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Changes in the Molecular Structure of Hair in Insulin-Dependent Diabetes

Veronica J. James,*[,1] Dennis K. Yue,† and Susan V. McLennan†

**Biophysics Department, School of Physics, The University of New South Wales, Sydney 2052 NSW, Australia; and †The Department of Endocrinology, School of Medicine, The University of Sydney, Sydney NSW, Australia*

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Low-angle synchrotron X-ray diffraction has revealed clear and consistent changes in the molecular structure of α-keratin of hair in insulin-dependent diabetes (IDDM) both for human IDDM subjects and for baboons with streptozocin induced diabetes. These changes in both meridional and equatorial intensity distributions are fully explained by a newly developed hexagonally packed model for keratin which locates the modification produced in hair in IDDM in the labile structure of the matrix at established intermediate filament linkage sites. The nature of the extracellular bonding suggests that the change is endogenous, occurring via the blood during the aggregation of the IFs in the follicle. The reproducibility of these changes indicate that hair may represent an easily accessible tissue for the study of how hyperglycaemia can modify extracellular matrix materials which lead to diabetic complications.

The suitability of scalp hair and its wide usage as a biopsy tissue for the determination of trace elements, for the identification of systemic heavy-metal intoxication and for the detection of therapeutic and abused drug usage has been fully summarised by Katz and Chatt, 1988 (1). Its usage as a diagnostic tool or indicator for various diseased states has made little progress to date, mainly because such diagnostic indicators require established standard reference values from normal and pathological material with appropriately small standard deviations.

A cross-section of hair is comprised of a thin outer envelope, the cuticle, a small inner section called the medulla and the major component, the cortex. (2,3). The macrofibrils which make up the cortex resemble cylinders of 200 nm diameter (4). Combining the results obtained from synchrotron X-ray diffraction studies and swelling experiments, Feughelman and James have developed a new model for α-keratin which satisfies all previously established results (5,6). This model shows that on the microscopic level, the macrofibrils are composed of hexagonal arrays of intermediate filaments (IFs), each of which is composed of 8 highly organised tetramers of low sulphur content, embedded in a cystine rich matrix. Each tetramer is in turn made up of four α-keratin molecules arranged in a coiled-coil. The 8 tetramers are illustrated by the longitudinally parallel lines of the cylinders of Figure 1 (A). This figure also illustrates that the tetramers are tilted at approximately 7° to the axial direction and are stepped relative to one another at distances seen in the axial projection of 7.83 nm. As a result, the interfibrillar linkages form a slow helix of pitch 47.0 nm, as illustrated in flat projection in Figure 1 (A) and in cylindrical form in Figure 1 (B).

Using the synchrotron sources on the Australian National Beamline Facility and the Japanese Beamline BL15a at the Photon Factory, one of us, VJJ, has shown that for normal hair, in addition to the infinite lattice associated with the 47.0 nm, there is a second repeat lattice of 62.6 nm, the axial projection of the repeat distance of the full length of any tetramer along the fibril. Although each tetramer is rotated through an angle of 120° along its length to accommodate the hexagonal packing, the arrangement of the intermediate filament linkages of neighbouring IFs gives rise to a linear repeat of 63.1 ± 0.1 nm. Figure 1(B) illustrates 4 consecutive points along this infinite lattice by the letters a,b,c,d (5,6). In projection this gives the repeat spacing of 62.6±0.1 nm.

High resolution synchrotron X-ray diffraction techniques studies of normal human and baboon hair have provided detailed information on their ultrastructure (4,7). These results indicated that a study

[1] Corresponding author. Fax: 61-2-96549795; E-mail: vjj@newt.phys.unsw.edu.au.

