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Study of the interaction between hair protein and organic acid that improves hair-set durability by near-infrared spectroscopy

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Synopsis

In this study, hydrogen bonds around hair proteins were analyzed by near-infrared spectroscopy to reveal the mechanism of improving hair-set durability by treatment with a specific organic acid. The improvement of set durability was confirmed by measurement on single hair fibers, suggesting that improvement is not because of the surface adhesion increase but because of the internal changes in the hair.

Through analysis by two-dimensional near-infrared correlation spectroscopy, it was found that a combination band of stretching NH and amide II is deconvoluted into three bands interacting with different hydrogen bonds. From the assignment of the three bands, the behavior of the organic acid in the hair was clarified as follows: it adsorbs at the site where water originally binds, even in extremely dry conditions, prevents water penetration, and makes strong and stable hydrogen bonds with hair proteins. The formation of such strong and stable hydrogen bonds suppresses the exchange of hydrogen bonds that is the cause of the breakage of set durability.

INTRODUCTION

It is well known that hair damage is a cause of lusterless appearance, rough feel, and mechanical breakdown. According to recent studies (1–3), hair damage by bleaching or dyeing, along with repeated daily hair care processes, including hair drying with a hot dryer, generates several types of light-scattering origins in the internal structure of the hair, such as splitting of cuticle layers, the generation of micropores in the cortex, and the generation of a porous medulla. The pores inside hair account for its dull appearance. For a technology to suppress the pores, it has been reported that a combination of specific organic acids and solvents are effective (3,4). In further research to confirm the change in appearance by the organic acid and solvent on various hairs, we

noticed that hair-set durability was also improved by the same treatment, as well as hair luster.

Drying a wet hair, held in a given shape, with a hot dryer or a curling iron makes the hair set. This process is usually called water set. It is well known, however, that the created hair style is easily broken down with time, especially quickly in high-humidity conditions. This is due to the exchange of hydrogen bonds in the hair fiber, according to the movement of water molecules (5).

Through treatment with an aqueous solution comprising malic acid (MA) and benzyloxyethanol (BOE), the setting durability of the hair was improved. This improvement was observed in the test using single hair fibers (6), suggesting that the effect is not on the basis of a surface-adhesion increase but a change in the internal property of hair. Consequently, the treatment makes it possible for hair to be set with a natural feel. This setting method is completely different from fixing by setting polymers or adhesive oils, which often are the causes of hair roughness or stickiness.

Since water set is closely related to the interaction of proteins with water, near-infrared (NIR) spectroscopy is one of the best methods (7) to analyze hydrogen bonds and has been used by other researchers. The NIR region corresponds to overtone and combination bands of infrared bands. The absorbance intensity of these bands is relatively weaker than that of normal modes in infrared spectroscopy, but the low absorbance leads to a high rate of transmission and gives internal information on the hair. The NIR spectrum is complex in general, but many bands for various materials, including proteins, have been assigned (8). For wool and human hair fibers, spectra in the NIR region have been measured and the main bands assigned (9–11).

By measurements with NIR spectroscopy (6), it has also been suggested that the mechanism of improvement of set durability by organic acid is due to the strengthening of hydrogen bond interaction around hair proteins. For further analysis, generalized two-dimensional (2D) correlation spectroscopy was applied in this study. This method, proposed by Noda (12), has been applied extensively to analyze the IR and NIR spectra of proteins (13–16). In this method, a perturbation is applied to a system and various spectra are measured under sequential conditions. The measured fluctuations of spectral signals are then transformed into 2D spectra by use of a correlation method.

The 2D correlation spectra can deconvolute overlapped bands, enabling us to know the correlations between bands due to different structures, and it provides information about the specific order of structural changes under perturbation. Any perturbation—mechanical, electrical, chemical, thermal, etc.—may be used, and temperature, concentration, or pH are well selected as variables. In this study, we selected the treatment time on hairs as the variable.

EXPERIMENTAL

HAIR SAMPLES

Chemically untreated Chinese hair washed with a plain shampoo was used. The average diameter, measured by a micrometer, was 62 μm.

For the acid treatment, hair fibers were immersed in an aqueous solution comprising MA (4%)/BOE (10%)/ethanol (15%) at 40°C for 20, 40, or 60 minutes, washed with water

for 30 sec, then naturally dried. For control samples, the hair was also treated with deionized water or an aqueous solution of 0.001 N HCl/BOE (10%)/ethanol (15%) in the same manner.

CURL RETENTION MEASUREMENT IN HIGH HUMIDITY

Single fibers (*ca.* 10-cm long) were wound around glass rods (10 mm ϕ) under a constant tension by pending a small weight at the tip end of the fibers. The samples were then wetted with a small amount of water and blow-dried. After storage under ambient conditions (*ca.* 25°C, 30% RH) overnight, the hair fibers were removed from the rods. All of the hair fibers were water set in the shape of the glass rods, taking the appearance of "springs." By being cut at one side of the spring, some single fibers, having an almost round shape, were obtained. About ten curls were obtained from five hair fibers. Their shape was recorded by a digital camera, and they were kept in a glove box controlled at 26°C, 90% RH. The fibers' shape was again recorded at time *t:* the time left in the box. After repeating series of the process above, the radii of the curvature of the hair fibers at *t*, R_t, were measured and the hair set index, $5/R_t$, where 5 is the radius of the glass rod in millimeters, was calculated. Since most of the hairs were not completely straight, the corrected hair set index was calculated according to the following equation;

$$(5/R_t)_{corr} = \{(5/R_t) - (5/R')\}/\{1 - (5/R')\}. \quad (1)$$

Here, R' is the radius of curvature of the hair fiber after being immersed into water, followed by natural drying.

