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Oxidation of Bovine Albumin by Hypochlorous and Hypobromous Acids: Structural and Functional Alterations

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Authors' contributions

This work was carried out in collaboration between all authors. Author VFX designed the study, wrote the protocol, managed the analyses of the study and wrote the first draft of the manuscript. Author MSP performed the experiments, managed the analysis and literature searches. Authors JRF and MLM performed the experiment. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: Hypochlorous (HOCl) and hypobromous (HOBr) acids are among the most powerful oxidants produced by the innate immune cells. Albumin is the predominant protein in most body fluids and is considered the most important antioxidant of blood plasma.
Study Design: Oxidation of bovine albumin (BSA) and study of its structural and functional alterations.
Place and Duration of Study: Faculty of Science and Faculty of Pharmaceutical Science, University of the State of Sao Paulo UNESP, between June and December 2012.
Methodology: BSA was oxidized with excess of HOCl or HOBr and its structural and functional alterations were analyzed by spectroscopic techniques as UV-Vis absorption, intrinsic and synchronous fluorescence, fluorescence quenching, Rayleigh scattering and

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circular dichroism.
Results: Both oxidants were able to deplete the intrinsic fluorescence of BSA, but HOBr was more effective than HOCl. The alterations in the synchronous fluorescence, UV-Vis absorption, and the appearance of a fluorescence band centered at 450 nm confirmed the difference between the oxidants. The oxidation did not induce aggregation of BSA as measured by Rayleigh scattering. The far-UV circular dichroism spectra showed a loss in the helical content and the near-UV-circular dichroism showed an alteration in the tertiary structure; HOBr was the more effective of the oxidants in this case. However, the oxidations did not induce significant alterations in the binding capacity of BSA, which was evaluated using hydrophobic (norfloxacin) and hydrophilic (ascorbic acid) drugs.
Conclusion: These results suggest that, although highly susceptible to oxidation, the alterations did not inhibit BSA's physiological function as a transport protein.

Keywords: Hypochlorous acid; hypobromous acid; albumin; oxidative stress; binding capacity.

1. INTRODUCTION

Hypochlorous (HOCl) and hypobromous (HOBr) acids are among the most powerful oxidants produced by the innate immune cells [1]. The leukocyte polymorphonuclear neutrophil is the primary source of HOCl in the immune system, and it is produced by the myeloperoxidase (MPO)-catalyzed oxidation of chloride by hydrogen peroxide (H_2O_2) [2]. HOBr is formed by the oxidation of bromide. In addition to MPO, eosinophil peroxidase (EPO), which is present in polymorphonuclear eosinophils, is able to oxidize bromide to HOBr [3]. Although these oxidants perform an essential physiological function as bactericidal agents, a growing body of experimental evidence suggests that HOCl and HOBr are involved in the pathogenesis and/or progression of inflammatory diseases such as atherosclerosis [4], asthma [5], rheumatoid arthritis [6], and cystic fibrosis [7].

Albumin is the most predominant protein in most body fluids and has many roles: it functions as the major transport protein by increasing the solubility of hydrophobic endogenous and exogenous molecules; it is a component of the solutes responsible for osmotic pressure of blood; and, it acts as a buffer for pH homeostasis [8]. Albumin is also considered the most important antioxidant of blood plasma, which is relevant since this extracellular fluid is exposed to oxidative stress and antioxidant enzymes are present in low concentration in this tissue [9]. Many studies have demonstrated the relationship between the levels of oxidized albumin and diseases for which oxidative stress is a relevant pathophysiological component such as IgA nephropathy [10], β-thalassemia major [11], acute ischaemic brain injury [12], chronic obstructive pulmonary disease [13], cardiovascular diseases [14], and diabetes [15].

The main oxidative modifications in proteins, including albumin, are related to the oxidation of free sulfhydryl groups to sulfenic, sulfinic and sulfonic acids, the formation of dityrosine cross-linking, 3-chlorotyrosine, proteins carbonyls and the oxidation of tryptophan residues [9,16-20]. Consequently, hypohalous acids are of fundamental importance, since all these modifications have been described in terms of their interactions with proteins [16-20].

For this investigation, we oxidized bovine albumin with HOCl and HOBr and studied the spectroscopic alterations that might reveal modifications in its secondary and tertiary structures and its drug-binding capacity.

