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A new method to restore the hair crystalline and amorphous structures: Combination treatments of glycine betaine and macromolecular hydrolyzed keratin proteins

ARIMINO

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Introduction:

Objectives

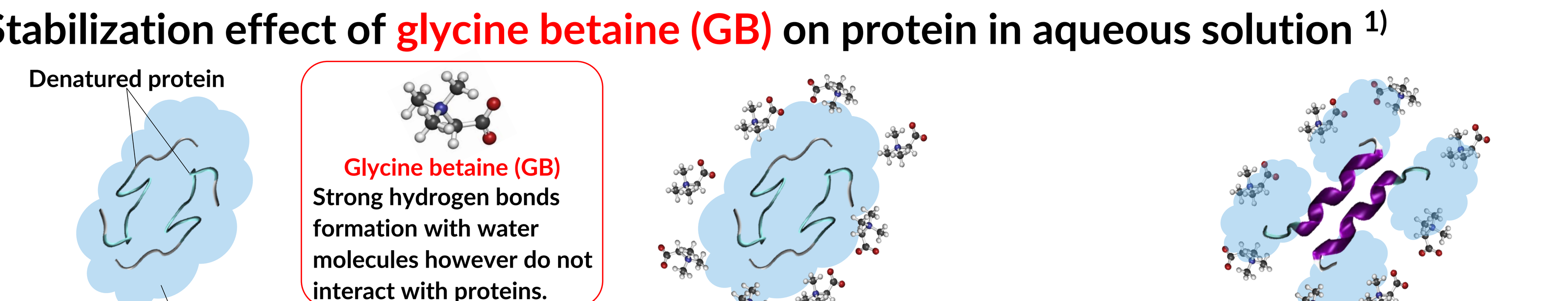
To elucidate how to repair the crystalline and amorphous structures constituting the hair cortex.

<The protein stabillization effect of glycine betaine [1] was applied to an effective reair method>

Backgrounds

- ► Proteins in the hair cortex form crystalline or amorphous conformations, creating flexibility and toughness. Chemical treatments like permanent waving cause damage to the conformations and deterioration of texture.
- ► We reported on a method glycine betaine (GB) used for restoring the crystalline structure denatured [2], however, we continued searching for methods to more efficiency repair the entire cortex structure.
- ► We found some keratin proteins to be a synergistic substance with GB and evaluated them in detail.

Stabilization effect of glycine betaine (GB) on protein in aqueous solution [1]



Denatured proteins lose secondary structures and interact with many water molecules (hydrophilization). ▶▶▶ **GB removes water around proteins. → Thermodynamically destabilized system (water-deprived part).** ▶▶▶ **Eliminating entropic instability by changing protein structure and discharging water. → Change to a compact, hydrophobic structure [≈ Structural Repair]**

Materials & Methods:

Materials

Hair sample: Chemically untreated hair was collected from a Japanese adult woman to make hair bundles of approximately 1.0 g. They were purified with an anionic surfactant solution and named Untreated.

Macromolecular keratin proteins: Intermediate filaments (IF)-derived (INCI name: Keratin; MW 40,000) and **keratin-associated proteins (KAP)** -derived (INCI name: Hydrolyzed Keratin; MW 20,000-40,000).

Preparation of hair samples

A variety of samples were prepared from permanent waving treatment followed by immersing in an aqueous solution containing GB and/or macromolecular keratin protein as a hair structural repair agent (see table below).

Samples	Permanent waving treatment a)	Single or combinatorial treatments: Single / First Step: GB b)	Single / First Step: Macromolecular keratin protein c)	Second Step: GB b)	Second Step: Macromolecular keratin protein c)
Untreated	—	—	—	—	—
Permed	✓	—	—	—	—
GB	✓	✓	—	—	—
Ifp	✓	—	✓ (IF -derived)	—	—
Kap	✓	—	✓ (KAP-derived)	—	—
Ifp-GB	✓	—	✓ (IF -derived)	✓	—
Kap-GB	✓	—	✓ (KAP-derived)	✓	—
GB-Ifp	✓	✓	—	—	✓ (IF -derived)
GB-Kap	✓	✓	—	—	✓ (KAP-derived)