NIR SPECTROSCOPY

NIR spectra were measured with a Yokogawa Infra Spec NR800 spectrometer. A total of 1024 scans were accumulated at an 8 cm^{-1} resolution for each measurement. The cell of the NIR was set under dry nitrogen atmosphere at 25°C. Hair samples, untreated and treated by an aqueous solution of MA/BOE/ethanol for 20, 40, and 60 min, were dried under vacuum overnight before the NIR measurement.

The NIR spectra obtained were subjected to smoothing, baseline correction, and intensity correction before further analysis. For the data processing, Grams software (Thermo Electron Corp.) was used.

GENERALIZED 2D CORRELATION SPECTROSCOPY

The mathematical background for 2D correlation spectroscopy has been described in detail by Noda (12). For the generalized 2D correlation analysis, we used a program named *2D POCHA* composed by D. Adachi (Kwansei-Gakuin University).

RESULTS AND DISCUSSION

HAIR-SET DURABILITY

Figure 1 shows the results of the curl retention measurement for untreated hairs and for those treated with aqueous solutions of MA/BOE/ethanol or HCl/BOE/ethanol. For the

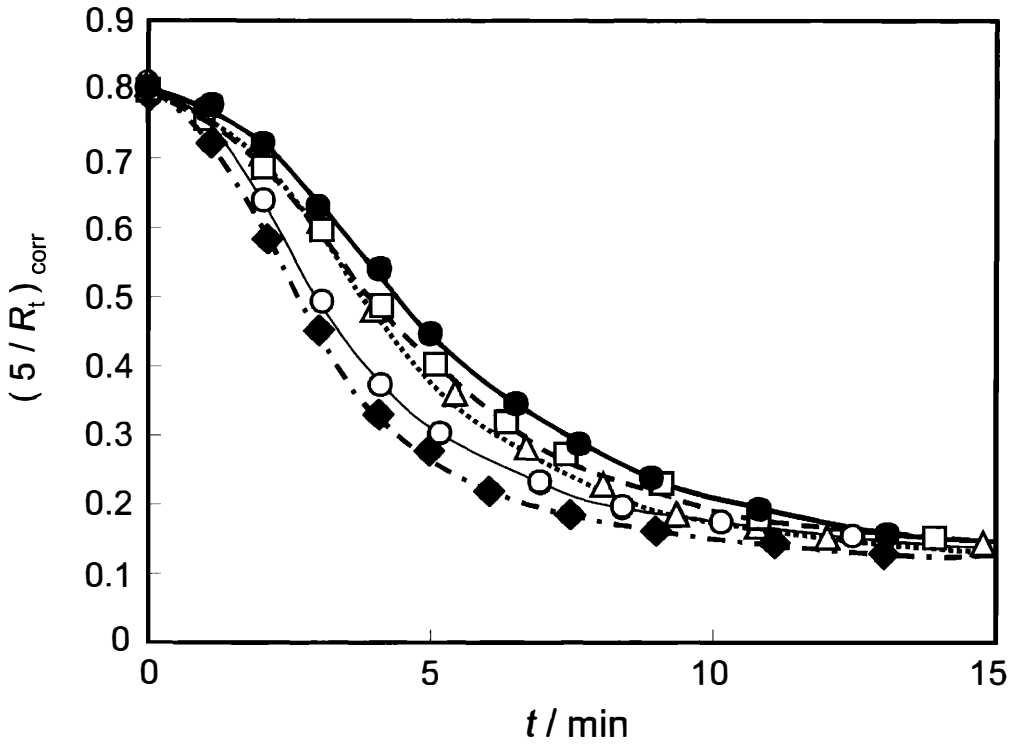

Figure 1. Change of the corrected hair-set index plotted against the time left, *t*, in the box controlled at 26°C, 90% RH. ○: untreated. △, □, ●: treated by an aqueous solution of malic acid (MA)/benzyloxyethanol (BOE)/ethanol for 20, 40, and 60 min, respectively. ◆: treated by an aqueous solution of HCl/BOE/ethanol for 60 min.

untreated hair, the initial curl set rapidly relaxed and returned to the original, almost straight, shape. Such change is seen as a rapid decrease of the hair set index in Figure 1. This means that the water set of hair is broken. For the MA/BOE/ethanol-treated hairs, the set durability improved with treatment time. It is again worth noting that this measurement was done on single fibers, not on a hair bundle. This suggests that the durability improvement comes from changes in the internal properties of the hair fiber. In Figure 1 the result for the HCl/BOE/ethanol-treated hair is also plotted, showing that setting durability did not improve. This means that the improvement of the set durability comes from MA, not from BOE.

