Protein Carbonylation and Decarbonylation
A New Twist to the Complex Response of Vascular Cells to Oxidative Stress

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Although the Greek physician Galenos (129 to 216 AD) recognized early that ventilation of the lungs is important for the transfer of an unknown substance from the air into the blood, it took surprisingly long to discover that oxygen is a major “fuel” for our metabolism that oxidizes nutrients to generate chemical energy. Even William Harvey in his famous work about the circulatory system published in 1628 thought that respiration was necessary solely for cooling down the blood, ie, preventing it from burning. He explicitly disputed that the lungs are responsible for the transport of “spirit,” a hypothetical substance thought to be essential for life. Not until 1788, shortly before his execution in the turmoil of the French revolution, the chemist Antoine Laurent de Lavoisier (who had discovered oxygen some 10 years before) stated “... that is, respiratory gas exchange is a combustion, like that of a candle burning”.2,3

However, apart from truly being the spirit of our life, oxygen is a rather harmful and dangerous compound that may be illustrated best by the fact that chemical reactions in which electrons are captured from a molecule are referred to as oxidations. In our body, biological macromolecules are effectively shielded from oxygen and oxygen-derived radicals by a multitude of both specific and nonspecific protective mechanisms, including uric acid, glutathione, the thioredoxins, or enzymes, including the superoxide dismutases or catalase, to name a few.4

Surprisingly, it was only 20 years ago or so when it was realized that molecular oxygen not only reacts with cellular components but also gives rise to the formation of reactive oxygen species (ROS), acting both as powerful defensive arms against invading microorganisms and as intercellular and intracellular signaling molecules. Most prominent among these ROS is the superoxide anion, generated by the growing list of NADPH oxidase isoenzymes, and its primary enzymatic or nonenzymatic dismutation product, hydrogen peroxide. Both molecules, either directly or indirectly, are capable of altering proteins chemically, thus influencing their function. Today, many signal transduction pathways have been characterized that operate at least in part through enzymatic ROS formation and consecutive protein modification, thereby eliciting changes in gene expression, cell migration, and proliferation.5

The main protein modifications originating from such an increase in oxidative stress comprise direct oxidation, namely that of amino acids with a thiol group, such as cysteine, oxidative glycation, and carbonylation. In this context, it is remarkable that oxidative protein carbonylation, apparently the most frequent type of protein modification in response to oxidative stress,6 is thought to be irreversible and destined only to induce protein degradation in a nonspecific manner.7 Chemically, oxidative carbonylation preferentially occurs at the amino acids proline, threonine, lysine, and arginine, presumably through a metal-catalyzed activation of hydrogen peroxide to a reactive intermediate. At least in the case of the basic amino acids arginine and lysine (and possibly histidine), this is not a simple oxidation reaction because carbonylation of these amino acids is accompanied by the loss of 1 or more nitrogen atoms similar to Schiff base reactions.

In this regard, the work of Wong et al8 provides the first insight into this putative novel signal transduction mechanism in mammalian cells. By using a systematic proteomics approach in rat pulmonary vascular smooth muscle cells, the authors convincingly show that the potent vasoconstrictor peptide endothelin (ET)-1, in a receptor-dependent manner, elicits the carbonylation of several distinct target proteins in these cells. Chiefly on the basis of these protein modifications, Wong et al propose the existence of this new redox-sensitive signaling pathway. The most stable, and hence least reactive ROS generated by ET-1 in vascular cells, is hydrogen peroxide. However, hydrogen peroxide may be reduced by metal ions to yield the highly reactive hydroxyl radical, and this is not only the basis for the toxic effects of peroxides but also has been shown to contribute to enzyme inactivation. In their article, Wong et al suggest such a Fenton-like reduction to be responsible also for the protein carbonylation observed by them. This modification, though, must not necessarily lead to inactivation or disintegration of the protein. Thus, a signaling pathway that contrariwise results in the activation of a protein through carbonylation has been described for the first time recently in bacteria. Here, the transcription factor PerR is only activated after carbonylation.9 According to Wong et al, they have discovered a similar mechanism of protein carbonylation in their rat vascular smooth muscle cells and, despite a rather vague perception of the functional consequences thereof, propose that this pathway may play an important role in pulmonary hypertension. What is the evidence?

The set of proteins that were carbonylated on exposure of the pulmonary vascular smooth muscle cells to ET-1 included antioxidative and protective factors such as peroxiredoxin-6 or...
DJ-1 but also proapoptotic factors like annexin A1. Most intriguingly and contrary to present concepts, ET-1 in parallel induced a fast and efficient decarbonylation of a few carbonylated proteins through activation of the thioredoxin pathway, whereas others remained carbonylated. Moreover, this different stability of the protein modification had rather clear-cut functional consequences: whereas stably carbonylated proteins such as annexin A1 were subjected to rapid proteasome-mediated degradation, decarbonylated proteins such as peroxiredoxin-6 seemed to be protected. Therefore, the different effects of ET-1 on the structure of these proteins may have effectively protected the smooth muscle cells from apoptosis. In their article, Wong et al further suggest that this particular ET-1–mediated redox signaling pathway may play a causal role in the typical phenotypic changes of smooth muscle cells in pulmonary arteries and arterioles in developing pulmonary hypertension.

Thus far, the data presented by Wong et al, which are schematically summarized in the Figure, without a doubt provide a compelling story. However, despite the evidence for the concept of the authors, several questions remain unanswered or have not yet been addressed. Given the limited space in the journal and the amount of additional work needed for this purpose, clearly the whole story cannot unfold in a single article. Apart from issues related directly to the work of Wong et al (such as the relevance of an ET-1–mediated generation of ROS in the lung, where there is most likely already a high exogenous exposure to these molecules or a more detailed analysis of the fate of the various decarbonylated proteins), there are more fundamental questions to be asked focusing, for example, on the potential consequences of protein decarbonylation. Below is an incomprehensive list of such questions that may not only provide Wong et al but also other researchers in the field with a strong incentive to further investigate this new aspect of redox signaling.

- What is the exact chemistry of the decarbonylation reaction? Are the decarbonylated amino acids simply restored or modified further?
- What is the nature of the reductive enzymatic system catalyzing this reaction? Thioredoxin-dependent reduction of oxidized cysteines by peroxiredoxins is well characterized. Because intracellular decarbonylation also seems to be thioredoxin-dependent, it will be of great interest to search for enzymes that are capable of reducing and hence possibly restoring carbonylated amino acids.
- Possibly the most interesting point to be studied is the fate of the decarbonylated proteins: Is their activity restored by regeneration of the oxidized amino acid side chains? Given the nature of amino acid modifications (see above), this would be rather surprising. Do they become inactivated and finally end up in the proteasome as well? Furthermore, in view of the example from bacteria, is redox signaling through oxidative carboxylation a pathway that alters protein function only quantitatively or even qualitatively?

In summary, the article by Wong et al demonstrates for the first time that mammalian vascular smooth muscle cells make use of protein carbonylation as a means of signal transduction, thus providing an intriguing new perspective to the study of redox signaling in the vascular system. Even though, naturally, more questions remain than answers, the article provides an interesting, perhaps controversial but nonetheless inspiring, reading.

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**References**

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