ELECTRON MICROSCOPICAL OBSERVATIONS OF FIBROUS INSULIN

by

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When insulin is heated with dilute acids it is converted into an insoluble form, termed by DU VIGNEAUD[1] the "heat precipitate". The conditions of formation and the nature of the precipitate have been studied most fully by WAUGH[2,3,4], who has concluded that the insulin is converted into fine fibrils (F-insulin) which subsequently clump leading to the visible precipitate. At the appropriate p_H the transformation into the fibrous form may lead to a clear thixotropic gel exhibiting double refraction. In this case it is supposed that the fibrils remain dispersed. Although the formation of fibrous insulin does not take place under physiological conditions, it nevertheless appears to provide a simple model system for the study of the transformation from soluble to insoluble fibrous protein.

An observation of great importance to the understanding of fibre formation is that F-insulin may be reconverted to soluble insulin (G-insulin) by the action of alkali, and at the same time there is a recovery of biological activity. This fact and others brought forward by WAUGH[4] suggest strongly that the conversion into the F-form cannot involve any far-reaching modification of the molecular architecture, such as the chain-unfolding proposed to explain the formation of artificial fibres from proteins. Rather it would appear that the insulin transformation is an example of an aggregation of corpuscular molecules (G–F transformation) which has been proposed as a fundamental step in the synthesis of natural fibres[5,6,7,8]. An attempt to explain the initiation of such linear aggregates in terms of colloidal forces has been made by REES[9].

We have examined in the electron microscope fibrils obtained from gels of F-insulin in the hope of finding some evidence concerning their formation. WAUGH[2] has reported that HALL has previously examined such material electron microscopically and found fine fibrils of widths varying between 100 and 180 A and many thousands of Ångströms long. So far as we know a full account of this work has not appeared and our own estimates of fibre width are smaller than those of HALL.

EXPERIMENTAL

A solution of 2% crystalline insulin was heated in a closed ampoule at 100° C with hydrochloric acid at p_H 2.3 for 20 minutes. These conditions lead to the production of a viscous solution or gel, which, from the point of view of microscopy, is more tractable

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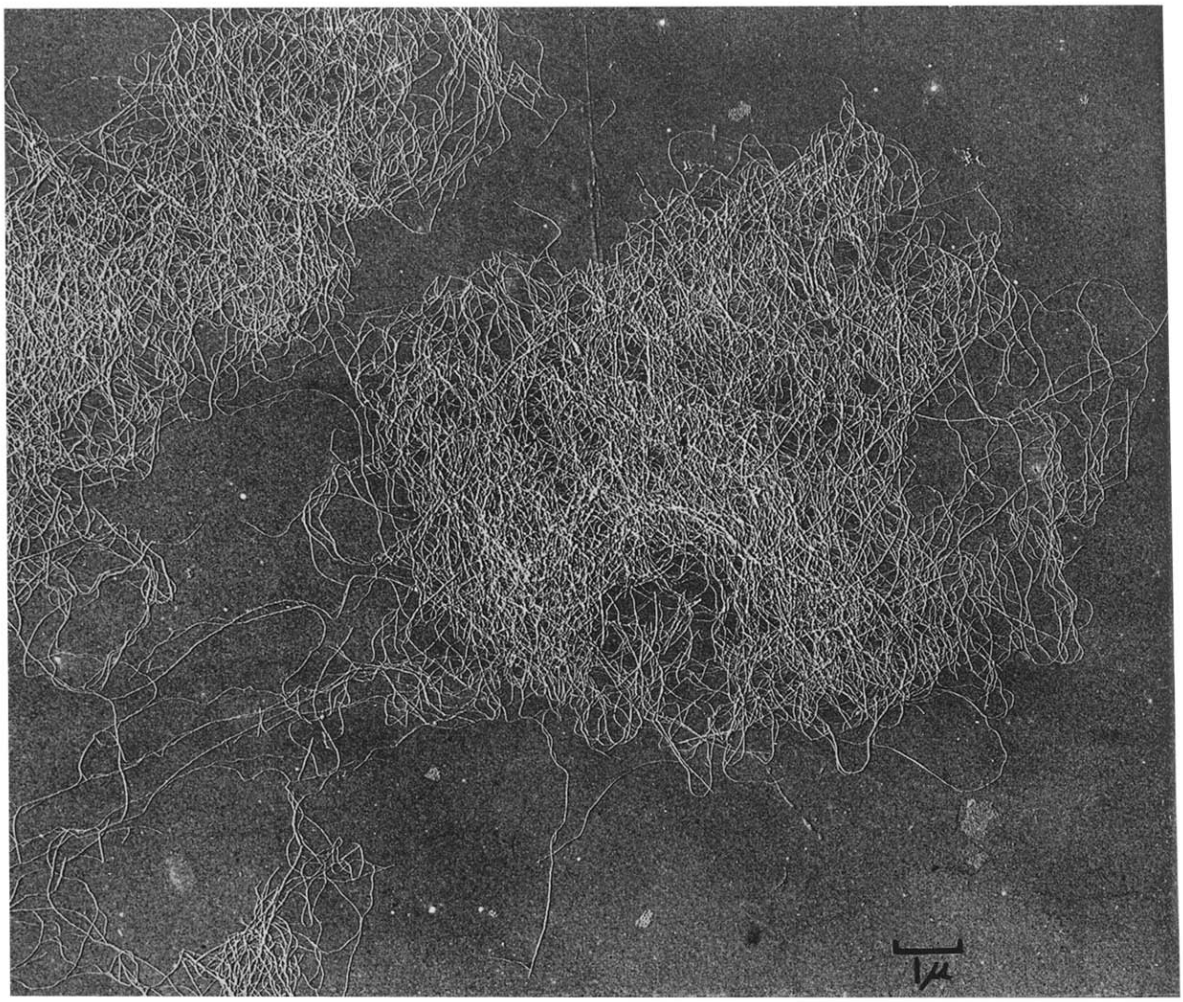