NIR SPECTRUM OF THE UNTREATED HAIR

Figure 2 displays an NIR spectrum from 7000 to 4000 cm^{-1} of the untreated hair after smoothing and baseline correction. Five main bands are observed. Three bands in the regions of 6800–6400 cm^{-1}, 6000–5600 $cm,^{-1}$ and 4950–4750 cm^{-1} have been assigned to be a first overtone of NH stretching, first overtones of CH stretching for CH_2 and CH_3 groups, and a combination of NH stretching and amide II, respectively (9,10). The band in the 4700–4500 cm^{-1} region has two possibilities (10): one is due to the combination of NH stretching and amide III, and the other is due to the ternary

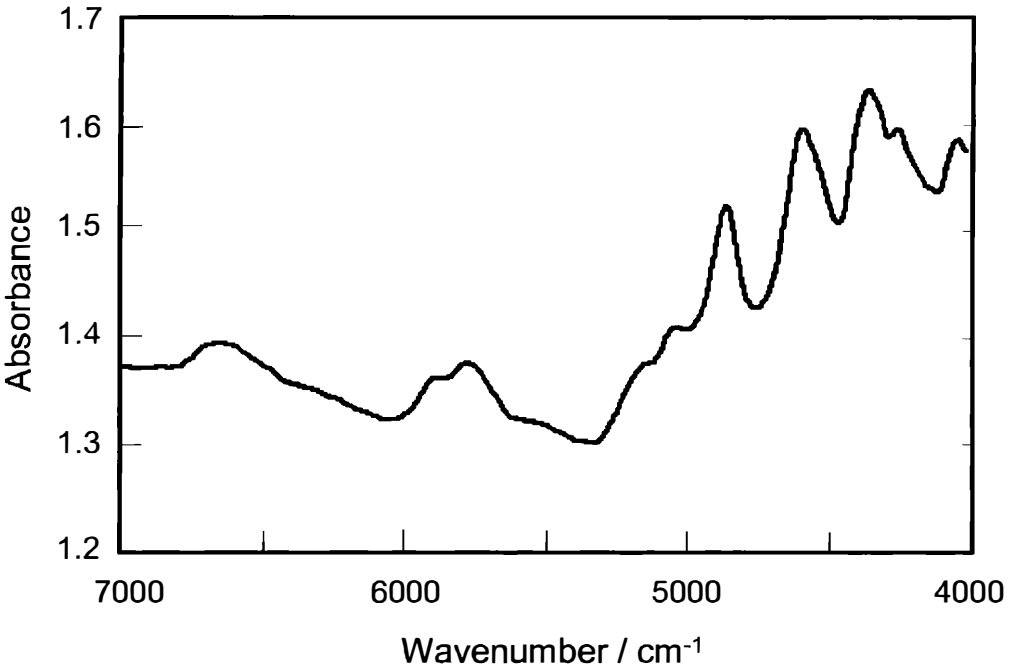

Figure 2. NIR spectrum of chemically untreated Chinese hair in nitrogen atmosphere at 25°C after being dried under vacuum overnight.

combination of 2 × amide I + amide III. The bands in the 4500–4200 cm^{-1} region seem to be combinations of CH stretching and CH deformation for CH_2 and CH_3 (9). Since the band of the first overtones of CH stretching for CH_2 and CH_3 seems not to be changed by the interaction with acids, this intensity of the band was used for the correction of band intensity.

The combination band of NH stretching and amide II was used for the following analysis.

NIR SPECTRA OF THE TREATED HAIR

Figure 3 shows NIR spectra in the 5300–4700 cm^{-1} regions for the untreated hair and that treated with the acid solution for 20, 40, and 60 min. In addition to the combination band of NH stretching and amide II around 4890 cm^{-1}, two other bands can be seen in this figure. The band at around 5170 cm^{-1} is due to the combination of OH stretching and OH deformation of water (8), and the other small band around 5050 cm^{-1} is due to the side-chain amide group (9,10,17).

INTENSITY OF THE WATER BAND

It can be seen that the intensity of the water band decreases with treatment by the acid solution. The trend of the decrease in water is clear in Figure 4, where the relative absorbance at 5170 cm^{-1} is plotted against the treatment time, T. The relative absorbance is the highest for the untreated hair. This means that the untreated hair contains