2. MATERIALS AND METHODS

2.1 Chemicals and Solutions

Bovine serum albumin (BSA) free of globulin and fatty acid, norfloxacin, ascorbic acid, sodium chloride, sodium bromide, 2,4-dinitrophenylhydrazine (DNPH), guanidine-hydrochloride and trifluoroacetic acid were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Hypochlorous acid (HOCl) was prepared by diluting a 5% concentrated solution, and the concentration was determined spectrophotometrically after dilution in 0.01 M NaOH at pH 12 (ε_{292nm} = 350 $M^{-1}cm^{-1}$). HOCl was diluted daily to give a work solution of 100 mM in water. HOBr was synthesized by combining 100 mM HOCl and 200 mM NaBr in water [21]. BSA was dissolved in 50 mM phosphate buffer at pH 7.0 to give a 1 mM stock solution and stored at 4ºC. Accurate protein concentration was determined by measuring its absorbance at 280 nm (ε280nm = 43,890 $M^{-1}cm^{-1}$) [22] on a Perkin Elmer Lambda 35 UV−visible spectrophotometer (Shelton, CT, USA).

2.2 Fluorescence, Synchronous Fluorescence and UV-Vis Studies

The fluorescence spectra of BSA, which were automatically corrected for emission, were obtained using a Perkin Elmer LS 55 spectrofluorimeter (Shelton, CT, USA) adjusted as follows: excitation at 280 nm and emission scanning between 310 and 450 nm for intrinsic fluorescence; and excitation at 360 nm and emission scanning between 400 and 550 nm for the new fluorescence band of the oxidized protein. The slit widths were 2.5 nm for excitation and 10 nm for emission wavelengths. A 3-mL quartz cuvette with a 10 mm path length and a magnetic stirrer were used in the evaluations. The reaction mixtures contained 10 μM BSA and 200 or 400 μM oxidant in 50 mM sodium phosphate buffer at pH 7.0 and 25ºC. The fluorescence measurements were performed 4h after the addition of oxidant. The synchronous fluorescence spectra were obtained by scanning simultaneously with a fixed wavelength between the excitation and emission monochromators ($\Delta\lambda$) of 15 or 60 nm. The UV-Vis experiments were performed using a Hewlett Packard 8452 Diode Array spectrophotometer (Agilent, Santa Clara, CA, USA).

2.3 Rayleigh Scattering Studies

The elastic scattering of light, an indication of protein aggregation [23], was measured on a Perkin Elmer LS 55 spectrofluorimeter adjusted as follows: excitation at 270 nm and emission scanning between 255 and 295 nm, with slit widths of 2.5 nm for excitation and 10 nm for emission. A 3-mL quartz cuvette with a 10 mm path length and a magnetic stirrer were used in the evaluations. The reaction mixtures contained 10 μM BSA and 200 μM oxidant in 50 mM sodium phosphate buffer at pH 7.0 were incubated at 25ºC for 4h. Next, the samples were divided and an aliquot was kept at 25ºC and another at 70ºC for an additional 4h.

2.4 Determination of Carbonyl Group Content

BSA (10 μM) was incubated with 200 or 400 μM oxidant in 50 mM sodium phosphate buffer at pH 7.0 for 4h at 25ºC with gentle agitation. Next, 0.5 mM methionine was added to scavenge residual oxidant, and the reaction was incubated for an additional 30 min. The formation of protein carbonyl groups produced by oxidation with haloamines or hypohalous

acids was analyzed as previously described [24] with minor modifications. Briefly, the reaction mixture containing oxidized BSA (0.5 mL) was added to 0.5 mL DNPH (10 mM in 2 M HCl) and was incubated for 1h at room temperature; the mixture was vortexed at 10 min intervals. The protein was then precipitated with 0.5 mL cold TCA (final concentration: 30% w/v) by incubation in an ice bath for 30 min. The solution was subjected to centrifugation at 11,000 rpm for 10 min, and the supernatant was discarded. The precipitated protein was washed three times with 1 mL of an ethanol:ethyl acetate mixture (1:1 v/v). The precipitated protein was then dissolved in 0.5 mL 6 M guanidine hydrochloride (prepared in 20 mM potassium phosphate at pH 2.3) and incubated at 37ºC for 1h. Next, the reaction mixture was centrifuged at 11,000 rpm for 10 min, and the carbonyl group content was determined spectrophotometrically (ε_{370nm} = 22,000 $M^{-1}cm^{-1}$) and expressed as nmol/mg protein.

