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Carbon-13 n.m.r. studies of keratin intermediate filament of human hair

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^{13}C n.m.r. spectra of low sulfur fraction in S-(carboxymethyl) keratine, (SCMKA), which corresponds to the hard keratin intermediate filament (KIF) in human hair were observed in aqueous solution. The spectra were compared with those of SCMKA in 8 M urea and high sulfur fraction in S-(carboxymetyl) keratine, (SCMKB), in aqueous solution. The circular dichroism spectrum of SCMKA indicated 40% α-helix in the aqueous solution, and changed to that of random coil in 8 M urea. The SCMKB in aqueous solution also took random coil. The observed peaks in the ^{13}C n.m.r. spectra of SCMKA in aqueous solution come mainly from the amino acid residues such as Thr, Ser, Pro and Gly in the N- and C-terminal domains with random coil structure. Thus it is suggested that these domains had high mobility. However, the amino acid residues, Leu, Ile, Glu, Arg and Lys, in the rod domain give essentially no peaks because of very restricted motion of the chain with coiled-coil structure. © 1997 Elsevier Science Ltd.

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Introduction

Human hair is a member of the mammalian α-keratins, as well as wool, nail, horn and so on[1]. The histological structure of hair fibre consists of cuticle and cortex, as well as wool. The cortex consists of spindle-shaped macrofibrils which have two main structures, microfibril and matrix, differentiated of structures and amino acid compositions[1–3]. The microfibril is a crystalline fibrous protein which is composed of mainly α-helical proteins with low cysteine content. The molecules are aligned along the fibre axis, and embedded in amorphous matrix with cysteine-rich content. Therefore, hair fibre is regarded as the oriented fibre in which crystalline filaments are aligned in amorphous matrix.

The microfibril in keratin fibre is classified into a class of intermediate filaments (IF) based on their sequences and structural homologies with the other IF proteins such as vimentine, desumin, glial filaments and neurofilaments. Hence, microfibril has recently been called α-keratin intermediate filament (KIF). All IF proteins consist of a central rod domain, and N- and C-terminal domains[2,4–9]. The rod domain has four helical segments in which two α-helix chains are associated to form a double-stranded coiled-coil chain. Besides, the structure of N- and C-terminal domains is considered to be disordered. The coiled-coil structure has been first proposed by Crick[10] and Pauling and Corey[11], and the detailed structure has been studied with X-ray and n.m.r. ânalyses for GCN4 leucine zipper in atomic level resolution[12,13].

In this study, the low sulfur fraction of S-carboxymethyl derivative (SCMKA)[14–16], from hair, is prepared as the microfibril (KIF) of hair, and the ^{13}C solution n.m.r. spectra are observed. The spectra are also compared with those of SCMKA in 8 M urea and the high sulfur fraction of S-carboxymethyl derivative (SCMKB) in aqueous solution. The circular dichroism (CD) spectra of these samples are also observed, to elucidate the overall conformation. On the other hand, the n.m.r. analysis enables study of the local structure in solution from the conformation-dependent chemical shift values. In addition, the peak width gives the information on local mobility in the chain. If the motion is highly restricted, we can no longer observe the peaks.

Experimental

Materials. Commercial black Asian hair from STAFFS Co. Ltd. was used for all experiments. The hair bundle was washed with 1% aqueous solution (wt/wt) of sodium dodecyl sulfate (SDS), thoroughly rinsed with deionized water, and then cleaned by extraction three times with hexane, ethanol and acetone. The bundle was air-dried at room temperature. S-(carboxymethyl) keratin (SCMK) was prepared from the cleaned hair according to the procedure of O'Donnel and Thompson[14], and then was separated into SCMKA (low-sulfur fraction, KIF) and SCMKB (high-sulfur fraction, KIFap/KIF associated protein) according to the procedure of Dowling and Crewther[15].

Helix-rich and amorphous-rich fractions separated from SCMKA were prepared by partial hydrolysis with α-chymotrypsin from bovine pancreas (purchased from Wako Pure Chemical Industries Ltd, Tokyo). Subsequently, each fraction was separated by adjusting the pH to 4.0. The precipitated fraction corresponds to the helix-rich portion, and the supernatant fraction to the amorphous-rich portion, respectively[17].

