

- cell-mediated immunity. *Annu Rev Immunol* 16:111–135
- Hertl M, Veldman C (2003) T-cellular autoimmunity against desmogleins in pemphigus, an autoantibody-mediated bullous disorder of the skin. *Autoimmun Rev* 2: 278–283
- Sayegh MH, Turka LA (1998) The role of T-cell costimulatory activation pathways in transplant rejection. *N Engl J Med* 338: 1813–1821
- Stanley JR, Yaar M, Hawley-Nelson P, Katz SI (1982) Pemphigus antibodies identify a cell surface glycoprotein synthesized by human and mouse keratinocytes. *J Clin Invest* 70:281–288
- Veldman C, Hohne A, Dieckmann D, Schuler G, Hertl M (2004) Type 1 regulatory T cells specific for desmoglein 3 are more frequently detected in healthy individuals than in patients with pemphigus vulgaris. *J Immunol* 172:6468–6475
- von Herrath MG, Harrison LC (2003) Antigen-induced regulatory T cells in autoimmunity. *Nat Rev Immunol* 3:223–232
- Waldmann H (2002) Reprogramming the immune system. *Immunol Rev* 185:227–235

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UVA-Irradiated Pheomelanin Alters the Structure of Catalase and Decreases Its Activity in Human Skin

John M. Wood¹ and Karin U. Schallreuter^{1,2}

More than 40 years ago Aronoff showed that catalase structure and activity is seriously affected by photo-oxidation of its own substrate, hydrogen peroxide, owing to cleavage of its porphyrin active site. Here we support the results of Maresca *et al.* (in this issue) and expand on them by using structural modeling of native catalase and its photo-oxidation product, where both methionine and tryptophan residues are oxidized, yielding a deactivated enzyme.

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In this issue of the *Journal of Investigative Dermatology*, Maresca and co-workers show that both the charge and the activity of epidermal catalase are severely affected by reactive oxygen species (ROS) generated by UVA-irradiated pheomelanin photochemistry (Maresca *et al.*, 2006, this issue). This work supports and expands on earlier research from Aronoff, who was the first to demonstrate that the porphyrin ring of the active center of catalase is photo-oxidized by UVR (Aronoff, 1965). Almost two decades later, Menon and colleagues showed that UV as well as visible light, together with pheomelanin, produced sufficient ROS, which in turn caused cytotoxicity of Ehrlich ascites carcinoma cells (Menon *et al.*, 1983).

Nowadays the production of short-lived singlet oxygen and its conversion to hydroxyl radicals by the photo-oxidation of pheomelanin or by porphyrin are well established (Menon *et al.*, 1983; Nordberg and Arner, 2001). The question arises: how do these ROS affect protein structures?

In this context, the two amino acids methionine and tryptophan in protein sequences are targets for oxidation, consequently leading to structural alterations and thus deactivation of proteins (for review see Schallreuter, 2005). Methionine is more vulnerable to oxidation by ROS than tryptophan; it forms two diastereomers of methionine sulfide (R and S), whereas tryptophan is converted to 5-OH-tryptophan (Neiers *et*

al., 2004). It has been shown that several important enzymes are deactivated by hydrogen peroxide because of oxidation of those two amino acid residues in their sequences, including pterin 4a-carbinolamine dehydratase (EC 4.2.1.96) and dihydropteridine reductase (EC 1.6.99.7), both involved in the recycling of the essential cofactor (6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (6BH₄), as well as acetylcholine esterase (EC 3.1.1.7) (for review see Schallreuter, 2005).

Maresca *et al.* identified 5-OH-tryptophan and methionine sulfoxide as oxidation products of UVA/pheomelanin photo-oxidation in the sequence of catalase by applying fluorescence spectroscopy and micro-Raman spectroscopy. The chosen reference standard for methionine sulfoxide has two peaks (see Figure 7 in Maresca *et al.*, 2006), indicating that this standard must be a mixture of two compounds. One would suspect that the standard is a mixture of methionine sulfoxide and methionine sulfone, but this assumption should be confirmed by amino acid analysis (Hasse *et al.*, 2004). Moreover, it is surprising that the porphyrin ring of catalase is not affected under the experimental conditions chosen.