2.5 Quenching Studies

The reaction mixtures containing 10 μM BSA and 200 μM oxidant in 50 mM sodium phosphate buffer at pH 7.0 were incubated at 25ºC for 4h. Next, 0.5 mM methionine was added to scavenge residual oxidant and incubated for an additional 30 min. Fluorescence quenching experiments were performed by the addition of varying amounts of norfloxacin (5 - 30 μM) to the protein or to the oxidized protein solution in 50 mM sodium phosphate buffer at pH 7.0; the mixtures were incubated for 1h at 25ºC before measurement. For the experiments with ascorbic acid, the reaction mixture was diluted 10-fold before the addition of the quencher (0–100 μM). Proteins samples were excited at 280 nm and the emission was recorded at the maximum of the fluorescence band (340-350 nm). The fluorescence intensities were corrected for the inner filter effect caused by attenuation of the excitation end emission signal because of absorption by the quencher and protein using the equation: $F_{corr} = F_{obs} \times 10^{(Ab_{em} + Ab_{ex})/2}$, where F_{corr} and F_{obs} are the fluorescence intensities corrected and observed, respectively, and Ab_{em} and Ab_{ex} are the absorption of the mixture at excitation and emission wavelength, respectively [25]. The Stern-Volmer plot was used to obtain the quenching parameters.

2.6 Circular Dichroism Studies

Circular Dichroism (CD) spectra were recorded with a Jasco J-815 spectropolarimeter (Jasco, Japan), equipped with a thermostatically controlled cell holder at 25ºC. The spectra were accumulated in triplicate with 1 nm step resolution at a scanning speed of 20 nm/min and a pathlength cell of 10 mm. The baseline (50 mM phosphate buffer) was subtracted from all measurements. Far-UV CD spectra were recorded at a protein concentration of 0.5 μM over the wavelength range of 200-250 nm and near-UV CD spectra were recorded at a protein concentration of 10 μM over the wavelength range of 250-300 nm. The results were expressed as mean residual ellipticity (MRE) in $degree.cm^2.dmol^{-1}$, calculated as: MRE = θobs/(10.n.l.C), where θobs is the CD in millidegrees, n is the number of amino acids residues (583 for BSA), l is the light path in cm, and C is the molar concentration of the protein in mol/L.. The helical content was calculated from the MRE value at 222 nm using the equation: % helix = [- (MRE_{222nm}+ 2340)/30300]x100 [26].

3. RESULTS AND DISCUSSION

BSA, one of the most studied and characterized proteins, has 20 tyrosine and two tryptophan residues, with the latter being the main responsible for its intrinsic fluorescence [27]. These amino acid residues are also among the main targets for oxidizing agents in

proteins and the bleaching of the intrinsic fluorescence of proteins is frequently used as an indicator of its oxidation [28-30]. Hence, we initiated the studies by measuring the alteration in the intrinsic fluorescence of BSA as an analytical parameter for assessing its oxidation. We found that a 40-fold molar excess of HOCl or HOBr was able to almost completely deplete the intrinsic fluorescence of BSA. However, a difference between the oxidants was revealed using a lower molar excess of the acids (20-fold). These findings are in agreement with the higher reactivity of HOBr and its haloamine derivatives (Fig. 1, a and b) [20].

