chains which were in a special configuration in the native state assume a configuration similar to that which occurs in β-keratin. To substantiate this suggestion Astbury, Dickinson and Bailey[1] attempted to align the peptide chains by stretching fibers and films made from denatured edestin, excelsin and egg albumin in order to obtain a typical fiber diagram. Their attempts to draw fibers were not very successful, but they were able to devise methods for producing films which, when stretched, showed partial orientation. The edestin and excelsin films showed the type of orientation to be expected if long peptide chains having a β-keratin configuration were present. Egg albumin, however, showed an unexpected type of orientation which they attributed to the shorter chain length in this protein as compared to edestin and excelsin.

A method has now been developed[2] for producing fibers from native egg albumin (and other corpuscular proteins) which exhibit a high degree of molecular orientation. The orientation is normal and is identical with that found with β-keratin fibers. The results of an X-ray diffraction study of these fibers and the relation between these results and those reported by Astbury, Dickinson and Bailey[1] are reported in this paper.

Experimental

The protein fibers used in this investigation were kindly furnished by Dr. H. P. Lundgren of this Laboratory. The method used in their preparation will be only briefly outlined as it has been described in detail elsewhere.[2]

The fibers discussed in this paper were made by mixing equal portions of 3% solutions of twice crystallized native egg albumin and the sodium salt of an alkylbenzene sulfonate.[3] The method of making fibers consists in precipitating the complex formed between the protein and detergent with a saturated solution of magnesium sulfate; pulling the resulting doughy precipitate into a fiber, washing the fiber in water to remove excess salt, and then extracting the detergent by immersing for twenty-four hours in a 60:40 acetone—water solution. The resulting pure protein fiber is then slowly elongated while in an atmosphere of live steam. The elongation in steam can be carried up to 500% without undue difficulty.[4]

The X-ray photographs were obtained in the usual way with filtered CuKα X-rays. All photographs except Fig. 1B were taken at 4 cm. The latter was taken at 10 cm.

Results

X-Ray photographs were taken of fibers in various stages of the process outlined above. Figure 1A is the reproduction of a diffraction pattern obtained from a fiber which had been pulled out from the doughy precipitate resulting from the addition of magnesium sulfate. This fiber has been washed in distilled water to remove excess salt, but still retains detergent. The diffuse ring gives a Bragg spacing of 4.7 Å. This spacing is typical of all molecules having a long hydrocarbon chain such as the detergent has and is also characteristic of proteins.

The much weaker diffuse inner ring gives a Bragg spacing of approximately 10 Å. This diffraction is due to the side chain spacing of the protein. A photograph taken at 10 cm. shows a single long spacing of 30 Å. (Fig. 1B). This spacing is identical with that obtained from the detergent.[5]

Figure 1C shows the result of extracting the above fiber with a 60:40 acetone-water mixture for twenty-four hours. Chemical analysis for total nitrogen has shown[2] that this treatment completely removes the detergent. A spectroscopic analysis[6] has shown that essentially all of the remaining magnesium ions are also removed by this treatment. Figure 1C is typical of disoriented peptide chains in the β-keratin configuration. This pattern is very similar to that obtained from heat denatured egg albumin shown in Fig. 1D. The spacings obtained from photographs 1C and 1D are listed in columns 2 and 3 of Table I. It is seen that they are in excellent agreement.

Figure 1E shows the result of drawing a similar fiber 400% while in steam. The direction of elongation is vertical. The molecular orientation is,

* Not copyrighted.

(1) W. T. Astbury, S. Dickinson and K. Bailey, *Biochem. J.*, **29**, 2351 (1935).

(2) H. P. Lundgren, THIS JOURNAL, **63**, 2854 (1941); H. P. Lundgren and R. O'Connell, *Ind. Eng. Chem.*, in press.

(3) The detergent used was Nacconol N.R.S.F., kindly furnished by the Allied Chemical and Dye Corporation.

