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Keratin intermediate filaments: Differences in the sequences of the Type I and Type II chains explain the origin of the stability of an enzyme-resistant four-chain fragment

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ABSTRACT

Previous studies have shown that a strong interaction exists between oppositely directed 1B molecular segments in the intermediate filaments of trichocyte keratins. A similar interaction has been identified as having a significant role in the formation of unit-length filaments, a precursor to intermediate filament formation. The present study is concerned with the spatial relationship of these interacting segments and its dependence on differences in the amino acid sequences of the two-chain regions that constitute the 1B molecular segment. It is shown that along a particular line of contact both chain segments possess an elevated concentration of residues with a high propensity for dimer formation. The transition from the reduced to the oxidized state involves a simple axial displacement of one molecular segment relative to the other, with no attendant rotation of either segment. This changes the inter-relationship of the two 1B molecular segments from a loosely packed form to a more compact one. After the slippage eight of the cysteine residues in the dimer are precisely aligned to link up and form the disulfide linkages as observed. The two remaining cysteine residues are located on the outside of the dimer and are presumably involved in inter-dimer bonding. The existence of a unique line of contact requires that two chains in the molecule have different amino acid compositions with the clustering of dimer-favoring residues phased by half the pitch length of the coiled coil.

1. Introduction

Intermediate filaments (IF) are ubiquitous structural components of the architecture of living cells and although they serve diverse functions their molecules are built to a common plan, with a rod-like central domain around 46 nm in length, and N-terminal (head) and C-terminal (tail) domains that vary widely in size and chemical composition depending on which of the five IF chain types are present. Keratins are formed from an obligate mixture of Type I and Type II chains; vimentin, desmin and glial fibrillary acidic proteins (amongst others) are formed from Type III chains; neurofilaments (for example) are constructed of Type IV chains, and the lamins are formed from Type V IF chains (Parry, 1990). In all cases the rod domains form the framework of the IF (see Chou and Buehler, 2012 for a detailed atomic model of a trichocyte keratin molecule). Each molecule, irrespective of chain type, has a rod domain containing two major segments of two-stranded coiled coil of closely similar lengths (about 22 nm). The first (segment 1) comprises coiled-coil segments 1A and 1B separated by linker L1. The latter can be α-helical (as in vimentin: Chernyatina et al., 2012; Aziz et al., 2012) but is unlikely to be so in some other IF proteins where the sequences are rich in amino acids that would strongly disfavor this conformation. The second (segment 2) is a two-stranded α-helical coiled coil over its entire length, though over its N-terminal end (about 30–35 residues) and at a short central region the strands lie closely parallel to one another in a bundle-like conformation (Parry, 2006; Nicolet et al., 2010).

Using crosslinking data it has been shown that the coiled-coil regions in the two chains that constitute the molecule have the same lengths and lie in axial register irrespective of chain type (Steinert et al., 1993a,b, 1999; Wang et al., 2000). In addition to the keratins some of the molecules in other types of IF can be formed from a mixture of chains and/or chain types though, more commonly, two identical chains constitute the molecular structure. Examples of mixed chain structures include NF-L(IV)/NF-M(IV), NF-L(IV)/NF-H(IV), NF-L(IV)/peripherin(III), GFAP(III)/NF-L(IV), nestin(IV)/vimentin(III), and nestin(IV)/α-internexin(IV) (see, for

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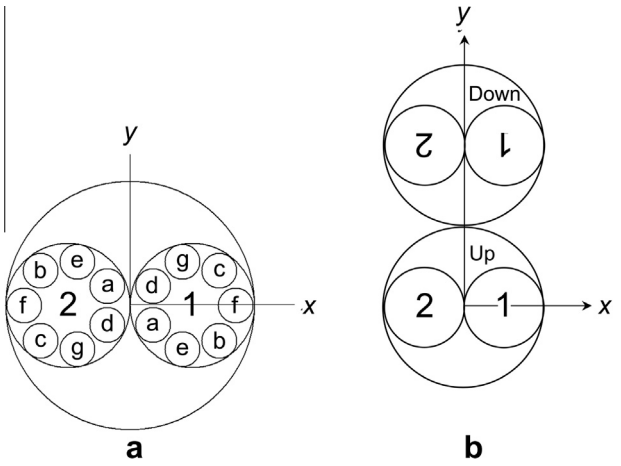

Fig.1. (a) A helical projection (Klug et al., 1958) onto the plane $z = 0$ of the residues in a two-strand coiled coil. In a finite length of coiled coil it is assumed that the β-carbon atom of the first residue in the *f*-position lies on the *x*-axis (Fraser et al., 1964). In the case of the 1B segment there are 101 residues in each chain and these are numbered from 1 to 101, commencing with the N-terminal residue. Residue 1 is in the *a*-position so that the *f*-position in the helical projection above includes residues 6, 13, 20, . . ., 97. (b) A section in the plane $z = 0$ of the model for the dimer; the 1B Down molecular segment is related to the 1B Up molecular segment by a diad which is tangential to the line of contact between the exscribed cylinders around the Up and the Down 1B segments. Both molecular segments are shown with zero rotation around their respective axes and the aim of the present study is to determine the rotation of the Up molecular segments that maximizes the propensity for dimer formation. The orientation in the Down molecular segments is then calculable by the operation of the diad.

example, Parry, 2005). In keratins, however, the two chains are always a Type I and a Type II.