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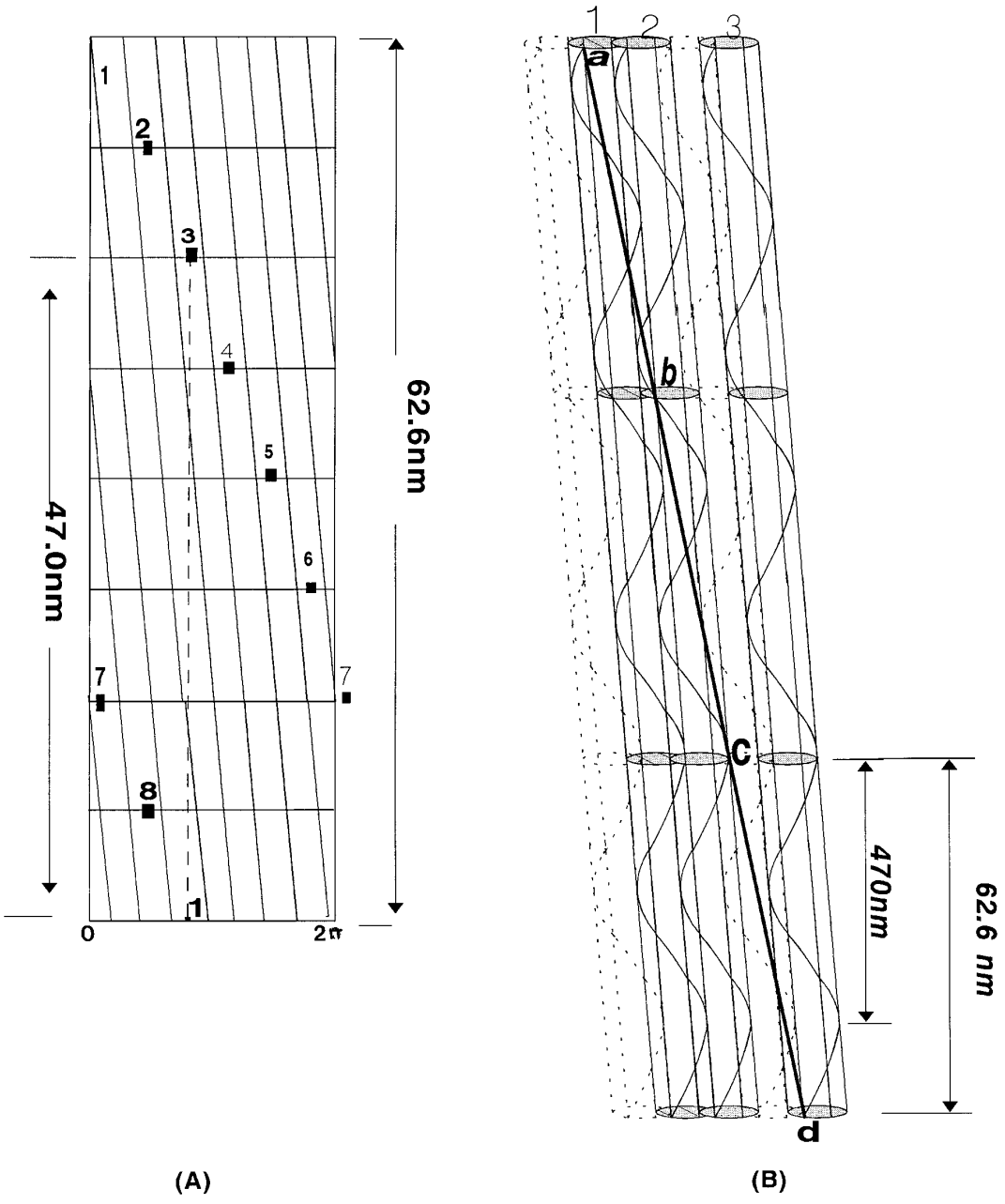

FIG. 1. Diagrammatic representations flat (1 A) and cylindrical (1B) of the 8 tetramers. The crosslink sites between intermediate filaments are numbered (1–8). The hexagonal packing is indicated by the fact that the bond 7 is axially above bond 1 as indicated. The repeat distance of any tetramer along the axis is 62.6 nm but rotated through 120°. Four consecutive repeat positions along the 62.6 nm lattice are indicated on Figure 1B by the letters a, b, c, d.

of hair by X-ray diffraction could provide interesting information in diabetes. Hair has the obvious advantage of ease of collection and transport, making repeated studies possible. The purpose of this investigation therefore was to determine whether changes occur in the structure of hair in IDDM and whether such changes could provide a new tool to investigate diabetic complications.

MATERIALS AND METHODS

Human scalp hair from the occipital region was used for the investigation. After the nature of the research had been discussed with them, 10 to 20 hairs were collected from each of 7 Caucasian persons, all in perfect health and from 6 persons with IDDM of more than one year duration. Their age and duration are shown in Table 1. Care was taken to ensure that no dyeing or bleaching of the hair had taken place prior to sample collection. The speci-

