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Models of α-keratin structure

In simple polymers it is generally accepted that the density predicted from a plausible model structure must be greater than the observed macroscopic density, since a certain proportion of non-crystalline material is always present. Despite their considerable molecular and histological complexity, it has long been assumed that the same criterion can be applied to α-keratins. If this assumption is correct, then the average volume, $\bar{v}$, occupied by an amino acid residue in a satisfactory model structure should be less than that calculated from the macroscopic density, d, and mean residue weight, $\bar{M}$, by means of the expression $\bar{v} = 1.66\ \bar{M}/d$, where $\bar{v}$ is expressed in A^3 and d in g·cm^{-3}.

As the macroscopic density of native α-keratins increases with crystallinity[1], it is clear that the more crystalline the specimen for which $\bar{v}$ is determined the more stringent the density criterion becomes. The most crystalline α-keratin structures recognised so far are the quill tips of various porcupines, but the calculation of $\bar{v}$ for these structures has been hampered in the past by the lack of precise analytical or density data.

On the basis of X-ray diffraction studies, we have selected the descaled quill tip from the North American porcupine (*Erethizon* sp.) as the most suitable subject for measurement and have determined both its amino acid composition and density. The analysis was carried out by ion exchange chromatography on columns of Dowex 50-X8[2] using the ninhydrin reagent described by MOORE AND STEIN[3]. Proline was determined by the method of CHINARD[4] after preliminary ion exchange chromatography[5] and cystine was estimated by amperometric titration. The results of the analysis are presented in Table I. The density was determined by flotation in a mixture of *o*-dichlorobenzene and bromobenzene, after drying at 100° C *in vacuo* for 3 h. A value of 1.317 was obtained.

TABLE I

AMINO ACID COMPOSITION OF PORCUPINE QUILL TIPS (*Erethizon* sp.)

Amino acid	*No. of Observations*	*Nitrogen as % of total nitrogen*		*g amino acid from 100 g quill tips*
		Mean	*S.E.*	
Alanine	2	4.26	0.36	4.62
Amide	2	8.99	0.70	1.75
Arginine	2	18.82	0.42	9.97
Aspartic acid	3	5.30	0.29	8.58
Cystine	1	6.77	—	9.92
Glutamic acid	3	9.52	0.56	17.05
Glycine	3	6.72	0.24	6.14
Histidine	2	1.32	0.33	0.83
Isoleucine	3	2.34	0.24	3.74
Leucine	3	5.78	0.20	9.23
Lysine	2	4.09	0.15	3.64
Phenylalanine	3	2.10	0.12	4.22
Proline	2	4.10	0.02	5.74
Serine	3	6.53	0.12	8.35
Threonine	3	3.59	0.22	5.23
Tyrosine	2	3.07	0.00	6.76
Valine	2	4.11	0.07	5.86

Unless the analytical figures account quantitatively for all the nitrogen present, the mean residue weight obtained depends upon the method of computation[1]. In the present case, a value of $\bar{M} = 109$ was obtained by the number average method used by ASTBURY AND WOODS[6] and a value $M = 111$ by applying the method due to CHIBNALL[7]. Neglect of tryptophan, for which analytical figures are not at present available, will give values of M which are about 1% too low, assuming a tryptophan content similar to that of other α-keratins. The true value of $\bar{M}$ is probably therefore close to 111, giving a value of 140 Å^3 for v.

It may be concluded from our results that, if the density criterion is applicable, the average volume per residue in an α-keratin model should not be greater than about 140 Å^3. It is important to note however that this applies only to dry keratin for it has been shown that the crystallites expand laterally on hydration[6,8]. Unfortunately, the degree of hydration is rarely specified in model structures or in measurements of X-ray reflections, thus hampering precise comparisons.

The only existing models of α-keratin structure which are sufficiently detailed to test by comparison with the observed value of the average volume per residue are those of PAULING AND COREY[9] and HUGGINS[10]. Both are based on hexagonal units which account semi-quantitatively for certain features of the X-ray diffraction diagram, in particular the equatorial reflections. The former has 9 residues in a volume of 1351 Å^3 and the latter 81 residues in 11,610 Å^3, assuming a translation per residue of 1.49 Å. Thus, the average volumes per residue are 150 Å^3 and 143 Å^3 respectively. When compared with the observed value of 140 Å^3 in a material which is not completely crystalline, neither structure would appear to be sufficiently compact to account for the observed density.

It is questionable however whether structures of either type are applicable to keratin, as the lower angle equatorial reflections are tacitly assumed to arise from the lateral repetition of comparatively simple structural units across the width of a crystallite, whereas evidence from electron microscopy suggests a distinct polyphase structure. In ultrathin sections of developing hair it is possible to distinguish aggregates of microfibrils embedded in an osmiophilic matrix[11], and together with the enormous intensification of the lower angle equatorial reflections in osmium treated wool[12] this suggests that these reflections are produced essentially by the lattice of microfibrils and are little dependent on their internal structure. Similar views based on other evidence have been expressed by MERCER[13] and KRATKY *et al.*[14].

If this interpretation of the X-ray diagram is correct, it is feasible that the osmiophilic matrix, which is not necessarily amorphous, has a chemical composition or polypeptide chain arrangement different from that of the microfibrils. In this case useful comparisons of observed and predicted densities or average volumes per residue can only be applied to models of the complete structure.

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