Two types of mammalian α-keratins have been characterized; the 'soft' α-keratins of skin (epidermal keratins) and the 'hard' α-keratins (trichocyte keratins) of epidermal appendages, such as hair, claws and quills. Both are stabilized around the time of cell death by disulfide bonds formed between pairs of cysteine residues, but the number of such bonds in trichocyte keratin is very much greater than in epidermal keratin. Electron micrographs of cross-sections of both types of keratin exhibit a filament/matrix texture: in the case of the epidermal keratins the matrix is believed to comprise some or all of the N- and C-terminal domains of the molecules, and the cores of the filaments are formed from the rod domains. An analogous situation exists in the keratins of birds and reptiles except that the secondary structure in the filaments is predominantly the β-sheet (Fraser and Parry, 2008).

A major difference between epidermal and the trichocyte keratins is that the matrix in the latter contains considerable amounts of both sulfur-rich and glycine–tyrosine-rich proteins. A second difference is that during the transition from the reduced to the oxidized state the framework of the IF undergoes both molecular slippage and compaction. This brings the cysteine residues in the 1B segment of neighboring molecular segments into axial alignment and enables the formation of disulfide linkages (Parry, 1996; Fraser and Parry, 2012). By comparison, epidermal proteins contain very little cysteine and the 1B segment in particular does not contain any at all, thereby indicating that any change in the framework of epidermal IF on oxidation will not depend on the change in free energy associated with the formation of disulfide linkages.

There is a clear functional relationship between the concentrations of disulfide bonds in the two types of keratin and their mechanical properties. Epidermal keratins have a sufficient concentration to ensure insolubility without sacrificing flexibility whereas trichocyte keratins, with their extensive cross-linking, are tailored to strengthen epidermal appendages to a degree consistent with their function.

The present study is concerned with the role of the 1B segment in determining the packing of the molecules in the IF framework of keratins. Trichocyte keratins, such as quill and hair, yield detailed X-ray diffraction patterns but the packing is not sufficiently crystalline to be able to employ standard protein crystallographic methods. An early X-ray diffraction study of trichocyte keratin by Fraser et al. (1965) concluded that the most likely molecular conformation was that of a two-strand coiled coil rope. Studies of an enzymatic fragment from wool (Crewther and Harrap, 1967) later revealed that it contained four chains (Ahmadi and Speakman, 1978). This implied that in the native IF there was a strong association between a pair of two-stranded coiled coil segments. In addition, the fragments were shown to form regular assemblies with an axial repeat of 16 nm (Suzuki et al., 1973) and this was also visualized in electron micrographs (Dobb et al., 1972). Later studies established that a fragment of this length from crosslinked IF consisted of a pair of oppositely directed, near in-register 1B segments (Wang et al., 2000). Mücke et al. (2004) also established that this same interaction occured in vimentin. Steinert (1991a,b) also produced evidence of the importance of antiparallel 1B segments in IF assembly. The linear dispositions of the acidic and the basic residues in segment 1B both exhibit a periodicity of 9.55 residues and Crewther et al. (1983) suggested that inter-chain ionic interactions would play a role in specifying the antiparallel axial alignment of the two 1B segments. There is thus a wealth of data indicating the importance of a close association between the 1B segments in oppositely directed molecules.

A great deal of evidence has been accumulated on the likelihood of finding particular residues in the contact areas between dimers as opposed to the rest of the surface (Jones and Thornton, 1996; Bahadur et al., 2003). In the present paper we explore the application of this knowledge to interactions between the 1B segments of keratin IF and how they are related to the changes that occur during keratinization. A useful measure of the propensity for dimer formation is given by $\ln(f_i/f_s)$ where f_i is the frequency with which a particular surface residue occurs in the contact area and f_s is the frequency with which it occurs in the complete surface. A useful property of this metric is that if a residue shows no preference for the contact area then $f_i = f_s$ and the dimer propensity is zero.

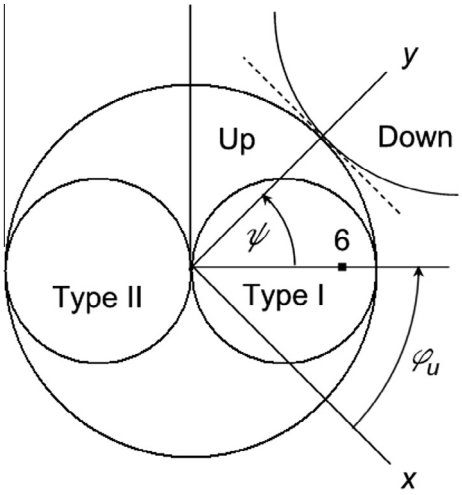

Fig.2. A section on the plane $z = 0$ showing the relationship between the Up and the Down molecular segments when the Up molecular segment is rotated about its axis. The projection of the perpendicular diad is shown as a dotted line, the *z*-coordinate of the diad is a function of the *z*-stagger between the Up and the Down molecular segments. ψ is the angular coordinate of the line of contact between the Up and Down molecular segments, and the variation in dimerization propensity for different values of $\psi = \pi/2 - \varphi_u$ were summed for 10° segments in the range 0°–360°.