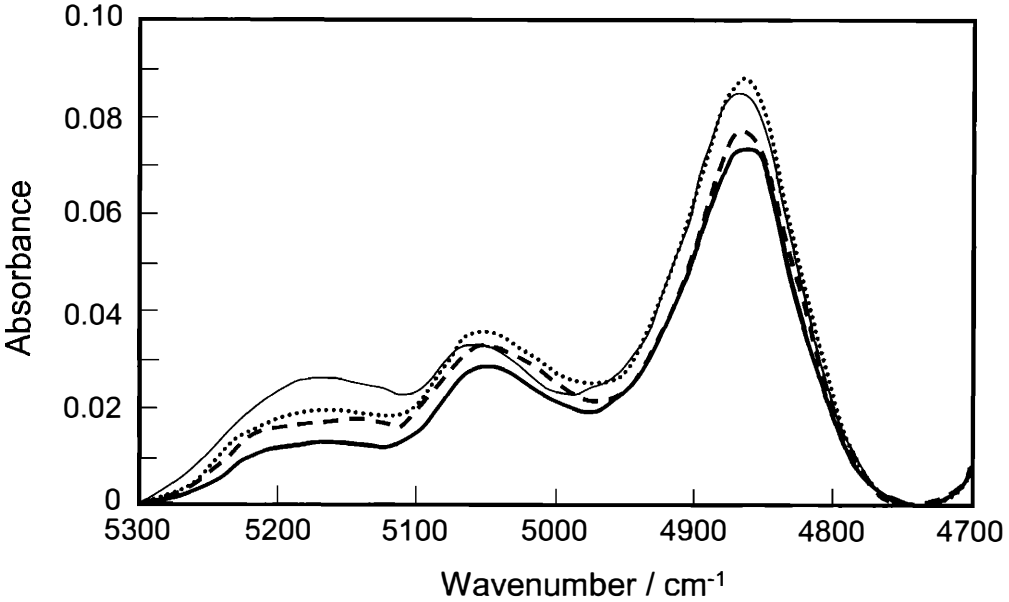

Figure 3. NIR spectra in the 5300–4700 cm^{-1} region corrected on the basis of the intensity of the CH stretching band around 5900 cm^{-1}. Thin solid line: untreated hair. Dotted line: hair treated with MA/BOE/ethanol solution for 20 min. Dashed line: treated for 40 min. thick solid line: treated for 60 min.

some water despite being dried under vacuum overnight and measured in the dry condition. The absorbance of the water band decreases with increasing treatment time in the acid solution, suggesting that the bound water is replaced by substances in the acid solution.

PEAK SHIFT OF PROTEIN BAND BY ACID

In Figure 3, the peak of the NH stretching and amide II combination band (protein band) appears to be shifted toward lower wavenumbers by the acid treatment. To display it in more detail, the second derivatives of the spectra in the range of 4950–4750 cm^{-1} are shown in Figure 5, showing clearly a peak shift toward lower wavenumbers with the acid treatment. In contrast, little shift was observed for the combination band in the case of the hair treated with an aqueous solution of HCl/BOE/ethanol (data not shown). Therefore, the peak shift should be caused by malic acid.

For the stretching mode, it is known that the band shifts toward lower wavenumbers if the bond is affected by the hydrogen bond. On the contrary, the amide II band, NH in-plane deformation mode, may shift toward higher wavenumbers by the hydrogen bond. The effect of the hydrogen bond on the peak shift is, however, generally higher for stretching modes than for deformation modes. Therefore, the peak shift toward lower wavenumbers for the combination band of NH stretching and amide II means that the hydrogen bond is strengthened.

A similar trend for the peak shift and that of set durability improvement was also found for bleached Japanese hair (6). Thus it seems that malic acid works in the same manner for damaged hair, although all kinds of damaged hair have not been investigated.