Methods. The CD spectra of SCMKA and SCMKB were observed as 0.01% (wt/wt) aqueous solutions together with

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a SCMKA sample in 8 M urea with a CD spectrometer (JASCO CD-1000). The spectra were recorded in a 0.1 cm light-path length. The α-helix content of SCMKA was evaluated from the value of molar ellipticity at 222 nm ($[\theta]_{222}$) by assuming 100% helicity when $[\theta]_{222} = 3.5 \times 10^4$.

The ^{13}C and ^{1}H n.m.r. spectra were recorded with a JEOL α-500 n.m.r. spectrometer. The typical spectral conditions for ^{13}C n.m.r. observation were as follows: observation frequency of 125 MHz, 11 000 scans, 34 KHz spectral width, and 3.0 s delay between pulses. The sample concentration was 5% (wt/wt) in D_2O, and measured at 50°C. The chemical shifts were measured relative to the internal reference (dioxan) and converted to the tetramethylsilane (TMS) reference.

The amino acid composition was determined for the SCMKA, SCMKB, the helix-rich and amorphous-rich fractions of SCMKA. 4 mg of each sample was hydrolysed with 6 N HCl at 110°C for 24 h, dried under N_2 gas flow and dissolved in 0.5 ml of citrate buffer (pH 2.2). Filtered solutions were analysed with an amino acid analyser (Mitsubishi Kasei Model-AA01).

Results and discussion

Figure 1 shows the CD spectra of SCMKA and SCMKB.

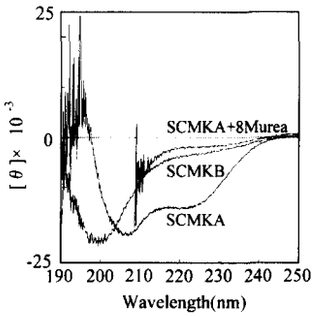

Figure 1 Circular dichroism spectra of SCMKA, SCMKB and [SCMKA + 8 M urea]. The spectrum from 190 to 210 nm of [SCMKA + 8 M urea] was deleted since noise caused by urea interference prevented the normal pattern

The former spectrum has a typical pattern of α-helix having peaks at 208 nm and 222 nm. The helix content was evaluated to be about 40% from the peak intensity at 222 nm. On the other hand, SCMKB showed a typical random-coil pattern. The peak at 222 nm in the CD spectrum of SCMKA disappeared in a protein denaturation reagent, 8 M urea, indicating helix–random coil conformational transition.

The ^{13}C n.m.r. spectra of SCMKB (1) and SCMKA (2) in aqueous solutions, and SCMKA in 8 M urea (3) are shown in *Figure 2*. The sample concentration, the accumulation times, observed temperature and other experimental condition are almost the same among the spectra. The peak assignment was performed from the distortion enhancement by polarisation transfer (DEPT) spectra, the chemical shift and the amino acid composition. The S/N ratio in spectrum (2) is relatively poor with broad base-line in the region 15~60 ppm. From the CD spectrum, the conformation of SCMKA was 40% α-helix. Therefore the relatively broad spectrum with low S/N ratio is due to the presence of α-helical chain with stranded coiled-coil structure. Actually, SCMKB in aqueous solution and SCMKA in 8 M urea give sharp spectra, which is in agreement with random coil structure obtained from CD study. A similar tendency concerning different spectral character between SCMKA and SCMKB was observed in the ^{1}H n.m.r. spectra (data not shown).

In order to analyse the spectra in detail, the amino acid compositions of SCMKA and SCMKB were determined and listed in *Table 1*.

In addition, helix-rich and amorphous-rich fractions in SCMKA were obtained and the amino acid compositions are also listed in *Table 1*. In the helix-rich fraction of SCMKA, the helix-favouring residues such as Glu and Leu are more abundant. On the other hand, in the amorphous-rich fraction of SCMKA and SCMKB, the relative amounts of the non-helix-favouring residues such as Cys, Pro, Ser, Thr and Gly were greater. In the SCMKA spectrum, the peak intensities from the Glu and Leu residues are clearly small compared with the values expected from the amino acid composition. It is shown that the peaks from amino acid

Table 1 Amino acid composition[a] of human hair, SCMKA, helix-rich SCMKA, amorphous-rich SCMKA and SCMKB