a) Permanent waving treatment that consists of reduction and oxidation steps, was performed once as follows.
Reduction step: The Untreated were immersed in a 0.75 M of ammonium thioglycolate solution (pH 9.25) at 35 °C for 15 min, washed with water for 3 min, and then towel-dried. Oxidation step: reduced hair was immersed in a 6 %(w/w) sodium bromate solution(pH 6.0) at 35 °C for 15 min, washed for 3 min, and air-dried.
b) The hair bundle was immersed in a 60 %(w/w) solution of GB (pH 8.0) at 35 °C for 30 min*.
c) The hair bundle was immersed in a 1 %(w/w) solution of IF- or KAP-derived macromolecular keratin protein at 35 °C for 30 min*.
*When the single treatment or the second step of the combinatorial treatment, the hair bundles immersed were washed with water for 3 min , and then air-dried.

1. Exploration of the crystalline and amorphous structural repair methods

From early exploration, we used **the tensile testing in-water** (20 °C) or **in-air** (20 °C, 60 %RH) as evaluation criteria for screening the combination of GB and various ingredients and treatment conditions.
The 15 % elongation stress in water approximates the characteristic value upon elongation of **IF** [3]. The tensile stress of **IF** components are almost constant both in-water and in-air [4]. Therefore, we considered comparing the 15 % tensile stress in the water and the air makes it possible to infer the effect of each treatment on **KAP**.

2. Interaction between glycine betaine and macromolecular keratin proteins

We analyzed the secondary structure changes when GB and macromolecular keratin proteins were mixed by **circular dichroism spectroscopy** (25 °C, in distilled water). Macromolecular keratin proteins derived from **IF** or **KAP** showed peak patterns of α-helix or random-coil, respectively.

3. Verification of the effect of combinatorial treatments on hair cortex structure

3.1 High-Pressure Differential Scanning Calorimetry (HPDSC)

We analyzed shredded hair samples containing excess water in sealed capsules to retain moisture during heating. In this method, the endothermic peak at 130 °C ∼160 °C is attributed to the denaturation enthalpy ΔH, depending on the structural integrity of the α-helix segment in the **IF** [5]. The denaturation peak temperature T_p is thought to be controlled by the crosslinking density of **KAP** surrounding the **IF** [6].

3.2 Synchrotron Small-Angle X-Ray Scattering (SAXS)

Based on the **IF** distribution model, the shapes of the first peak on the equator at q = 0.7 nm^{-1} provide information on the regular structure of the **IF** in the fiber axis [7]. In addition, the scattering intensities that were integrated along the azimuthal angle at the position of the first peak (q = 0.7 nm^{-1}) from the two-dimensional SAXS patterns. The full width at half maximum ***W*** of each orientation peak reflects the mean **IF** inclination to the hair fiber axis, and the orientation degree ***F*** (%) was calculated as follows [8].

$$F\ [\%] = (360 - \Sigma W) / 360 \times 100$$

References:

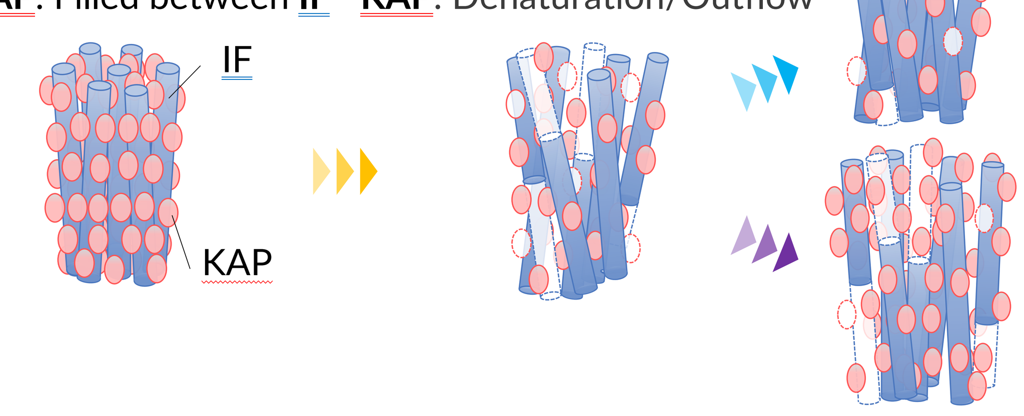
1) Arakawa, T., Timasheff, S. N. (1985) *Biophysical Journal*, 47:411-414.
2) Togashi, T. (2021) *Fragrance Journal*, 49:23-28.
3) Feughelman, M., Robinson, M. S. (1971) *Textile Research Journal*, 41:469-474.
4) Wortmann, F-J., Stapels, M. et al., (2006) *Biopolymer*, 81:371-375.
5) Feughelman, M., Mitchell, T. W. (1966) *Textile Research Journal*, 36: 578–579.
6) Wortmann, F-J., Sendelbach, G. et al., (2007) *Journal of Cosmetic Science*, 58:311-317.
7) Briki, F., Busson, B. et al., (1998) *Biochimica et Biophysica Acta*, 1429:57-68.
8) Kajiura, Y., Watanabe, S. et al., (2006) *Journal of Structural Biology*, 155:438-444.