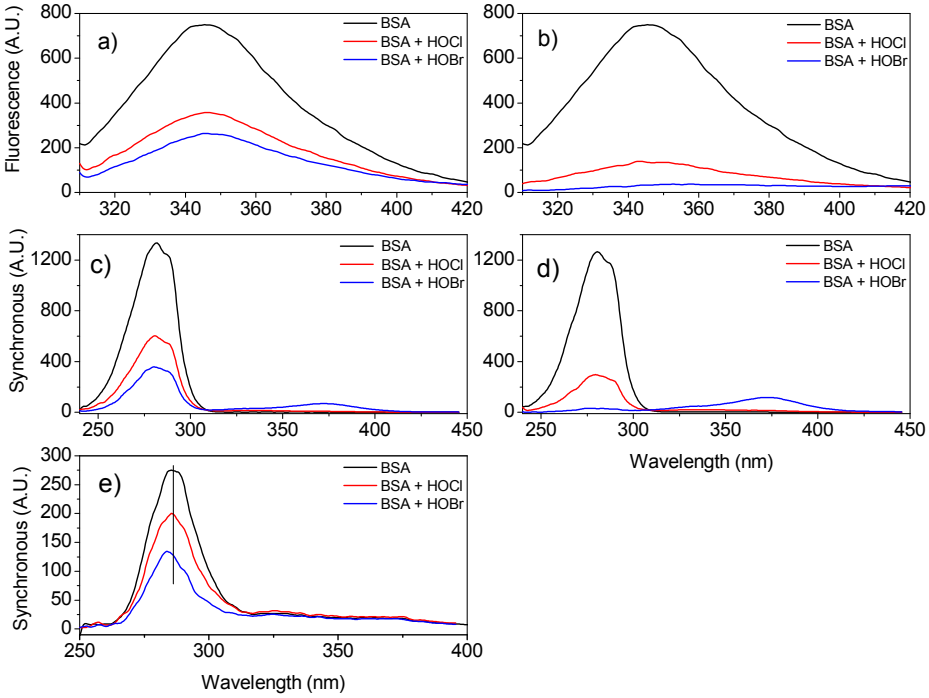

Fig. 1. Effect of hypohalous acid on BSA intrinsic and synchronous fluorescence

a) Depletion of intrinsic fluorescence using 20-fold excess of oxidant. b) Depletion of intrinsic fluorescence using 40-fold excess of oxidant. c) Depletion of synchronous fluorescence using 20-fold excess of oxidant (scanning at fixed $\Delta\lambda$=60 nm). d) Depletion of synchronous fluorescence using 40-fold excess of oxidant (scanning at fixed $\Delta\lambda$=60 nm). e) Depletion of synchronous fluorescence using 20-fold excess of oxidant (scanning at fixed $\Delta\lambda$=15 nm). The reaction mixtures consisted of 10 μM BSA and 200 or 400 μM oxidant in 0.05 M phosphate buffer, pH 7.0 at 25ºC. The spectra (λex 280) were taken 4h after addition of the oxidant

The relative efficacy of the oxidants was also evaluated by the formation of carbonyl groups and analyzed by derivatization with DNPH. In fact, among the pathways for production of carbonyl residues in proteins, the decomposition of chloramines generated by the interaction of HOCl with amino groups is well established [2]. In the experimental condition used here, both oxidants were able to produce carbonyl residues, but, contrary to the bleaching of intrinsic fluorescence, HOCl was more efficient than HOBr (Fig. 2). Taken together, these results suggest a higher efficacy of HOBr as an oxidant of tryptophan residues compared to HOCl, which is also in agreement with previous results using taurine bromamine [20].

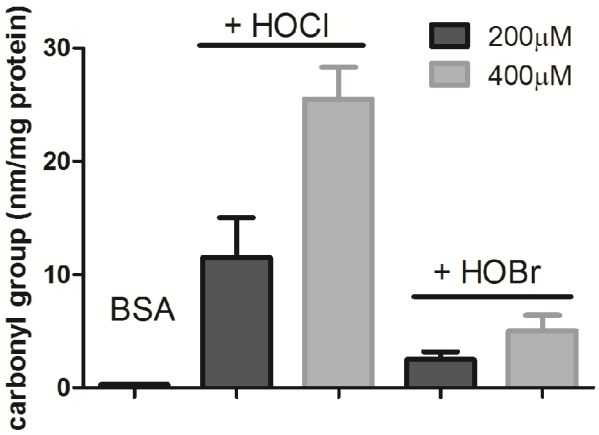

Fig. 2. Production of carbonyl groups in BSA.

The reaction mixtures consisted of 10 μM lysozyme and 200 or 400 μM oxidant in 0.05 M phosphate buffer, pH 7.0 at 25ºC. Carbonyl groups were measured as DNPH hydrazone derivatives and expressed as nmol/mg protein.