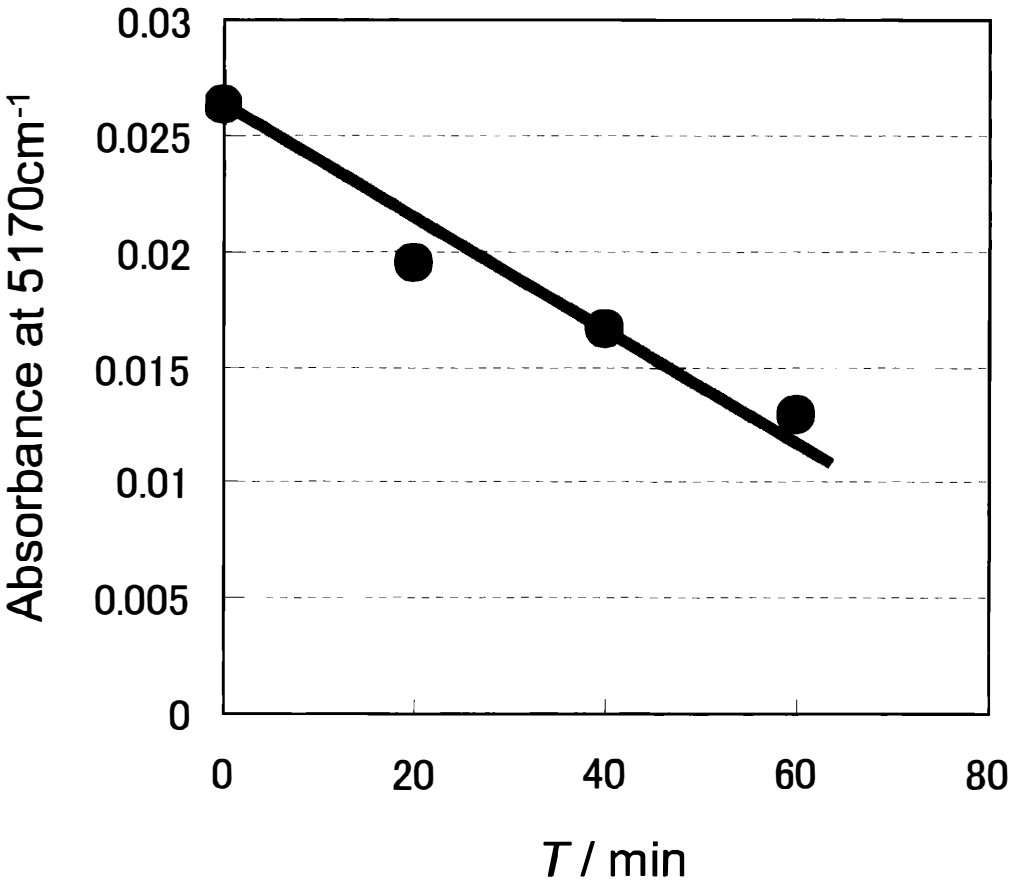

Figure 4. Absorbance of the water band (5170 cm^{-1}) plotted against the treatment time, T.

THE SYNCHRONOUS 2D CORRELATION MAP

Figure 6 displays a synchronous 2D NIR correlation map of the hair, constructed from the treatment time-dependent (0, 20, 40, and 60 min) spectral variations in the 5300–4750 cm^{-1} region. The one-dimensional spectra drawn at the top and at the left side of the 2D map are reference spectra obtained by averaging the spectra set. The synchronous correlation spectrum represents the simultaneous or coincidental change of spectral intensity variations measured at ν_1 and ν_2: here, ν_1 and ν_2 are spectral variables (wavenumbers in this paper). A synchronous spectrum is symmetrical with respect to the diagonal line (dashed line).

The obtained synchronous correlation map is characterized by two autopeaks (on the diagonal line) near 5170 and 4890 cm^{-1} and cross peaks located at the off-diagonal positions between them. The intensity of autopeaks of a synchronous correlation spectrum represents the overall extent of dynamic fluctuations of spectral signals (12,16). Therefore, it is found that the change in the water band and the protein band is large.

The cross peaks shows the relation between the 5170 cm^{-1} and 4890 cm^{-1} bands. The 5170 cm^{-1} is almost at the center of the water band, but 4890 cm^{-1} is a little deviated

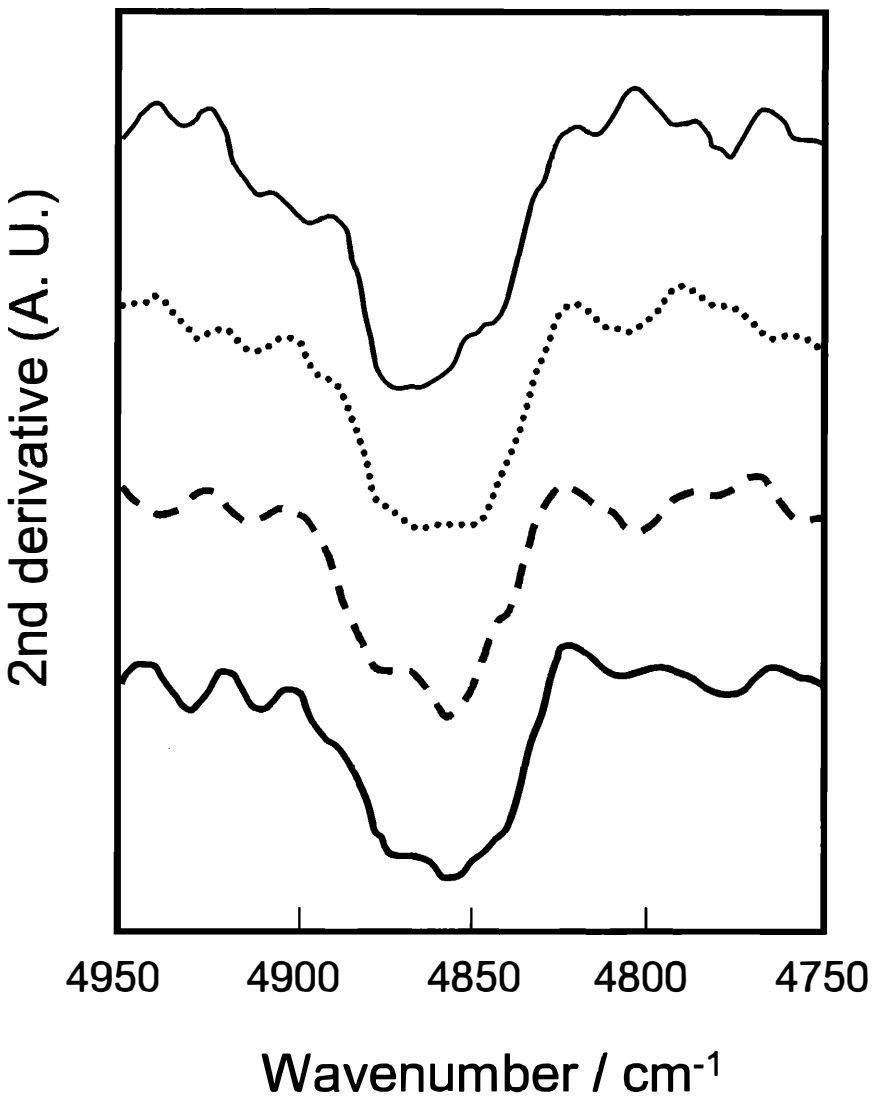

Figure 5. Second derivaties of the spectra shown in Figure 3 in the 4950–4750 cm^{-1} region. Thin solid line: untreated hair. Dotted line: hair treated with MA/BOE/ethanol solution for 20 min. Dashed line: hair treated for 40 min. Thick solid line: hair treated for 60 min.

to the higher wavenumber from the center of the protein band. This suggests the existence of other components, having lower wavenumbers in the protein band, which are to be identified in the following asynchronous 2D correlation study.