TABLE 1

Details of Samples

Human			Baboons		
	Diabetic			Diabetic	
Non Diabetic Age (years)	Age (years)	Duration of diabetes (years)	Non Diabetic Age (years)	Age (years)	Duration of diabetes (months)
4	6	1	2.00	2.00	2
19	19	7	2.35	2.00	6
23	22	10	3.00	3.00	6
30	28	20	3.75	3.00	10
35	30	15		3.60	20
43	31	2			
66					

mens for the baboon research consisted of hair cut from the scapular region of baboons (*papio hamadryas*). Details of the induction of diabetes, husbandry and diabetic complications status of this colony of insulin dependent diabetic baboons have been reported in previous publications (8, 9). Baboons, five diabetic and three age-matched non-diabetic controls, were chosen for this research to enable long term studies of changes in the molecular structure of hair in insulin dependent diabetes with duration. This study eliminates any possible effects of hair treatment and provides the perfect age and environmentally matched controlled sets. The age and duration of diabetes for the baboons are shown in Table 1.

The X-ray diffraction experiments were carried out using the low angle synchrotron facility BL15a at the Photon Factory, using a wavelength of 0.15 nm. The sample to image plate distances used were approximately 400mm for human samples and 248 mm for baboon samples. The closer distance was chosen for the baboon samples to obtain an extended data set required to confirm the interhelical spacing. Full details of sample preparation, experimental procedures and data handling are given elsewhere (8). All experiments and procedures have been approved by the appropriate Ethics Review Committee of the Institutions.

RESULTS AND DISCUSSION

Since the packing arrangement of hair is basically sets of cylinders within cylinders, a Bessel Function analysis was used to compute the mean diameters and the average separations of the various cylinders. Full details of this analysis and the determination of the reflections related to the various radii together with a typical equatorial diffraction pattern for normal hair is given in (4). No significant differences were found between normal and diabetic samples for the radii or centre to centre distances of the tetramers or of the helices. This indicates that there are no changes in the internal structure of the IFs even with long term IDDM. The radii of the tetramers for the human samples was 2.44 ± 0.03nm for normal samples and 2.45±0.03nm for IDDM samples. The mean centre to centre spacing for the keratin molecules was 1.03±0.01nm for every sample irrespective of the presence of diabetes. This is close to the accepted lateral dimension of the α-helices indicating that the helices are tightly coiled.

The Bessel Function analysis did however show that the IF radii increased for all IDDM samples relative to the controls for both human and baboon samples. These results are shown in Figure 2 (A) and in Figure 2 (B) respectively. In addition, these results indicate that whilst the changes with age are not significant, there is a very slight logarithmic trend downwards with increasing age. These facts confirm

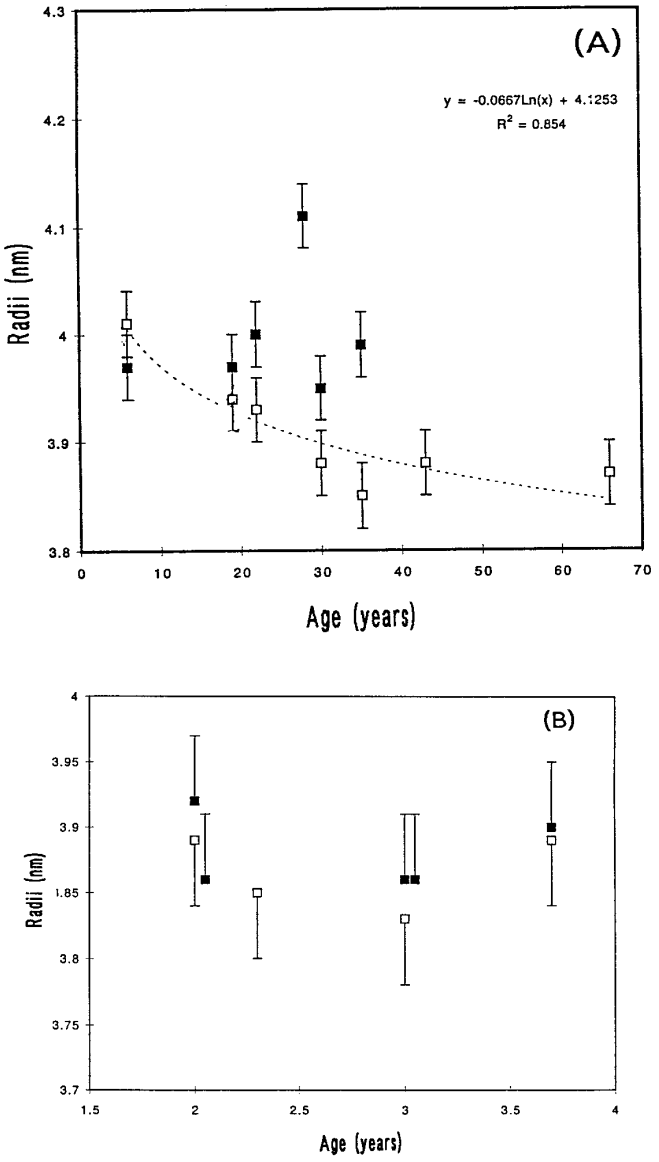

FIG. 2. Relationship of intermediate filament radii with age in human (A) and baboon (B) hair samples. Nondiabetic control (□) and diabetic samples (■).

that the change with IDDM cannot be inferred as accelerated ageing.