The spectral alteration provoked by the oxidation of BSA and the specific features of each oxidant were also noted by analyzing the alteration in its UV-Vis absorption band. As depicted in Fig. 3a, the oxidation of BSA causes molecular modifications in its amino acid residues, which are reflected in its UV-Vis absorption spectrum. The new absorption band at 315-360 nm for the oxidized protein was more intense using HOBr compared to HOCl. Similarly, a new fluorescent band for the oxidized BSA was also observed when excited at 360 nm (Fig. 3b). These results are in agreement with recently reported results of oxidation of free tryptophan [31], hence reinforcing the increased efficacy of HOBr as an oxidizing agent of tryptophan residues in BSA. A comparative study was also performed using synchronous fluorescence, a technique in which the excitation and emission wavelengths are scanned simultaneously at a fixed wavelength increment ($\Delta\lambda$). The synchronous fluorescence spectra provide information on the microenvironment in the vicinity of the fluorophore functional group. Particularly for proteins, $\Delta\lambda$ can be fixed at 15 or 60 nm and the results will reflect the alteration in the microenvironment next to tyrosine or tryptophan residues, respectively [32]. As depicted in Figs. 1c and 1d, the effects of the oxidants on the synchronous fluorescence of BSA with $\Delta\lambda$ fixed at 60 nm confirmed the experiments of intrinsic fluorescence, and, again, HOBr was more effective in its depletion. There was no alteration in the maximum fluorescence, which may indicate a change in the hydrophobicity of the microenvironment around the remaining tryptophan residues. However, the appearance of a new synchronous fluorescence band centered at 375 nm when the protein was oxidized by HOBr, but not by HOCl, can be considered an additional confirmation of the higher efficacy of HOBr as an oxidant of tryptophan residues. When $\Delta\lambda$ was fixed at 15 nm, besides the more efficient bleaching, the oxidation by HOBr also caused a slight blue shift (1.5 nm) in the maximum synchronous fluorescence, which indicates that the remaining tyrosine residues are located in a slightly more hydrophobic environment (Fig. 1e).

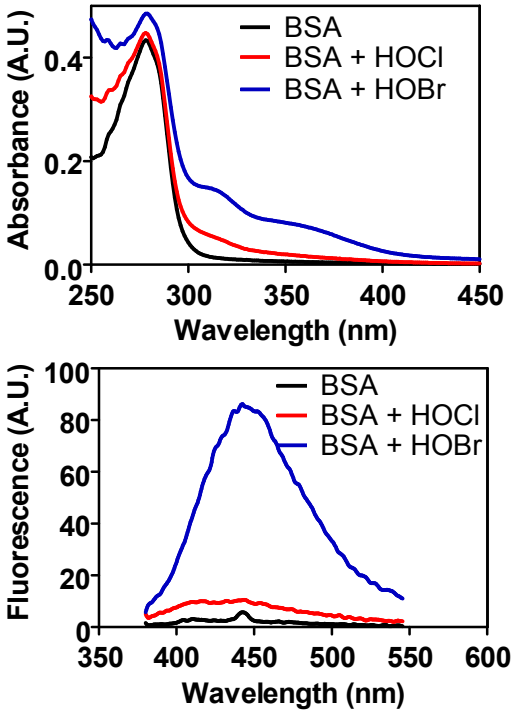

Fig. 3. Alteration of UV-Vis absorption and formation of new fluorescence band for oxidized BSA

The reaction mixtures consisted of 10 μM BSA and 200 μM oxidant in 0.05 M phosphate buffer, pH 7.0 at 25ºC. The spectra were taken 4h after addition of the oxidant. The fluorescence spectra were obtained by excitation at 360 nm.

The oxidation of some proteins induces the formation of insoluble aggregates, which can be detected by Rayleigh scattering by measuring the maximum of the elastic peaks of excitation light at 270 nm [23]. This is the case for lysozyme, a protein extremely susceptible to HOCl and HOBr. In fact, the use of only 4-fold molar excess of these oxidants was sufficient for aggregation of lysozyme, which caused a significant increase in the Rayleigh scattering peak [33]. Here, we found that, different from lysozyme, BSA did not aggregate after oxidation (Fig. 4). It is noteworthy that we used 40-fold molar excess of oxidants, which is significantly higher than in the lysozyme studies. Additionally, the stability of the protein was also studied by thermal treatment. At 70ºC, BSA undergoes a progressive aggregation caused by alterations in secondary and tertiary structures [24]. Here, after oxidation, we incubated the protein solution at 70ºC for 8h and measured the Rayleigh peak scattering. As shown in Fig. 4, the thermal treatment caused the aggregation of BSA; however, the oxidation did not contribute to further protein destabilization.