THE ASYNCHRONOUS 2D CORRELATION MAP

The asynchronous cross peaks develop only if the basic trends of dynamic spectral variations observed at two different wavenumbers of the cross peaks are dissimilar (12,16). Figure 7 depicts an asynchronous 2D NIR correlation map corresponding to the synchronous map of Figure 6. The wavelength range is limited from 4950 to 4750 cm^{-1} for the sake of detailed analysis of the protein band here. From the asynchronous cross

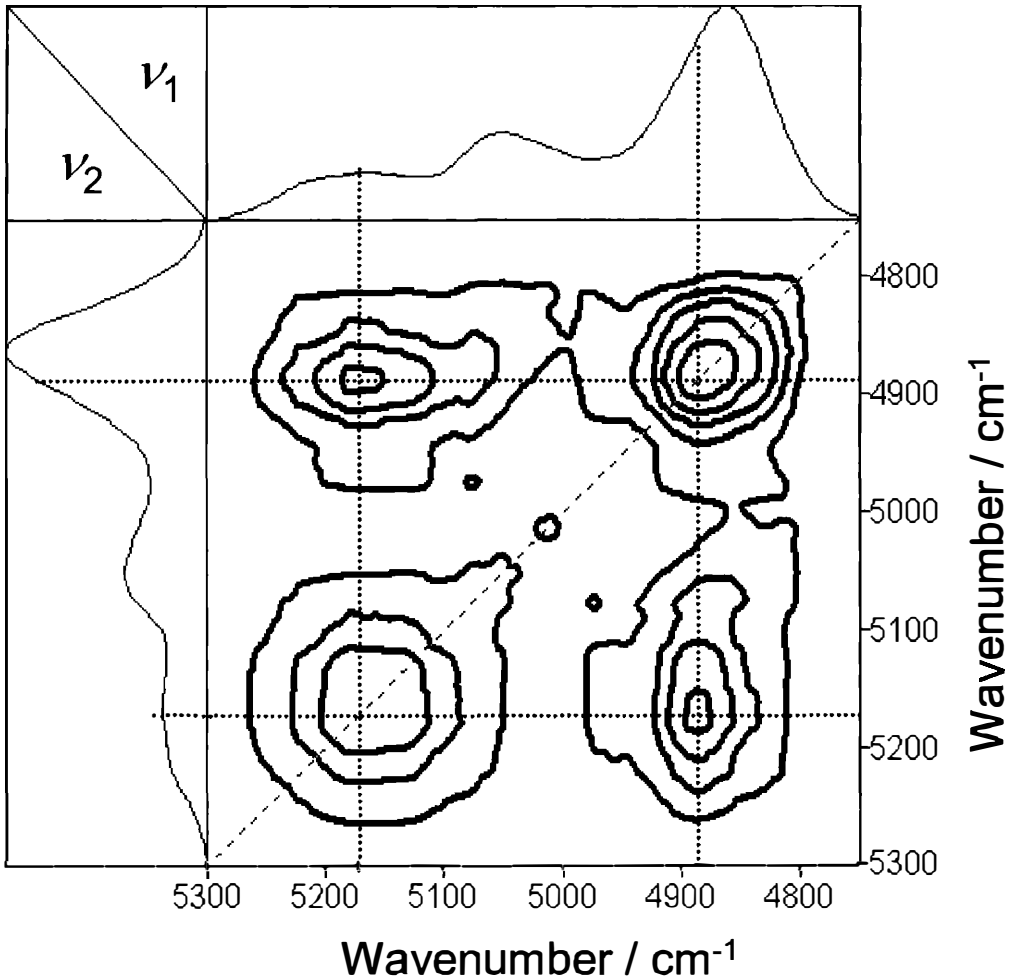

Figure 6. Synchronous 2D NIR correlation spectrum in the 5300–4750 cm^{-1} region constructed from treatment-time-dependent spectral changes in hair. Dotted lines through the tops of the cross peaks show which bands correlate with each other. All the correlation peaks are positive.

peaks, it can be clearly seen that the protein band, which is apparently one peak, is composed of more than three bands. The three bands, around 4890, 4850, and 4810 cm^{-1} are referred to as band A, band B and band C, respectively. The map shows clear correlations between A and B, and A and C.

These three bands seem to be different in the strength of their hydrogen bonds with the NH of the protein amides. According to the consideration described before, it can be said that band A is the band having no or the weakest hydrogen bond, band B is in the middle, and band C is the strongest.