As the arrangement of the IFs is only pseudo crystalline, the resulting reflections in the X-ray diffraction patterns are diffuse. The centre-to-centre spacings of the IFs, for all normal human samples measured, were in a range of 11 ± 1 nm. No values could be obtained for IDDM samples as the related peaks fell behind the beam-stop. Since the internal structure of the individual IFs remains unchanged in diabetes, the larger radii of the IFs must result from the attachment of material along the IFs. This bonding may be direct or via high-sulphur globules present in the matrix.

This conclusion is confirmed by the location of the striking and consistent changes in the meridional intensity distributions for hair from diabetic patients as compared with similar patterns from normal hair. The meridional intensity distribution for hair is extremely complicated as it amounts to the superposition of 6 separate lattices which by the relationships between their spacings give rise to a number of composite peaks but there are sufficient peaks unique to each lattice to confirm the existence of each separate lattice.(8) The intensity changes observed with IDDM relate only to the infinite lattices of spacing 47.0nm and 62.6 nm. Reflections from these lattices appear and disappear. In some cases, the positions of the maxima in composite reflections are changed and thereby accentuate a different component from one of these two infinite lattices. It was the appearance of 4 strong reflections in the meridional intensity distributions for every IDDM sample that confirmed undeniably the presence of the 62.6 nm lattice which enabled the structure of keratin to be determined (5,6). Figures 3(A) and 3(B) show sections of typical meridional intensity distributions, for a normal and an IDDM sample. These are sections of especial interest around the very strong nineteenth and thirty-eighth orders of the 47.0 nm lattice. In all IDDM cases, the intensity of the nineteenth order increases whilst that of the thirty-eighth order decreases relative to the values observed for normal samples. A plot of the relative values for the ratio of the intensities of the 38th order to the 19th order is given in Figure 4. Similar changes in the meridional intensities were obtained between normal and diabetic baboons but the data here was not as well resolved so that individual peaks in some cases could not be indexed.

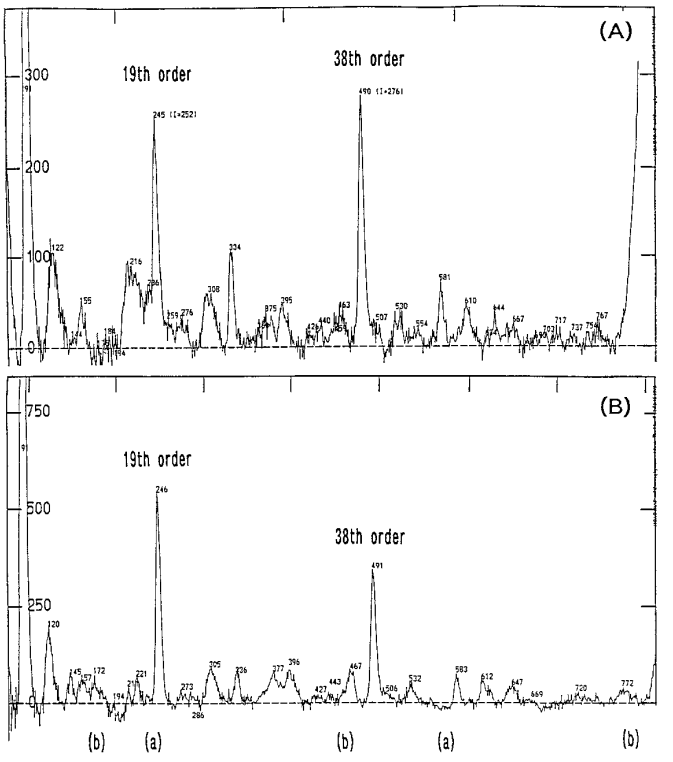

FIG. 3. Typical meridional intensity distributions after background removal for a normal (A) and a diabetic (B) human hair sample. A comparison of intensities for particular pixel values shows peaks which disappear (a) and appear (b) as well as change in intensity relative to one another.

