Supplementary data

Hypochlorite-modified albumin colocalizes with RAGE in the artery wall and promotes MCP-1 expression via the RAGE-Erk1/2 MAP-kinase pathway

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Characterization of modified albumin preparations

Albumin was modified by HOCl (added as reagent or generated by the MPO-H_2O_2-chloride system) or by D-glucose as described in the "Methods" section. Amino acid analyses revealed that Cys and Met are the most sensitive amino acids towards HOCl-modification followed by tyrosine (Table **I**). Less reactive were His, Arg, and Lys that are consumed in a dose-dependent fashion up to 20-50% at the highest HOCl concentration used. As expected, MPO-modification of albumin led to similar amino acid changes as seen with HOCl-albumin (oxidant:protein molar ratio of 50:1); only lysine residues are more readily modified by the MPO-system. The loss of Lys, His, and Arg, respectively, was more pronounced in AGE-albumin.

Coomassie staining of modified albumin preparations (subjected to SDS-PAGE) revealed a gradual loss of monomeric albumin with concomitant formation of dimeric and high molecular mass aggregates after treatment with increasing HOCl-concentrations (Fig. **I**). The same pattern was seen with MPO-albumin. Modification of albumin by

glucose led to a slight increase in the molecular mass of monomeric albumin (data in line with others (1)) with concomitant formation of high molecular mass aggregates. Immunochemical detection of HOCl-modified epitopes in the modified albumin preparations (subjected to SDS-PAGE and Western blotting) confirms data obtained with Coomassie staining (Fig. I). An increasing oxidant:protein molar ratio was paralleled by increased staining intensity of HOCl-modified monomeric, dimeric and trimeric albumin bands. The same pattern was observed with MPO-albumin. As expected no HOCl-modified epitopes were detected in AGE-albumin (Fig. I).

References

1. Valencia, J.V., Stephen, C.W., Douglas, Q., Geesje, H.K., DeGroot, J., TeKoppele, J.M., Hughes, T.E. (2004) Advanced glycation end product ligands for the receptor for advanced glycation end products: biochemical characterization and formation kinetics. *Anal. Biochem.* **324**, 68-78

Table I

Amino acid analyses of albumin preparations

Amino acid analyses of native albumin (Alb), HOCl-Alb (at indicated oxidant:protein molar ratio), MPO-Alb (assuming quantitative conversion of H_2O_2 into HOCl, an oxidant:protein molar ratio of ~50:1 could be expected), and AGE-Alb was performed as described in the "Methods" section. The relative elctrophoretic mobility of HOCl-Alb was 1.68 (25:1), 1.77 (50:1), and 1.95 (100:1), respectively. Data shown represent mean values from two experiments performed in duplicates. Results are expressed in mol of amino acids/mol of albumin. n.d. = not detectable.

Figure I

Characterization of HOCl-albumin and AGE-albumin

Five μg of native albumin (Alb), HOCl-Alb (oxidant:protein molar ratio of 25:1, 50:1, and 100:1), MPO-Alb (assuming quantitative conversion of H_2O_2 into HOCl, an oxidant:protein molar ratio of ~50:1 could be expected), and AGE-Alb were subjected to SDS-PAGE (3,75-20% gradient gels). Proteins were either (**A**) Coomassie stained or (**B**) transferred to nitrocellulose for immunochemical detection of HOCl-modified epitopes using clone 2D10G9 as primary antibody. Immunoreactive bands were visualized with HRP-conjugated chicken anti-mouse IgG. The molecular mass of the marker proteins is indicated at the left; the arrow indicates the position of native Alb.

Figure II

Blocking of HOCl-albumin binding by anti-sRAGE IgG

RAGE-HEK cells were incubated with 10 μg/ml ^{125}I-HOCl-Alb (oxidant:protein molar ratio of 100:1) or ^{125}I-AGE-Alb for 2 h at 37°C in culture medium in the absence or presence of 50 μg/ml non immune or polyclonal anti-sRAGE IgG. Binding of ^{125}I-HOCl/AGE-albumin to HEK cells was measured as described in the Methods section.

Table I

	Alb	HOCl-Alb (25:1)	HOCl-Alb (50:1)	HOCl-Alb (100:1)	MPO-Alb	AGE-Alb
Cysteine	2.8	5.0	3.3	0.0	1.5	0.0
Methionine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tyrosine	32.8	26.2	21.6	10.2	19.8	20.5
Lysine	85.5	77.7	77.0	71.3	56.5	34.2
Histidine	25.8	26.3	24.8	20.4	23.4	16.8
Arginine	38.9	35.2	33.5	30.6	41.4	23.1