(4) The per cent. elongations mentioned in the remainder of this paper refer only to the extension which occurs in the steam-bath and not to the initial elongation of the doughy precipitate. The latter appears to have no effect on the resulting molecular orientation, but does determine the final diameter of the fiber.

(5) A detailed X-ray investigation of the precipitates formed by the addition of magnesium sulfate to solutions containing different relative amounts of protein and detergent has shown that two phases are present. One is denatured albumin and the other the magnesium salt of the detergent. The results of this investigation will be published elsewhere.

(6) The authors are indebted to Dr. E. J. Eastmond of this Laboratory for making the spectroscopic analysis.

TABLE I

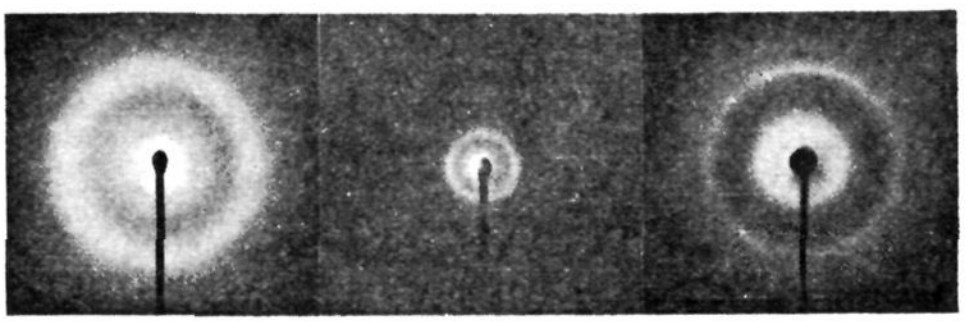
X-RAY SPACINGS OBTAINED FROM EGG ALBUMIN FIBERS, HEAT DENATURED EGG ALBUMIN AND β-KERATIN

Spacings are in Ångström Units

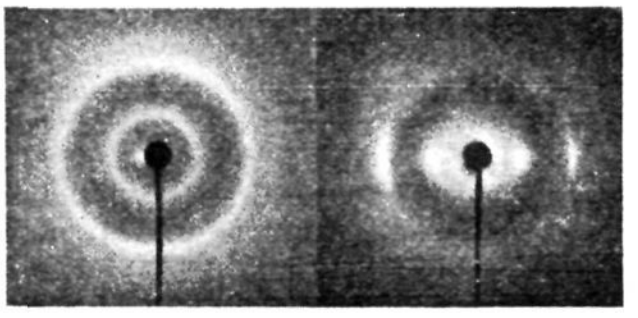
hkl	Egg albumin fiber, acetone extracted, not steam stretched	Heat denatured egg albumin	Egg albumin fiber—400% elongation	β-Keratin[a]
(001)	10.3	10.23	10.27	9.8
(200)	4.65	4.66	4.64	4.65
(210)	3.76	3.75	3.75	3.75
(020)	...	...	3.32	3.32

[a] W. T. Astbury and W. J. Woods, *Phil. Trans. Roy. Soc. Lond.*, **A232**, 333 (1933).

therefore, normal, *i. e.*, the peptide chains are parallel to the fiber axis, and is exceedingly good. It compares favorably with that observed from stretched natural keratin fibers.[7] The interplanar spacings obtained from this latter photograph are listed in column 4 of Table I. In column 5 are listed the values given by Astbury and Woods[7] for β-keratin.



A B C



D E

Fig. 1.—(A) X-Ray photograph of a fiber pulled from the doughy precipitate resulting from the precipitation of the egg albumin-detergent complex with magnesium sulfate. Camera distance (L) = 4.0 cm. (B) X-Ray photograph of the same fiber as (A) with L = 10.0 cm. (C) X-Ray photograph of an egg albumin fiber after removal of detergent, L = 4.0 cm. (D) X-Ray photograph of heat denatured egg albumin, L = 4.0 cm. (E) X-Ray photograph of an egg albumin fiber elongated 400% in steam. Fiber axis is vertical; L = 4.0 cm.