Amino acid	Human hair[b]	SCMKA	Helix-rich SCMKA	Amorphous-rich SCMKA	SCMKB
Lys	2.7	3.7	4.8	2.4	0.6
His	0.9	0.1	0.9	0.5	0.9
Arg	5.8	7.7	6.2	4.7	5.4
SCMC[c]	–	7.7	3.4	9.1	24.8
Asx[d]	4.9	9.6	9.6	7.8	2.6
Thr	6.8	6.0	3.9	6.6	10.7
Ser	11.7	10.6	7.7	10.8	13.2
Glx[e]	11.4	19.1	19.9	12.5	8.4
Pro	8.4	1.9	2.0	10.7	12.2
Gly	6.4	5.9	2.9	6.6	5.7
Ala	4.6	6.8	6.8	5.7	2.1
1/2Cys[f]	17.8	0.2	–	–	–
Val	5.8	6.5	5.8	5.8	5.0
Met	0.6	0.6	0.3	0.2	0.1
Ile	2.6	4.2	5.2	3.5	1.9
Leu	5.8	11.0	15.4	9.2	3.2
Tyr	2.0	3.2	3.2	2.4	1.5
Phe	1.6	2.2	1.9	1.6	1.2

[a] Expressed as residues per 100 residues
[b] Ref. [1]
[c] SCMC, S-(carboxymethyl)cysteine
[d] Asx, Asp and Asn
[e] Glx, Glu and Gln
[f] 1/2Cys, half-cystine