Conclusions:

Combinatorial treatments of glycine betaine with IF- or KAP-derived macromolecular keratin protein showed high repair effects on crystalline and amorphous hair cortex structure.

↑

1. **IF**-derived **protein** showed synergistic recovery of tensile stress in water and **KAP**-derived **protein** in air.
2. The secondary structure of **macromolecular keratin proteins** was regenerated by mixing with **GB**, which may have improved permeability and fixation and showed synergistic stress recovery effects.
3. Combination treatment with **IF**-derived **protein** showed high crystalline structure recovery (**HPDSC**) and restored crystal regularity (**SAXS**). While combination treatment with **KAP**-derived **protein** promoted **IF** orientation (**SAXS**).



GB-Ifp; The Interaction between **GB** and **IF**-derived **protein**, which occurs near or inside the Permed, effectively restored **IF** crystallinity.
⇒ Restoration of **IF** regular structure

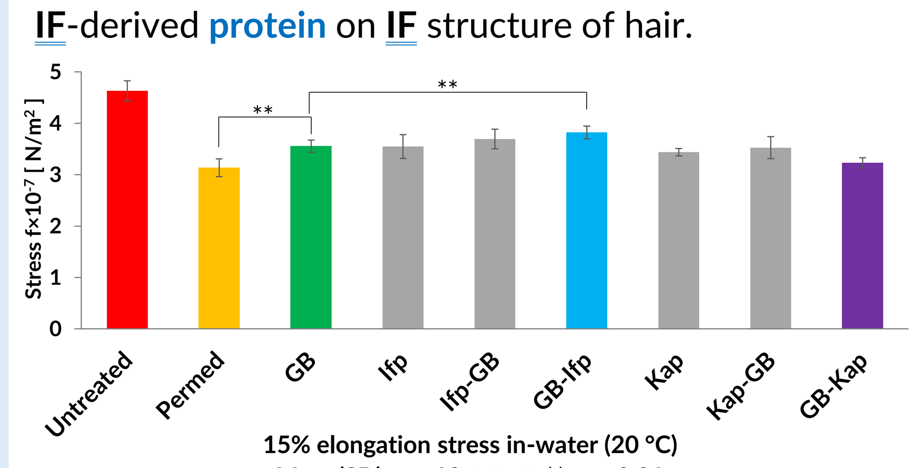
GB-Kap; The Interaction between **GB** and **KAP**-derived **protein** , which occurs near or inside the Permed, effectively fills in between **IF**.
⇒ Restoration of the **IF** orientation