ASSIGNMENT OF THE DECONVOLUTED PROTEIN BANDS

The wavenumber of band A, 4890 cm^{-1}, is the same as that of the band correlating with the water band in the synchronous correlation map (Figure 6). The sign of the cross peaks

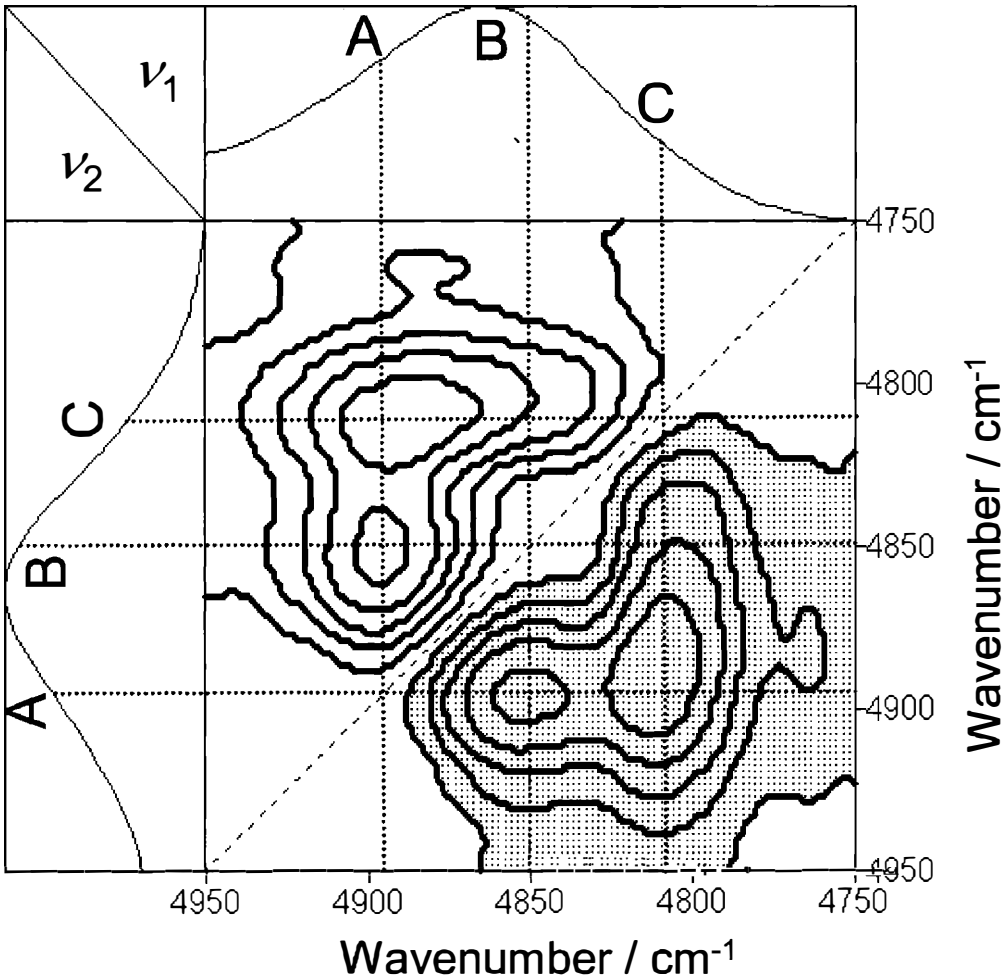

Figure 7. Asynchronous 2D NIR correlation spectrum in the 4950–4750 cm^{-1} region constructed from treatment-time-dependent spectral changes of hair. Dotted lines through the tops of the cross peaks show components A, B, and C of the protein band. Shaded peaks are negative.

in the synchronous correlation map are positive, indicating that the NIR spectral intensities of the water band and band A are either increasing or decreasing together. By taking into account the finding that the spectral intensity of the water band decreases with the treatment time, as shown in Figure 4, it can be said that band A decreases with the treatment time together with the decrease in the water band. As a result, band A is assigned to the NH protein linked by a hydrogen bond to water.

The other two bands cannot be clearly assigned by the data of these measurements, but band B is the most abundant and can be identified as the protein NH group interacting with other protein residues. Finally, band C seems to arise from the protein NH group interacting with the anionic carboxylic group of MA, because band C relates to the strongest hydrogen bond (18).

