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Bleach in the Diabetic Kidney Destabilizes Basement Membrane Collagen



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Bleach, or hypochlorous acid (HOCl), is a very old chemical that is widely used as a decolorizing and disinfectant agent. The addition of a few drops of bleach to a cashmere sweater not only decolorizes the stain within 24 h at room temperature but also oxidizes the wool protein itself, such that the fine meshwork is replaced by a hole within a few days (Fig. 1, left panel). The chemistry behind this phenomenon begins with a reaction between chlorine (or bromine) gas and water, leading to the powerful oxidizing agent, HOCl. At physiological pH, HOCl is in equilibrium with hypochlorite anion (ClO^-), and both chemical species are oxidizing agents, with HOCl being much more potent than ClO^- . Cysteine and methionine residues are attacked first, followed by slower reactions with lysine, tryptophan, terminal amines, and histidine. Other targets include backbone amides, arginine, tyrosine, and other residues or even DNA (1). Products of these reactions include chlorinated compounds, such as 3-chlorotyrosine, 2-chlorotryptophan, or chlorinated lysine residues, as well as hydroxylated compounds such as 3-hydroxyphenylalanine and hydroxytryptophan. Importantly, some of these modifications are labile and eventually form fully oxidized residues such as 2-aminoadipic acid, N-formylkynurenine, or kynurenine. Thus, many of the oxidized amino acids are nonspecific advanced oxidation products of proteins (AOPPs) (2). Unless one is able to detect the chlorinated or brominated intermediate species, identifying the mechanism and source of the oxidizing agent is difficult.

The importance of bleach in biology has been known for almost 50 years, starting with the discovery of myeloperoxidase (MPO), an enzyme that uses H_2O_2 to oxidize chloride, yielding the potent bactericidal agent, HOCl (3). The mechanism of oxidation proceeds via a highly oxidizing Fe(IV)-oxo complex (Fig. 1, right panel). The discovery of MPO in high amounts in leukocytes, monocytes, macrophages, and microglia has triggered many studies of its role in inflammation, not only in classical types of septic inflammation but more so in the broader concept of inflammation. Thus, MPO is implicated in diseases such as atherosclerosis,

arthritis, neurodegeneration, and even kidney disease (4,5). For oxidation to occur, H_2O_2 is needed. In diabetes, the H_2O_2 comes from mitochondria or NADPH oxidase, as well as from plasma amine oxidase and xanthine oxidase.

While some oxidative protein damage in diabetes is associated with transition metal catalyzed oxidation (6) and MPO (7), Brown et al. (8) now propose a novel additional mechanism for the renal glomerular basement membrane. This mechanism involves bleach, H_2O_2 , and VPO-1/peroxidase, an enzyme that was discovered in *Drosophila* just 10 years ago (9). The current proposal is the most recent of a series of elegant studies from the Vanderbilt University Medical Center's Center for Matrix Biology headed by Billy Hudson, which started with the identification of the novel and intriguing sulfilimine cross-link between methionine and hydroxylysine in the NC1 hexamer domain of type IV collagen (10). Early mechanistic studies revealed that sulfilimine synthesis required Cl^- or Br^- and H_2O_2 (i.e., a mechanism reminiscent of MPO). This triggered the question of whether collateral damage to other amino acids of the NC1 hexamer complex occurs in the diabetic kidney.

To investigate the potential impact of hypochlorous acids on renal basement membrane collagen in diabetes, Brown et al. (8) first probed diabetic rat tissue sections with an antibody specific for chlorinated or brominated proteins. They found an increase in the levels of halogenated proteins in the renal glomerular and tubular regions in two mouse models of type 2 and type 1 diabetes, respectively. They also found that binding of $\alpha\beta 1$ integrin to HOCl-treated or native diabetic type IV collagen was impaired in a dose-dependent manner, suggesting that damage to integrin binding sites of collagen that include tyrosine, arginine, and phenylalanine residues had occurred. They then carried out extensive proteomic studies to clarify the precise sites and types of damage to type IV collagen NC1 hexamers from diabetic mice or NC1 hexamers treated *in vitro* with HOCl. Strikingly, they found two major targets of specific damage ("hot spots"): chlorination and

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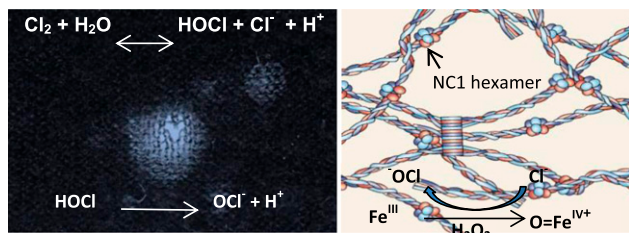


Figure 1—Effect of bleach (hypochlorous acid) on the author's cashmere sweater (left) as a model for type IV collagen from renal glomerular basement membrane (right, reproduced with permission from Kalluri [12]). Application of a drop of bleach destroyed the fine cashmere wool proteins fibers within 24 h, and a hole in the network developed within 4 days. In vivo, bleach formation requires an enzyme bound Fe(IV)-oxo complex, H_2O_2 , and halogen ions. The NC1 hexamer, which is key to the assembly of the type IV collagen network, is proposed to be a major target of bleach, perhaps produced by peroxidase in the diabetic kidney.

oxidation of tryptophan W^{192} in the $\alpha 1\text{NC1}$ domain and W^{28} in the $\alpha 2\text{NC1}$ domain. These modifications were increased two- to fivefold in the diabetic animals compared with controls, affecting up to 20 molar % of tryptophan residues. Importantly, the HOCl-treated NC1 hexamers became sensitized to proteolysis. Sophisticated molecular modeling studies revealed structural perturbations at domain interface regions critical for NC1 hexamer assembly and ultimately basement membrane stability. Overall, damage by oxidation was found to be more important than damage due to chlorination.

These exciting studies raise a number of questions as to whether hypochlorous acid is generated by peroxidase or MPO and the relative contribution of these enzymes to NC1 damage in human diabetic nephropathy. First, Brown et al. (8) found a bromotyrosine residue in the NC1 domain, compatible with peroxidase activity. Nevertheless, HOCl and hypobromous acid can also be produced by MPO. Surprisingly, no chlorotyrosine was detected, in contrast to its well-documented presence in atherosclerotic lesions (11). Clarification of the major culprit might be difficult but is important. Perhaps treatment of diabetic rats with specific inhibitors of MPO or peroxidase could help identify the key enzyme. Second, no detailed data on the human kidney were presented, except for preliminary evidence of tubular immunoreactivity to

chlorinated proteins and chlorinated tryptophan W^{192} in $\alpha 1\text{NC1}$ from a nondiabetic human kidney. Presumably a large number of human diabetic kidneys will have to be surveyed using renal biopsies to unequivocally establish a link between peroxidase, basement membrane destabilization, and clinical nephropathy. Ironically, both under- and overexpression of peroxidase might contribute to the disease, making a genetic approach in mice problematic. Clearly, a lot of important work is awaiting the authors.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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