Discussion

The agreement between the spacings listed in Table I for the steam-stretched egg albumin fiber and β-keratin is seen to be excellent. There is no question that these two fibers, one made from a corpuscular protein and the other obtained from a fibrous protein, have the same molecular configuration. The slightly larger value for the side chain spacing for egg albumin as compared to β-keratin, is due, according to the usual interpretation, to the slightly greater magnitude of the average length of the side chains in the former protein.

The identity period in the fiber direction of the albumin fiber is 6.64 Å., or 3.32 Å. per amino acid residue. This is identical with the value observed for β-keratin and indicates that the peptide chain in the albumin fiber is almost completely extended. The position and sharpness of the diffraction spots are indicative of the fact that the peptide chains in the albumin fiber are very nearly parallel to one another and to the fiber axis, and furthermore that they are packed together with a high degree of crystalline regularity. As would be expected, this fiber is birefringent, the sign of elongation being positive.

As a result of the close similarity in the diffraction patterns obtained from the egg albumin fiber and β-keratin, there can be no doubt that peptide chains occur in the denatured state of egg albumin which is a typical corpuscular protein. Whether these chains existed in the native protein has not been demonstrated, but the ease by which denaturation can be accomplished, as for example by small amounts of detergent, can best be interpreted on the basis of the hypothesis that they do occur as such in native proteins.

Since the X-ray study of the precipitated complex indicates that it breaks up into two phases on drying, it is interesting to inquire just what role the detergent plays besides unfolding of the corpuscular protein in the fiber-making process. At the *p*H used (6.5), both detergent and egg albumin have a charge of the same sign. The complex, therefore, probably involves non-polar association.[8] Upon the addition of magnesium sulfate, denatured egg albumin and the magnesium salt of the detergent are precipitated together. During the precipitation the detergent appears to act as a filler preventing the peptide chains from crystallizing together to form a hard brittle mass. This is evident from the diffuse character of the 4.7 Å. ring obtained from the egg albumin detergent mixture, Fig. 1A, as compared to the sharp ring at 4.66 Å. obtained from heat

(7) W. T. Astbury and A. Street, *Phil. Trans. Roy. Soc. Lond.* **A230**, 75 (1931); W. T. Astbury and W. J. Woods, *ibid.*, **A232**, 333 (1933).

(8) A more detailed discussion of complex formation between egg albumin and this detergent will be published in the near future.

denatured egg albumin, Fig. 1D. As soon as the detergent is removed the peptide chains are free to crystallize, and the resulting photograph, Fig. 1C, is identical with that obtained from heat denatured egg albumin, Fig. 1D.

The precipitate resulting from the addition of magnesium sulfate can be pulled out into fibers because the long peptide chains give it sufficient strength while the detergent apparently acts as a lubricant. Although the peptide chains are probably brought into partial alignment by this initial elongation process the X-ray photograph gives no evidence of molecular orientation (Fig. 1A).

The detergent thus plays at least three important roles: (1) It unfolds the corpuscular proteins. (2) It makes possible the precipitation of the protein by addition of small amounts of inorganic salt. (3) It prevents the peptide chains from crystallizing and also appears to act as a lubricant during the initial drawing process. After removal of the detergent, the peptide chains can be further oriented by slow elongation in steam. The steam presumably hydrates the polar groups and prevents strong interactions from occurring between adjacent peptide chains. This apparently enables them to slip over one another and eventually take up positions of least potential energy, *i. e.*, they become oriented parallel to the direction of elongation.

X-Ray photographs of fibers which have undergone various degrees of drawing in steam show that molecular orientation can first be detected at about 100% elongation. At 300% elongation the orientation is quite good, and at 400% it is excellent (Fig. 1E). Drawing the fiber to 500% did not improve the orientation noticeably over that observed for 400% elongation.