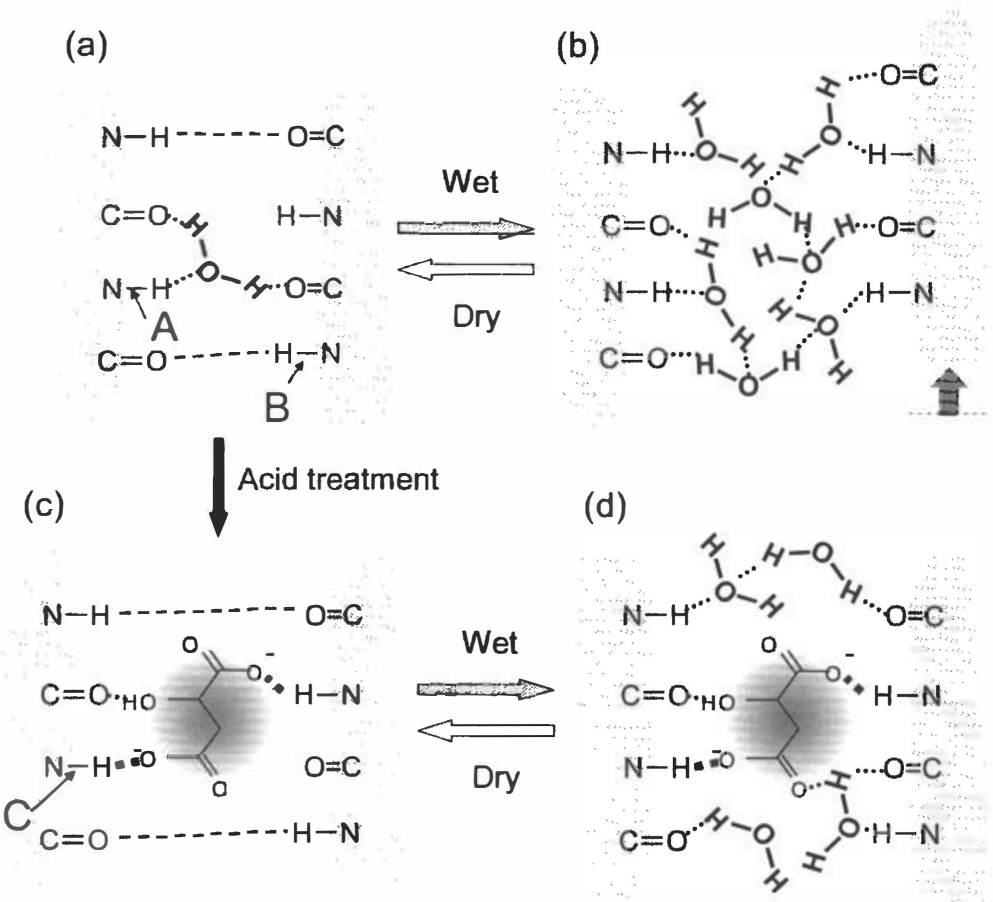

Figure 8. Schema to explain water set of hair and its improvement by treatment with an organic acid. (a, b) Untreated hair in dry and high-humidity conditions, respectively. (c, d) Organic acid treated hair in dry and high-humidity conditions, respectively. Shaded objects represent parts of internal hair proteins. Dotted and dashed lines represent hydrogen bonds. An arrow in (b) means that proteins can move with each other by the breakdown of hydrogen bond linkage between proteins.

A MODEL TO EXPLAIN THE IMPROVEMENT OF HAIR-SET DURABILITY BY ORGANIC ACID

Figure 8 illustrates a scheme to explain how organic acid works to improve hair-set durability. This figure does not represent a specific part of the internal hair structure, but represents the hydrophilic region that contributes to the water set and its relaxation by the permeation of water molecules, followed by the exchange of hydrogen bonds. This part is not identified yet, but may be in the cell membrane complex, endocuticle, or the space between globular keratin-associated proteins in cortical cells.

Figure 8a shows hydrogen bonds of the untreated hair in dry condition as dotted and dashed lines. Even in the dry condition, some water molecules remain and bind to the hair proteins. There are many hydrogen bonds between the proteins, retaining the water set. In high-humidity conditions, water molecules steadily permeate into the hair (Figure 8b). The hydrogen bonds between proteins are then easily replaced by those of

protein–water because they are not so strong. In the case of acid-treated hairs, an organic acid penetrating into the same area occupies the position where water originally existed (Figure 8c). Consequently, the band of water and band A of the protein band in the NIR spectra decrease simultaneously as described above. The anionic carboxyl group of the organic acid makes stronger hydrogen bonds with protein NH than protein–water (A) and protein–protein (B) hydrogen bonds. Even under high humidity, water molecules also permeate, but the strong hydrogen bonds with the organic acid are not easily replaced by water, and they prevent proteins from moving. As a result, the hair shape is maintained.

It is known that treatment with some acids decreases the water uptake of wool (19) and that naphthalenesulfonic acid improves hair-set durability (20). It is seen from Figure 4 that MA/BOE/ethanol treatment also reduces the water uptake of hair. The present NIR results suggest, however, a new, additional, mechanism of set durability improvement in the strengthening of hydrogen bonds by carboxyl groups of acids such as malic acid.

CONCLUSIONS

Hair-set durability against high humidity is improved by treatment with malic acid. From the fact that the improvement was confirmed even for single hair fibers, it is concluded that this improvement is due to internal changes in the hair fiber. This makes natural setting possible, and not due to the common technologies based on adhesion or fixing by oils or polymers.

By the analysis of 2D NIR correlation spectroscopy, the behavior of organic acid was determined. It adsorbs at the site where water originally binds, prevents water penetration, and makes strong and stable hydrogen bonds with hair proteins. The formation of such strong and stable hydrogen bonds suppresses the exchange of hydrogen bonds that is the cause of the breakage of set durability.

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