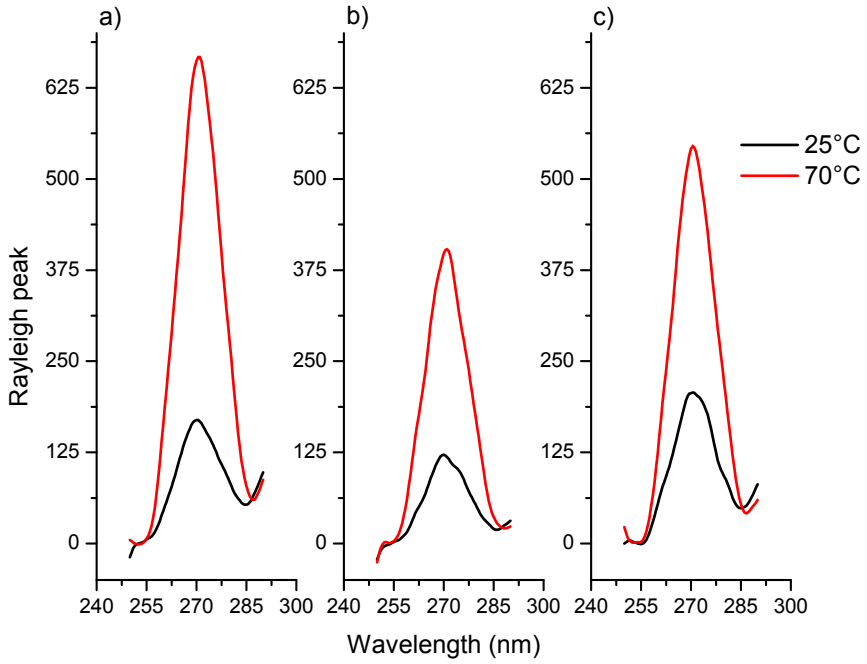

Fig. 4. Rayleigh light scattering.
The reaction mixtures consisted of 10 μM BSA and 200 μM oxidant in 50 mM phosphate buffer, pH 7.0 at 25ºC. a) BSA, b) BSA + HOBr, c) BSA + HOCl.

In order to understand the secondary structural changes induced by the oxidation of BSA, the far-UV CD spectra of the protein were measured before and after oxidations. The results depicted in Fig. 5 show the typical CD spectrum (converted to MRE) of a protein with the minimums at 208 and 222 nm, respectively. The results, which are also shown in Fig. 5, reveal that these elements of secondary structure were maintained after oxidation, but the intensities were decreased. Fig. 5 also shows that at 20-fold molar excess, the difference was not significant for HOCl, but, for the oxidation using HOBr, a very small decrease in the α-helical content was observed. However, at 40-fold molar excess of hypohalous acid, a small but significant loss in the helical content was observed for both oxidants. The α-helical content was calculated as described in the Materials and Methods section and the results were: BSA 49.5%, BSA + HOCl 44.4% and BSA + HOBr 42.4%. These results demonstrate that the oxidative alteration of the side chain of the amino acids has some effect on the tridimensional structure of BSA. Again, HOBr was more effective compared to HOCl, suggesting that the oxidation of tryptophan residues has a significant role in its secondary stability. Next, the near-UV CD spectra were analyzed. The near-UV CD spectra of proteins are linked to aromatic amino acids and are more sensitive to alteration in the tertiary structure of the proteins [34]. The near-UV CD spectra of BSA has two minima at 261 and 268 nm, as well as two shoulders at 277 and 284 nm [35]. Here, this spectral fingerprint was also noted for pure BSA. After oxidation, the general features of the spectra were maintained, but a significant decrease in the intensity was observed (Fig. 5, b and d), which can be interpreted as a loss in the aromatic amino acid content, which is in agreement with the previous fluorescence-based experiments.