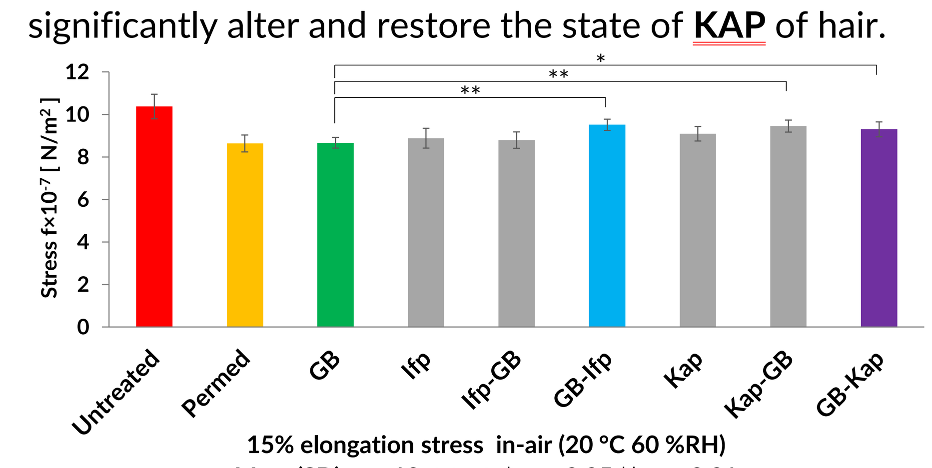
Results & Discussion:

1. Exploration of the crystalline and amorphous structural repair methods

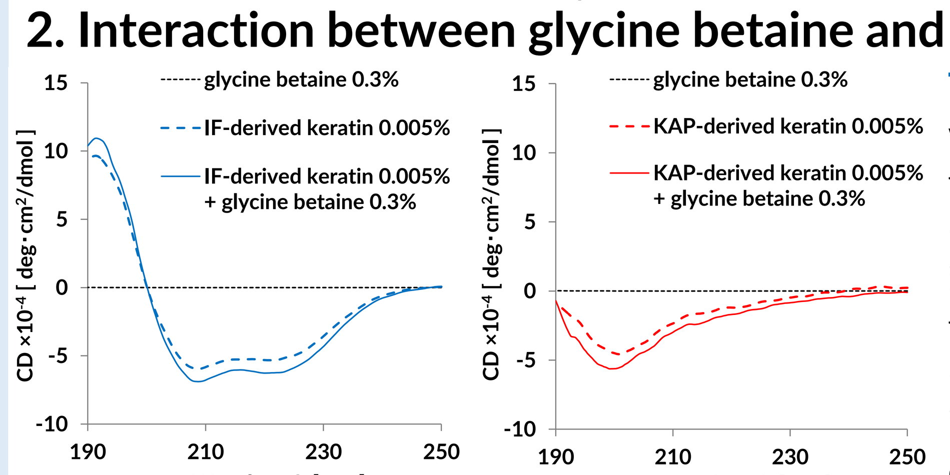
The GB-Ifp restored elongation stress significantly more than the GB.
→ We inferred a synergistic repair effect of GB and **IF**-derived **protein** on **IF** structure of hair.

The GB-Kap and **the Kap-GB** showed significant stress recovery not seen in the water.
→ The combination of GB and **KAP**-derived **protein** can significantly alter and restore the state of **KAP** of hair.





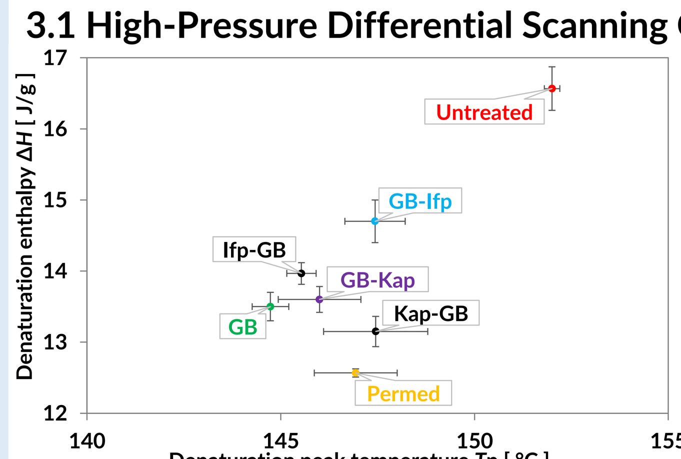
2. Interaction between glycine betaine and macromolecular keratin proteins



The molecular keratin proteins derived from **IF** or **KAP** were regenerated into a compact secondary structure through interaction with **GB**.
→ The regeneration must promote the penetration into the hair and its affinity for the cortex, therefore, the synergistic restoration of tensile stress will occur.
→ The regeneration that occurs around the hair will be important for hair structural repair because no repair effect was observed with pre-mix (data not shown).