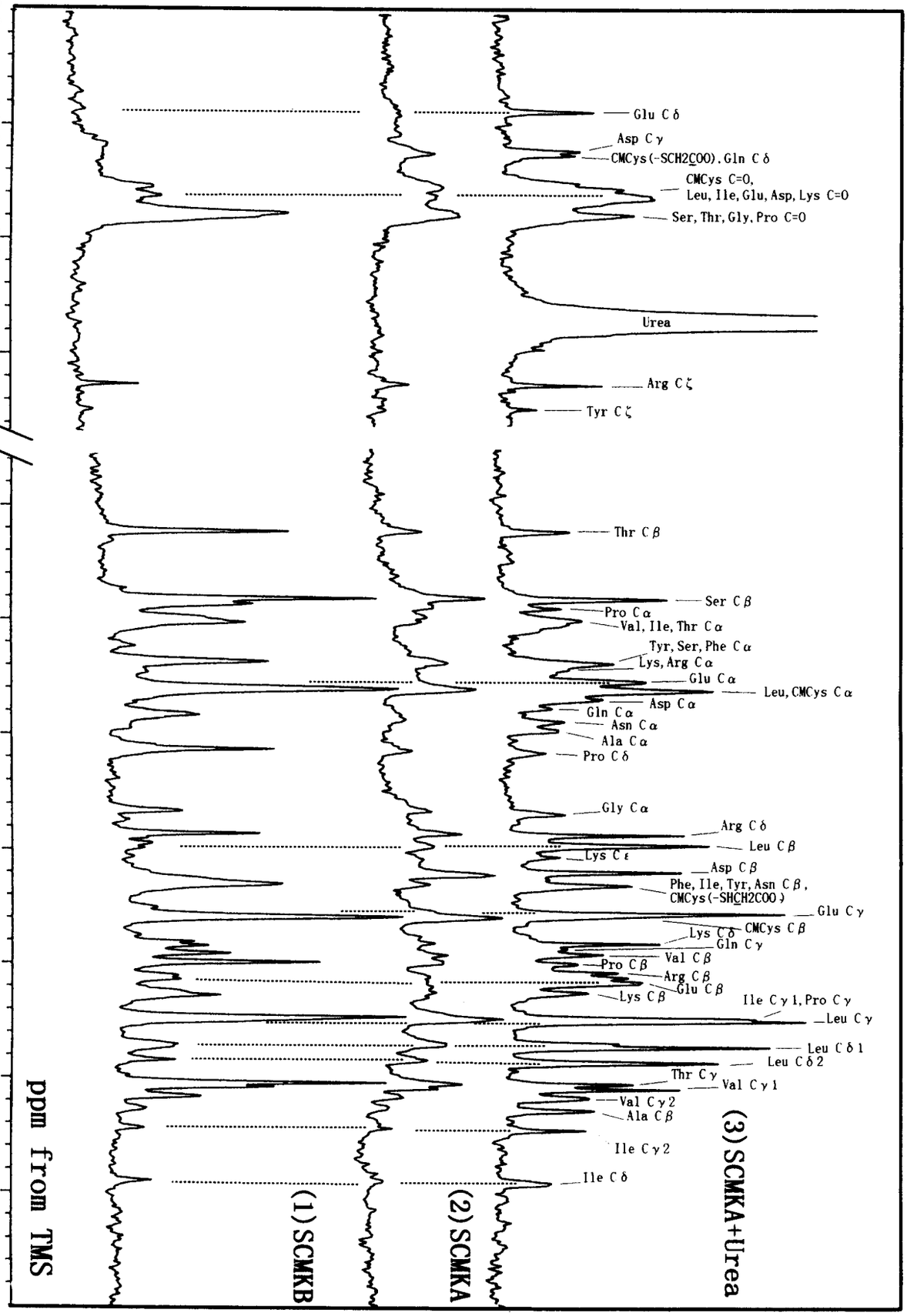

Figure 2 ^{13}C n.m.r. spectra of SCMKA, SCMKB in aquerous solution and SCMKA in 8 M urea solution. Peaks represented by the dotted line are carbons of amino acid such as Leu and Glu existing mostly in rod domain.

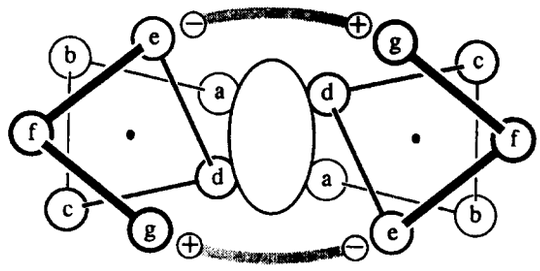

Figure 3 Model of coiled-coil structure[18,19]. The view is down the helical axis of a KIF and shows one heptad repeat for each polypeptide chain. Positions **a** and **d** are apolar residues, and an oval indicates the hydrohobic interaction area. Opposite charged residues in **e** and **g** positions are able to form ion pairing (gray line)

residues in nonhelical N- and C-terminal domains are mainly detected in the spectra, and those of the residues in the helical region with coiled-coil structure are not detected. Actually, in 8 M urea, the amino acid composition estimated from the relative peak intensities was approximately in agreement with those which were expected from the amino acid composition determined by amino acid analysis. In the structural model for the stranded coiled-coil structure as shown in *Figure 3*, the Leu residues are expected to occupy the positions **a** and **d**, by the amino acid sequence homology[18,19].

The relatively broad peaks of two methyl Cδ carbons in Leu are observed at 21.5 ppm and 22.9 ppm. The broad methyl peaks of Ile Cγ and Cδ carbons occuping at **a** and **d** are also observed. These peaks are due to the end methyl groups of the long alkyl side chains, and therefore the motion should be relatively high if there is no steric hindrance. It is hard to tell whether or not these peaks come from the residues located at **a** and **d** in the coiled-coil model (*Figure 3*). However, the presence of severe steric hindrance is clear in the chain in the viewpoint of highly resricted motion of these methyl groups.

The dramatic change between no-urea and 8 M urea solutions is observed for the peaks from Glu residue which is the most abundant one of SCMKA. A very broad peak was observed for the Cδ carbonyl carbon of the side chain of Glu in the aqueous solution. In the high field region, the Glu Cα and Cβ carbon peaks were also not observed clearly. The peak at 34.0 ppm of SCMKA in aqueous solution was due to a CMCys Cβ carbon peak, and was not a Glu Cγ peak. This is clear in the spectrum in 8 M urea; the Glu Cγ peak was observed at slightly lower field, 34.3 ppm. In the coiled-coil model in *Figure 3* the Glu residues are expected to occupy the positions **e** or **g**. Thus, it is suggested that the anion of the carboxylic group of the side chain contributes to the stability of the coiled-coil structure through the electrostatic interaction. Namely, the presence of such an interaction between the anion and the cation, for example, the Lys side chain, is speculated. Lys Cα, Cβ, Cδ and Cε were certainly not detected. On the other hand, the peaks of the residues, Cys, Pro, Ser, Thr and Gly in the N- and C-terminal domains which correspond to the amorphous-rich fraction in *Table 1* are relatively sharp in the aqueous solution of SCMKA, indicating random coil structure with high mobility. The unique physical properties of keratin fibre such as hair and wool may be related with such a dynamic structure of KIF.

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