Computer-assisted analysis of the catalase protein structure using HyperChem (Hyperchem Inc., Gainesville, FL, USA) and DeepView (Swiss Institute of Bioinformatics, Lausanne) indicates a significant change in the three-dimensional structure of catalase after oxidation of the eight methionine (at positions 61, 181, 212, 284, 339, 350, 392, and 394) and five tryptophan residues (at positions 143, 183, 186, 227, and 303) in the sequence of each subunit. Taking into consideration that each methionine and each tryptophan gain one oxygen atom upon oxidation, it can be calculated that the molecular weight of one oxidized catalase subunit would increase by 208 daltons. As the fully active enzyme is assembled by four subunits, the net gain would give a molecular weight of 1.232 kilodaltons, consequently changing the tertiary structure with partial deletion of some α -helices and some β -pleated sheets, which in turn would alter the isoelectric point, explaining the results of the Western blot in the report by Maresca *et*

¹Clinical and Experimental Dermatology/Department of Biomedical Sciences, University of Bradford, Bradford, UK; and ²Institute for Pigmentary Disorders in Association with the E.M. Arndt University, Greifswald, Germany, and the University of Bradford, Bradford, UK

Correspondence: Professor John M. Wood, Clinical and Experimental Dermatology/Department of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire BD7 1DP, UK.
Email: J.M.Wood@bradford.ac.uk

al. Moreover, this oxidation also leads to deactivation of the enzyme because of the structural changes in the active site, explaining the decreased enzyme activity observed by Ogawa *et al.* (2006; this issue). Methionine sulfoxide residues are repaired by reduction back to methionine by two complementary methionine sulfoxide reductases (MSRA and MSRB), which have been shown recently to be present in the human epidermis (Ogawa *et al.*, 2006; Schallreuter, 2006). At this point it would be useful to know whether catalase activities and protein expression or the repair enzymes differ in fair and dark skin. This is certainly the case for other antioxidant enzymes, such as thioredoxin reductase, which reduces hydrogen peroxide to water (Schallreuter and Wood, 2001). Taking the above results together, it is not surprising that, after excessive UVA exposure, catalase is deactivated, especially in fair-skinned/blond/red-headed phenotypes, because of the presence of pheomelanin, which itself generates ROS. However, it should be recognized that catalase represents the first line of defense against hydrogen peroxide-mediated damage in all organs and tissues. The importance of this enzyme was just recently highlighted when the overexpression of

mitochondrial catalase in the murine model increased the lifespan of the mice by 40%; this emphasizes the involvement of the enzyme in the aging process (Schriner *et al.*, 2005). Decreased levels, activity, and protein expression of epidermal catalase have indeed been extensively documented in patients with acute vitiligo, in whom it was shown that hydrogen peroxide levels in the millimolar range lead to deactivation and low protein expression of this enzyme (Schallreuter *et al.*, 1991; Schallreuter, 2005). However, it is important to recognize that ROS can cause both inactivation/deactivation and activation of enzymes, depending on their concentration (for review see Schallreuter, 2005).

CONFLICT OF INTEREST

The author states no conflict of interest.

REFERENCES

Aronoff S (1965) Catalase: kinetics of photooxidation. *Science* 150:72–73

Hasse S, Gibbons NC, Rokos H, Marles LK, Schallreuter KU (2004) Perturbed 6-tetrahydrobiopterin recycling via decreased dihydropteridine reductase in vitiligo: more evidence for H₂O₂ stress. *J Invest Dermatol* 122:307–313

Maresca V, Flori E, Briganti S, Camera E, Cario-André M, Taïeb A, *et al.* (2006) UVA-induced modification of catalase charge properties in the epidermis is correlated with the skin phototype.

J Invest Dermatol 126:32–41

Menon IA, Persad S, Ranadive NS, Haberman HF (1983) Effects of ultraviolet-visible irradiation in the presence of melanin isolated from human black or red hair upon Ehrlich ascites carcinoma cells. *Cancer Res* 43:3165–3169

Neiers F, Kriznik A, Boschi-Muller S, Branlant G (2004) Evidence for a new sub-class of methionine sulfoxide reductases B with an alternative thioredoxin recognition signature. *J Biol Chem* 279:42462–42468

Nordberg J, Arner ES (2001) Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radic Biol Med* 31:1287–1312

Ogawa S, Sander CS, Hansel A, *et al.* (2006) The repair enzyme peptide methionine-S-sulfoxide reductase is expressed in human epidermis and upregulated by UVA-radiation. *J Invest Dermatol* 126 (in press)

Schallreuter KU (2005) Vitiligo In: Hertl M (ed). *Autoimmune Diseases of the Skin: Pathogenesis, Diagnosis, Management*. Springer: Vienna, 367–384

Schallreuter KU (2006) Functioning methionine sulfoxide reductase A and B are present in human skin. *J Invest Dermatol* (in press)

Schallreuter KU, Wood JM (2001) Thioredoxin reductase: its role in epidermal redox status. *J Photochem Photobiol B* 64:179–184

Schallreuter KU, Wood JM, Berger J (1991) Low catalase levels in the epidermis of patients with vitiligo. *J Invest Dermatol* 97:1081–1085

Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, *et al.* (2005) Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308:1909–1911