Figure I

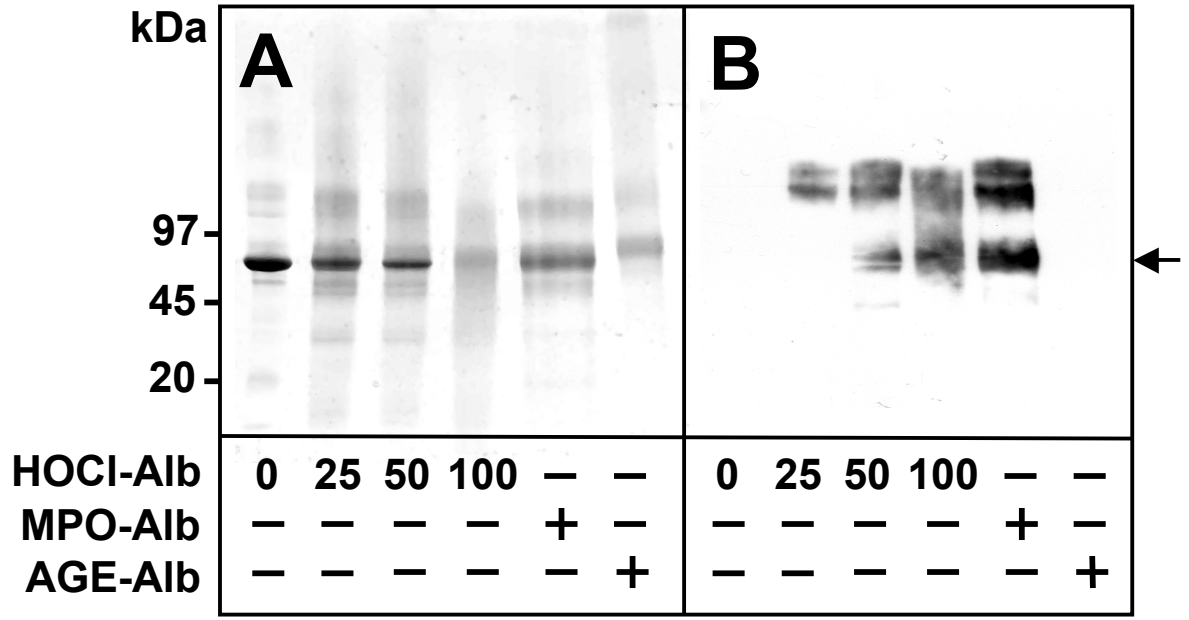
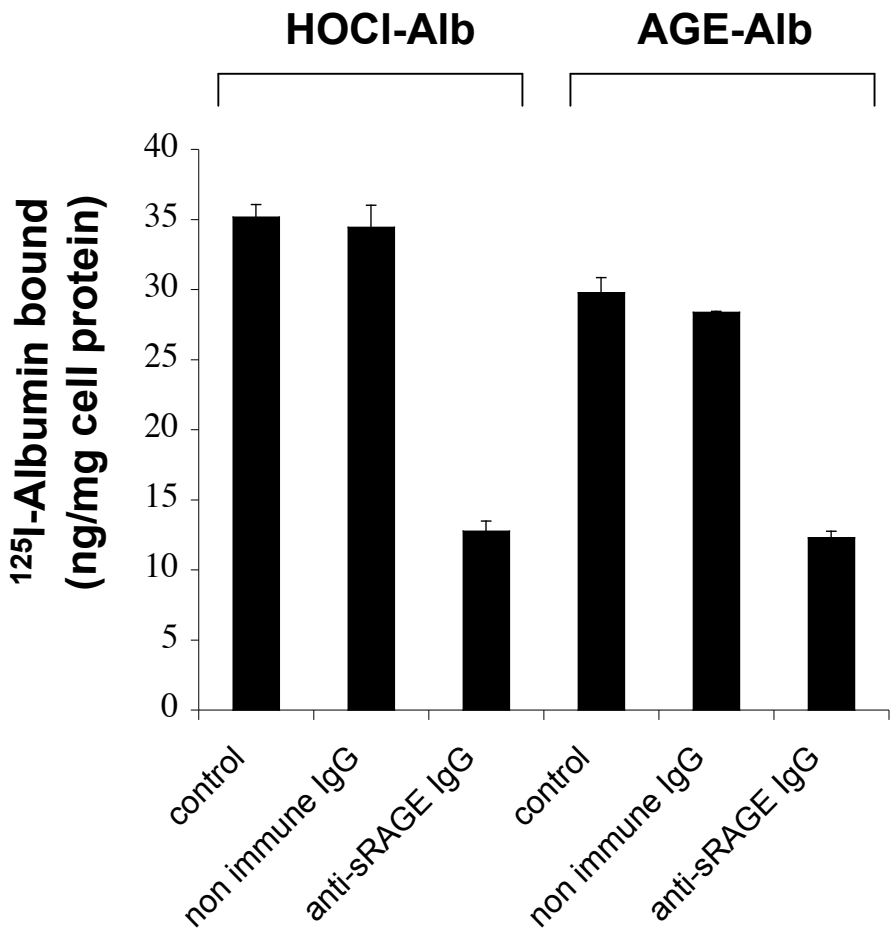


Figure II


HOCl-Alb
AGE-Alb
^{125}I-Albumin bound (ng/mg cell protein)
40
35
30
25
20
15
10
5
0
control
non immune IgG
anti-sRAGE IgG
control
non immune IgG
anti-sRAGE IgG