Fig. 1. × 9,000. Fibrils of F-insulin. Platinum shadowed. Photographed at 1350 ×. The occasional impression of periodicity in the fibrils is due to metal aggregation as may be seen in high resolution micrographs (Fig. 2).

than the visible precipitate produced at lower p_H values. The resulting gel was diluted about 100 times, drops were dried on collodion films and shadowed with platinum (*ca.* 5 A layer). These preparations were then examined by means of an R.C.A. Type E.M.U. electron microscope. An example of the electron micrographs obtained is shown in Fig. 1.

F-insulin prepared in this way clearly exists in the form of beautiful fibrils many microns long, a most striking transformation from the unresolved particles of soluble insulin. There was no evidence of a periodicity in these fibrils. At the time when these micrographs were taken the resolving power of the microscope was better than 20 A and no difficulty was experienced in separating the single crystallites of the order of

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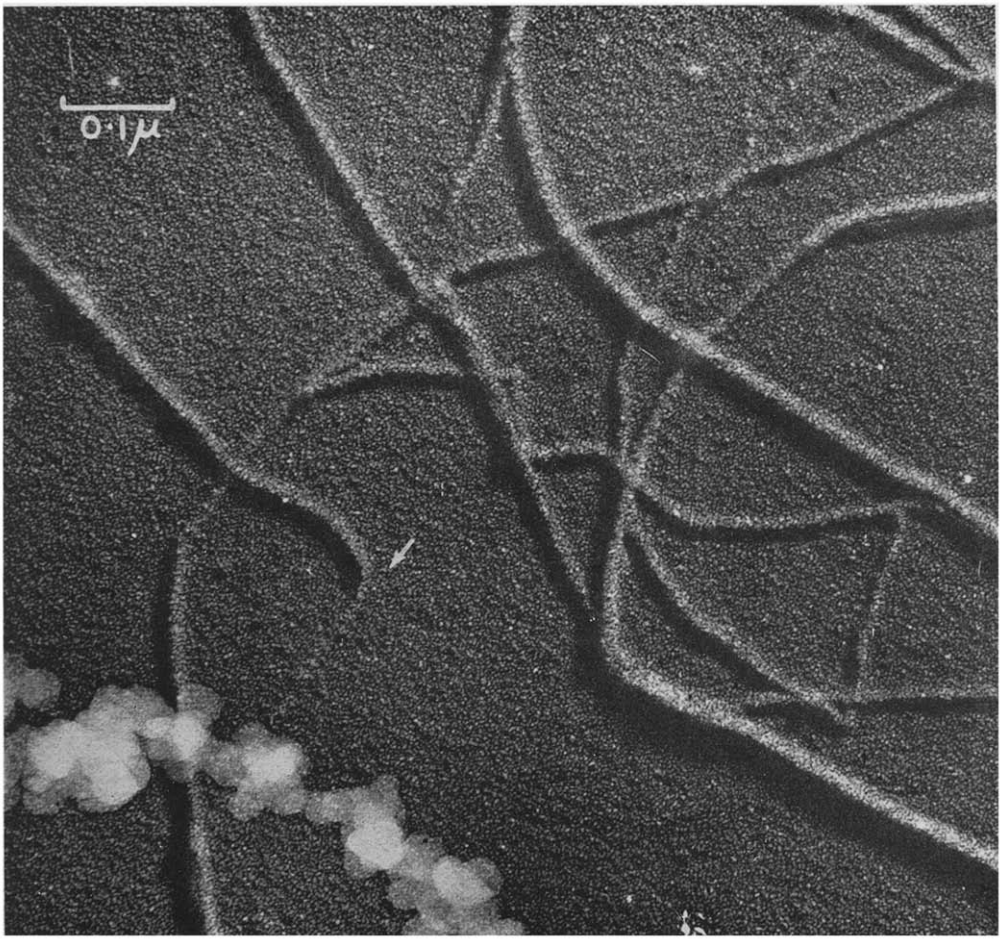