CONCLUSION

The increase in the radii of the IFs indicates the addition of material to the IF. No changes are observed in the internal structure of the IFs, since the dimensions and packing for the tetramers and for the α-helices are unaltered. Any bound material must therefore bind onto the exterior of the IFs and the increase observed must reflect the average change in radius that results from such additions. This is confirmed by the changes in the meridional intensities which relate to the 47.0 nm and 62.6 nm infinite lattices only. Such changes can only occur if additional material is bound at regular intervals along the keratin molecules and if the location of binding is at the sites of linkages between neighbouring IFs, since these sites give rise to the 47.0 nm and 62.6 nm infinite lattices. Even with this relatively small number of samples it is clear that definite and consistent changes do occur in the molecular structure of hair in IDDM. At this stage we can only speculate on the nature of the bound material. Whether it is a product of glycation or advanced glycation endproducts is unknown but in either case the additional material would attach at the time of formation of the fibril in the follicle. Further studies of this area can provide information on how hyperglycaemia can modify extracellular matrix materials and lead to diabetic complications.

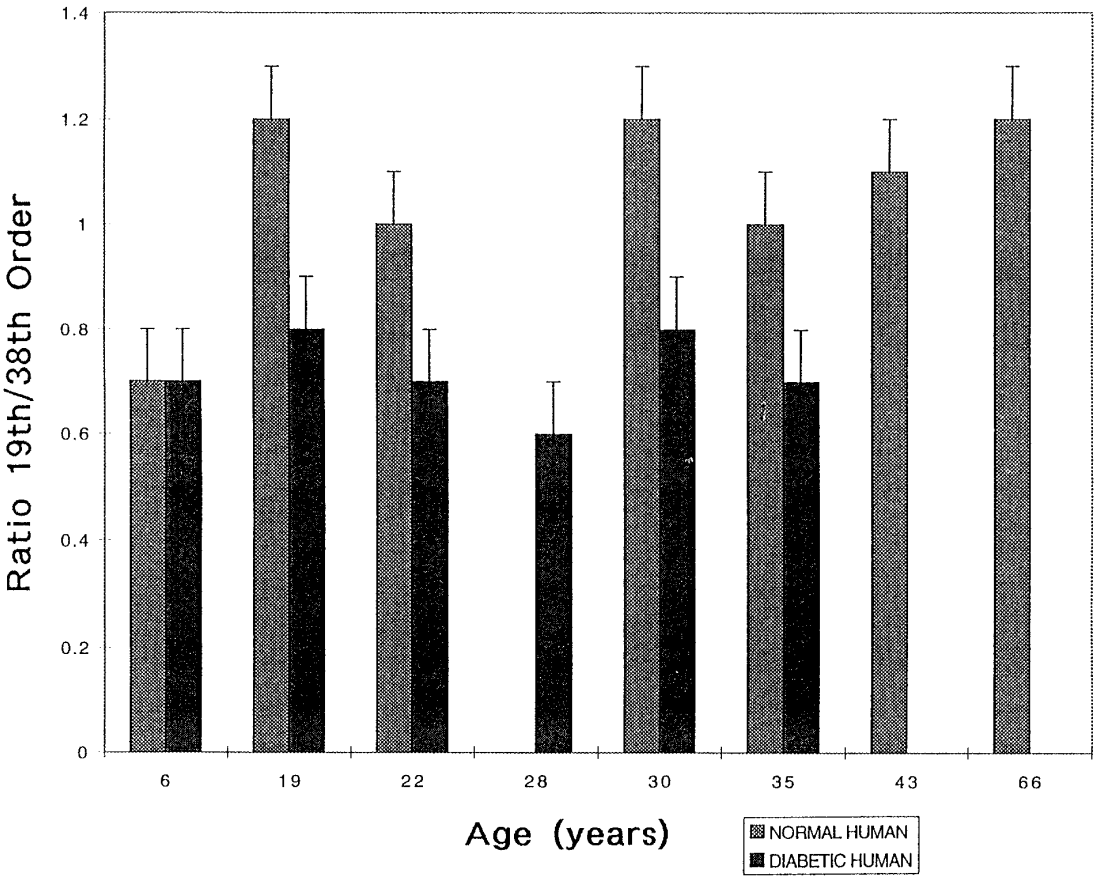

FIG. 4. Ratio of the intensities of the 38th order to that of the 19th order, the two very strong reflections, for normal and diabetic samples.

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