3. Verification of the effect of combinatorial treatments on hair cortex structure

3.1 High-Pressure Differential Scanning Calorimetry (HPDSC)

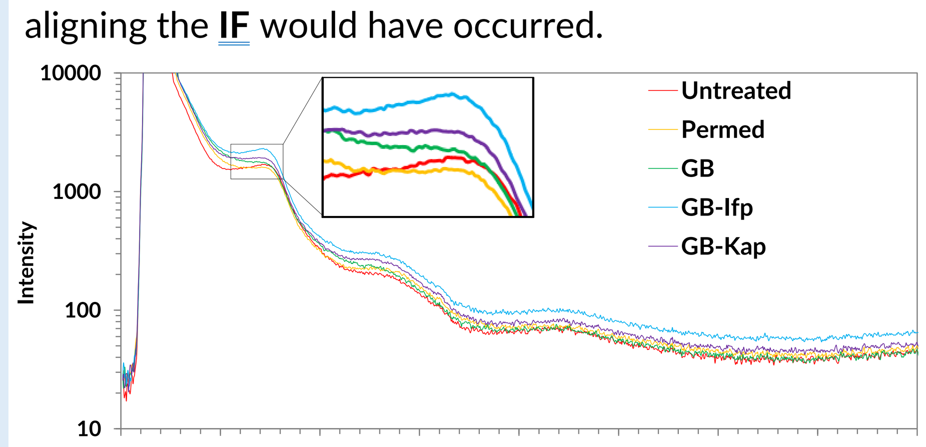


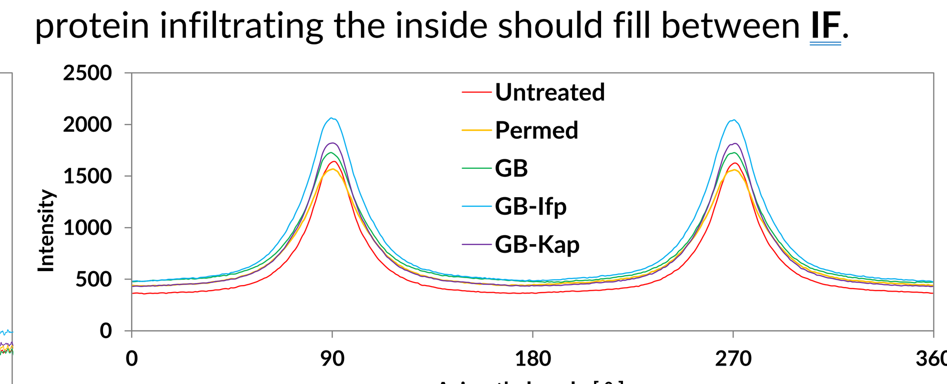
The GB-Ifp and **the Ifp-GB**; ΔH and T_P were elevated than **the GB**. The combination treatments appear to greatly restore crystal abundance and crystallinity, that melt at relatively high temperatures.
The GB-Kap and **the Kap-GB**; T_P increased, but the difference from **the GB** was not large. This may be because the HPDSC is in a state of excess water and the combination treatments do not repair the cross-links. However, since the combination treatments have the effect of increasing T_P, the effect on **IF** structure was evaluated by SAXS.

3.2 Synchrotron Small-Angle X-Ray Scattering (SAXS)

The Permed: The first peak became unclear shoulder peak. This must be a disturbance in the **IF** interval.
The GB-Ifp: The first peak was clearly restored, suggesting the regular structure of the **IF** was restored.
The GB-Kap: A slight peak shape recovered and aligning the **IF** would have occurred.

The Permed: The orientation degree ***F*** was reduced, and the **IF** was misaligned with the fiber axis.
The GB-Ifp: The ***F*** was clearly restored because the original crystalline structure of **IF** was recovered.
The GB-Kap: The ***F*** was most restored. Because the protein infiltrating the inside should fill between **IF**.





Hair sample	Untreated	Permed	GB	GB-Ifp	GB-Kap
F [%]	85.1	82.5	83.2	84.1	84.5

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