Fig. 2. × 150,000 high resolution micrograph. Platinum shadowed field. Photographed at 20,000 ×. Resolution *ca.* 20 Å. Single discrete crystals of the shadowing metal are obvious. The F-insulin fibrils reveal no structural details. Note the manner in which the fibrils blend with the field when shadowed longitudinally. The carbon particles in the lower left hand corner were used for focusing.

15–30 A width of the platinum used as a shadowing agent (see Fig. 2). We think therefore that any external features of the fibrils larger than *ca.* 60 A would have been revealed. The width of the finest fibrils running in the direction of shadowing was of the order of 50–80 A. (See Fig. 1). This is smaller than that found by HALL although coarser fibrils and multiple fibrils consisting of two or three single threads were occasionally present having gross diameters of 100–200 A.

The fine fibrils are flexible but seem to break readily by a transverse split leading to the formation of many shorter rodlets. The manner of breakdown seems to be of some significance. As shown in Fig. 3, the rupture takes place along transverse faults, the two separating ends being displaced laterally over each other.

The transformation into the fibrous form proceeds more rapidly in the presence of

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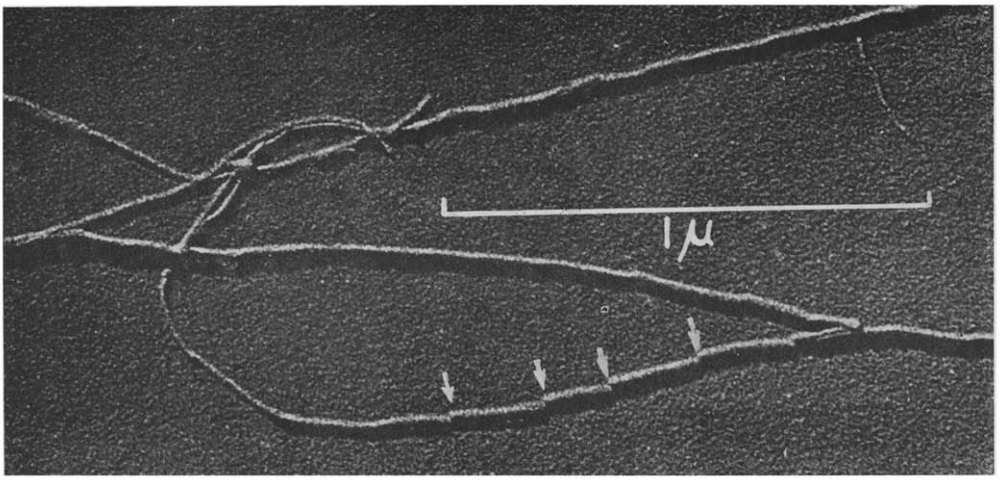

