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Combined proteomics and structural analyses of hair keratins for early detection of hair shafts damage/protection

424

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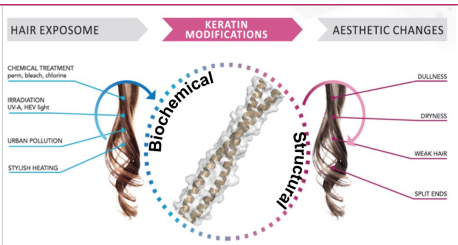
Introduction:

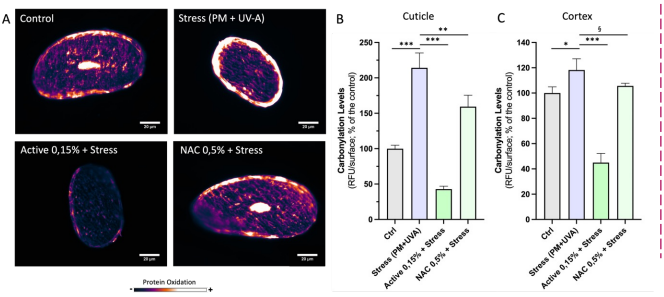
Hair damage induced by external aggressions, such as UV irradiation, fine dust pollution, heat-styling tools, chemical treatments, are tradionnaly assessed by spectroscopic and microscopic imaging or hair physical characteristics tests (shine, strength, suppleness, etc.).
However, these approches are not suitable to evaluate hair damage at daily equivalent stress doses precluding the evaluation of the efficacy of novel products or active compounds in conditions that are closed to consumer's uses. Protein carbonylation, a harmful oxidative protein modification, is considered as a major hallmark and a reliable biomarker of oxidative stress.
Being the principal components of hair shafts (65% to 95%), proteins are a primary target of oxidative damage upon stress, explaining, at least in part, the molecular basis of the chemical damage to both the hair and scalp.
The integration of results from the molecular and structural methods of analysis leads to a 360° vision of early damages on hair provoke by daily life stressor.



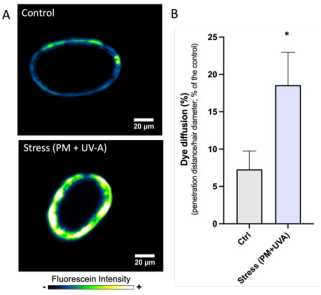
Results & Discussion:

Specific *ex-vivo* experimental models of daily above mentionned stress, reproducing closed to real life exposures, have been developped using hair tresses combined to proteomics approaches for **carbonylated (oxidized) proteins visualization & quantification (on the left)** and biophysical method for **hair structural integrity & porosity analysis (on the right).**

Hair fibers carbonylation by anti-oxidants



Hair fibers structural integrity



The *in situ* carbonylation levels on hair cross sections are visualized as a color range (from dark color for low levels to bright colors for high levels of carbonylation). The presence of the stress (PM+UV-A) induced significant increase of carbonylation in both cortex and cuticle regions. The presence of NiBDPP (Active 0.15%) or N-acetyl cysteine (NAC 0.5%) preserved hair fibers from stress-induced carbonylation. A significant dose-dependent increase in protein oxidation (carbonylation), related to both keratins, and keratins associated proteins, was observed upon exposures of hair to stress factors (*data not shown*).
The stress exposure induced a significant increase of protein oxidation in cortex and cuticle regions. However, protein carbonylation was prevented in the presence of anti-oxidant compounds.

Due diffusion is presented as a color range of fluorescent. The diffusion of the dye within the hair fiber is presented here as % of Permeability and results from the dye penetration distance along the long axis of hair fiber cross section.
The stress exposure induced a significant increase of the permeability of the dye due to a damage in hair structural integrity.

Conclusions:

Taking together these results lead to a new generations of high-sensitivity and wide-range tools for the assessment of hair protection from daily equivalent stress-induced damages. The protection of hair proteins from carbonylation could be an efficient approach for hair protection against airborne pollutants and UV radiation.

Materials & Methods:

Hair shafts & stress exposures.
For the exposure to airborne pollutants followed by UV radiation, hair strands were gently cut into small pieces (1 cm) and Particulate Matter HAPs from European Reference certified Material (CZ100) were applied (150 µg/cm2) followed by immediate exposure to UV radiation 84 J/cm2 as described above.
Carbonylated proteins assessments.
In situ detection of carbonylated proteins: Cross-section of hair fibers (perpendicular of hair axis) of 5µm were obtained (LEICA Crystat). Carbonylated proteins were labeled in situ on hair strands with a specific fluorescent probe. Hair images collected by Epi-Fluorescence Microscopy were integrated to obtain the carbonylation levels (intensity R.F.U. over surface) on the cuticle region or cortex.
Hair fibers permeation studies – Fuorscein diffusion.
Hair fibers were incubated in a 0.1% aqueous fluorescein solution (pH 5,6) at 37°C for 3 h, then rinsed in ultra-pure water and prepared for analysis. Cross-section of hair fibers of 5µm were obtained (LEICA Crystat). Hair images were collected by Epi-Fluorescence Microscopy (Ex = 498 nm / Em = 517 nm) and integrated. The diffusion of the dye was evaluated reporting a % of diffusion value, obtained from the dye diffusion distances along the diameter (on the long axis) of hair fiber cross section.
Hair fibers anti-oxidant treatments.
Hair strands were incubated into a solution of 0.5% of N-acetyl-cysteine or 0.15% of Nickel Bis (Hydroxy Diphenyl Methyl Pyrrolidino Methyl) Pyridinediyl T-Butylisocyano Perchlorate.

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