The average values for the tensile strength of fibers which have been drawn 100, 200, 300 and 400% in steam are listed in Table II. These values were obtained at 70° F. and with a relative humidity of 50%, and are for pure protein fibers, *i. e.*, no fixing or tanning agents have been added. These average values for the tensile strength are approximate because they are the result of only a few measurements. In spite of this the trend is significant and indicates how rapidly tensile strength increases with increase in orientation of the polypeptide chains. The correlation between molecular orientation and tensile strength is being more thoroughly investigated and will be published in the near future.

TABLE II

THE AVERAGE VALUE OF THE TENSILE STRENGTH OF FIBERS MADE FROM NATIVE EGG ALBUMIN COMPARED WITH THE PER CENT. ELONGATION UNDER STEAM

Per cent. elongation under steam	Average value of tensile strength lb./sq. in.
100	18,230
200	25,265
300	27,880
400	38,075

Astbury and co-workers[1] have studied the molecular orientation produced by stretching films of denatured edestin, excelsin and egg albumin. Edestin and excelsin films showed normal orientation, that is, the peptide chains ran parallel to the direction of stretch, but egg albumin gave an unexpected result. In the latter case the (020) and (200) reflections occurred on the equator and meridian, respectively, instead of *vice versa* as in β-keratin. In other words, the peptide chains were lying perpendicular to the direction of elongation. They attributed this to the presence of crystallites in which "the average length of the crystallites in the direction of the main-chains is shorter than their thickness in the direction of the backbone spacing."

The egg albumin fibers made in this Laboratory have always exhibited the normal type of orientation. It is probable that the crystallites observed by Astbury, Dickinson and Bailey are formed as a result of the heat treatment to which they subjected the egg albumin film before stretching. Once formed, these crystallites were evidently sufficiently stable to resist rupture when the film was elongated. The force resulting from the elongation, therefore, acted on the crystallites rather than on the individual peptide chains. The shape of the crystallites was such that the final position assumed by them left the peptide chains at right angles to the direction of elongation. By the application of tension in the presence of steam as was done in making the albumin fibers discussed in this paper, the formation of these crystallites is apparently prevented at least until after the peptide chains have become parallel to the direction of elongation.

Astbury interpreted his results as indicating that the peptide chains in egg albumin were short as compared to the chains in edestin and excelsin. Although this is probably true, the excellent orientation found for the fibers made from egg albumin indicate that the polypeptide chains in this protein are sufficiently long to enable them to become aligned parallel to the direction of elongation when subjected to suitable treatment, such as stretching in steam.

Concerning the immediate environment existing around any given peptide chain, it is probably the same in both the fiber discussed in this paper and Astbury's film. That is, in both cases the chains are nearly fully extended and close-packed. There are two essential differences however. One is that the fiber shows a much higher degree of molecular orientation than the film, as is evident by the differences in the arc lengths of the (200) reflection in the two cases. The second difference is that the peptide chains in the fiber are oriented parallel to the direction of stress rather than perpendicular to it as in the film. These two differences are reflected in the physical properties observed. The film was reported to be brittle and weak, whereas the fibers are remark-

ably pliable and have tensile strengths which average about 38,000 pounds per square inch (Table II).

Acknowledgment.—We wish to express our sincere thanks to Dr. Harold Lundgren, who very generously supplied us with fibers used, and to Dr. C. H. Kunsman for his encouragement and advice during the course of this investigation.

Summary

Fibers which were made from native egg albumin by a process which involves complex formation with detergent and drawing under steam are shown to be composed of parallel bundles of polypeptide chains running parallel to the fiber axis. The peptide chains are shown to have the β-keratin configuration as is evident from the similar appearance of the X-ray patterns obtained from the albumin fibers as compared with those obtained from well oriented β-keratin. The tensile strength of the synthetic pure protein fiber is shown to be dependent upon the degree of molecular orientation and reaches a value of 38,000 pounds per square inch.