Fig. 3. Illustrating transverse rupture of fibrils of F-insulin (see text).

salt as was found by WAUGH. Preliminary experiments show that in the presence of salt the fibrils aggregate immediately leading to the production of a visible precipitate of spherulites which is difficult to study by electron microscopy on account of its thickness. At edges it is possible to see that the fibrils form bundles and that the diameter of the individual filaments is greater (*ca.* 200 A) than was the case with dispersed fibrils. This may account in part for the greater diameter quoted by HALL. From the point of view of fibre genesis the relation between diameter and conditions of formation is important and is being further investigated.

THE PROCESS OF FIBRIL FORMATION

Soluble insulin (G-insulin) has a molecular weight of 48,000 and appears to exist as an aggregate of four molecules of molecular weight 12,000. It dissociates into the smaller molecules at low p_H values[10,11]. The appearance of the finest fibrils of F-insulin is compatible with WAUGH's view that the fibrils arise from an endwise linkage of corpuscular units, which receives particular support from the manner in which the fibres undergo transverse cleavage. The zone of weakness in this direction revealed by the manner of breaking may lie between two of the participating corpuscules which are not resolved themselves.

The minimum fibril width observed by us is of the order of 50–80 A and if this represents a true lower limit of fibril diameter it is too large to allow us to think that we have to deal with a simple linear aggregation of particles of M.W. of 12,000, *i.e.*, of diameter perhaps 30 A. WAUGH's view may be sustained however if it is assumed that prior to the linear polymerisation, which leads directly to the fibrous modification, there occurs a preliminary association of several molecules to produce a 50–80 A unit, the immediate precursor to the linear aggregate. The situation is similar to that proposed in the case of actin where a polymerising unit of about 200 × 300 A (M.W. about 1,500,000 seems to arise as an aggregation of G-actin molecules of M.W. 70,000[5]).

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SUMMARY

Electron micrographs of the fibrils of F-insulin, produced by heating soluble insulin at low p_H, show that these consist of fine fibrils of minimum width 50–80 A and indefinite length. No periodic structure was found in the fibrils, but the tendency to split transversely is in agreement with the view that they are formed by linear aggregation of particles.

The particle, which participates in the actual linear aggregation leading to fibril formation, seems to be larger than the 12,000 M.W. insulin molecule present in acid solution, and this suggests that a preliminary association of several molecules occurs before the actual linear polymerisation.

RÉSUMÉ

Des micrographies électroniques de fibrilles d'insuline-F, préparées en chauffant de l'insuline soluble à faible p_H, ont révélé des fibrilles fines d'une largeur minimale de 50–80 A et de longueur indéfinie. Nous n'avons trouvé dans ces fibrilles aucune structure périodique, mais leur tendance au clivage transversal est en accord avec l'idée que ces fibrilles se forment par aggrégation linéaire de particules.

La particule qui prend part à l'aggrégation linéaire conduisant à la formation des fibrilles semble être plus grosse que la molécule d'insuline de poids moléculaire 12,000 existant en solution acide; ceci suggère l'idée qu'une association de plusieurs molécules aurait lieu avant la polymérisation linéaire en question.

ZUSAMMENFASSUNG

Elektronenmikrographien von F-Insulin-Fibrillen, durch Erhitzen von löslichem Insulin bei niedrigem p_H hergestellt, zeigen feine Fibrillen von minimaler Breite 50–80 A und von unbestimmter Länge. Es wurde keine periodische Struktur in den Fibrillen gefunden, aber die Neigung zu transversaler Spaltung ist in Übereinstimmung mit der Ansicht, dass die Fibrillen durch lineare Aggregation von Teilchen entstehen.

Die Teilchen, welche sich an einander reihen, scheinen grösser zu sein als das Insulinmolekül (M.W. 12,000), welches in saurer Lösung existiert; es scheint, dass vor der eigentlichen linearen Polymerisation erst eine Assoziation von mehreren Molekülen stattfindet.

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