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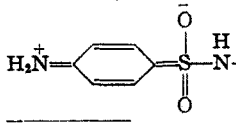
The Relation between Chemical Structure and Bacteriostatic Activity of Sulfanilamide Type Compounds

BY W. D. KUMLER AND T. C. DANIELS

The mechanism of the action of sulfanilamide type compounds[1] as proposed by Woods[2] and Fildes[3] in which the compound competes for the essential metabolite *p*-aminobenzoic acid seems to be well established and generally accepted.[4] The nature of the competitive action between *p*-aminobenzoic acid and the sulfonamides is an important factor in considering the relation between structure and bacteriostatic activity in these compounds. Some investigators[5,6] have correlated the activity of the sulfanilamide derivatives with their acid dissociation constants. Others[7] have suggested that the activity is related to the basic dissociation constants.

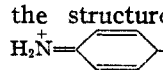
Kumler and Halverstadt[8] have suggested that the activity of sulfanilamide compounds is associated with the contribution of the resonating form with a separation of charge



They showed that this form makes about the same contribution in sulfanilamide as the analogous resonating form with a separation of charge makes in the methyl ester of *p*-aminobenzoic acid. The same thing holds true for free *p*-aminobenzoic acid.

Recently, Bell and Roblin[10] have reported that the bacteriostatic activity of sulfanilamide type compounds can be correlated with the negative character of the SO_2 group and with the acid dissociation constants. They present evidence to show that the more negative the SO_2 group, the more active the compound. They also point out that the more negative the SO_2 group, the more nearly it resembles the CO_2 group in *p*-aminobenzoic acid at *p*H 7. At the *p*H of 7 the carboxyl group in *p*-aminobenzoic acid is over 99% ionized and consequently the CO_2 group carries a negative charge.

In this paper we present evidence, first, that a fundamental factor for activity is the contribution of the resonating form with a coplanar amino group; second, that the negative character of the SO_2 group is a concomitant factor associated with the resonating form; third, that compounds which appear to be exceptions to Bell and Roblin's theory or do not fall within the scope of their theory, can be adequately accounted for on the basis of resonance, and fourth, that these ideas in part apply to certain other bacteriostatic compounds including the mono-aminoacridines.

In aniline some contribution is made to the structure of the molecule by the form  . If a group which has a greater tendency than hydrogen or carbon to accept a negative charge is placed in the para position, the contribution of such a form is greatly in-

(1) In accordance with the suggestions of Green and Bielschowsky, *Brit. J. Exptl. Path.*, XIII, 1, 13 (1942) sulfanilamide type compounds are here considered to be those that are antagonized by *p*-aminobenzoic acid.

(2) Woods, *ibid.*, **21**, 74 (1940).

(3) Fildes, *Lancet*, **238**, I, 955 (1940).

(4) Rubbo and Gillespie, *Nature*, **146**, 838 (1940); Lampen and Peterson, THIS JOURNAL, **63**, 2283 (1941); Landy and Wyeno, *Proc. Soc. Exptl. Biol. Med.*, **46**, 54 (1941); Wood, *J. Exptl. Med.*, **75**, 369 (1942).

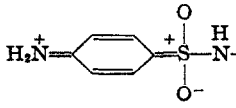
(5) Schmelkes, Wyss, Marks, Ludwig and Strandskov, *Proc. Soc. Exptl. Biol. Med.*, **50**, 145 (1942).

(6) Fox and Rose, *ibid.*, **50**, 142 (1942).

(7) Tolstoouhov, Paper presented at the Buffalo meeting of the American Chemical Society, Sept., 1942.

(8) Kumler and Halverstadt, THIS JOURNAL, **63**, 2182 (1941).

(9) Whether this structure is written as above, or as



is immaterial. The sulfur–oxygen bonds are mainly single, but they have some double-bond character. In this paper we have chosen to use double bonds because the separation of charge associated with the resonance with which we are mainly concerned is then illustrated more clearly.

(10) Bell and Roblin, THIS JOURNAL, **64**, 2905 (1942).