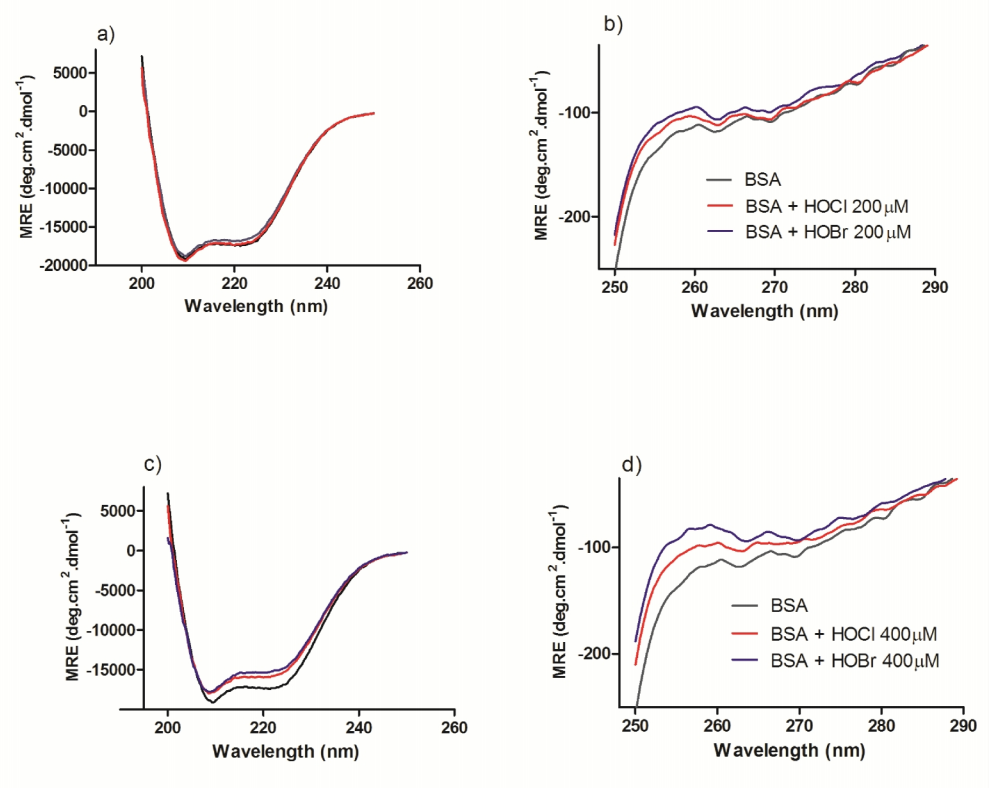

Fig. 5. Effect of oxidation on far-CD (a,b) and near-CD (c,d) spectra of BSA

The reaction mixtures consisted of 10 μM BSA and 200 or 400 μM oxidant in 0.05 M phosphate buffer, pH 7.0 at 25ºC. The spectra were taken 4h after addition of the oxidant

Albumin is the most abundant protein in the circulatory system of many organisms and one of its major functions is to carry endogenous and exogenous compounds by reversible binding, hence increasing their solubility and distribution in the body. Therefore, we also studied how the oxidation of BSA affects its binding capacity. We used norfloxacin and ascorbic acid as models of hydrophobic and hydrophilic drugs, respectively, which are able to bind to BSA [36,37]. As an analytical parameter for assessing binding capacity, we used the fluorescence quenching method, which can reveal mechanisms of quenching and binding constant. Fluorescence quenching is a phenomenon associated with the decrease in the quantum yield of the fluorophore caused by energy transfer, excited state reaction, ground state complex and collisional deactivation [27]. A static mechanism of quenching, which is an indication of the formation of a ground state complex with albumin, has been reported for norfloxacin [36]. The results depicted in Fig. 6 confirm this characteristic of the interaction between BSA and norfloxacin and also show a well-defined isoactinic point at 386 nm, which is an additional indication of free and bound forms of norfloxacin. Here, we found that, except for the lower initial fluorescence intensity, the previous oxidation of BSA did not cause alteration in the spectral changes during the titration by norfloxacin. Fig. 6 also shows the Stern-Volmer plot, from which we calculated the Stern-Volmer constant (Ksv) using the equation: $F0/F = 1 + Ksv[Q]$, where F0 is the fluorescence intensity in the absence of quencher and F is the fluorescence at the given concentration of the quencher. The Ksv values obtained for the interaction between BSA and norfloxacin were similar to recently-

reported values [36]. The results depicted in Table 1 show that the oxidation caused a minimum alteration in the binding capacity of BSA. The same general findings were obtained when the fluorescence of BSA was quenched with ascorbic acid (Fig. 7, Table 1).

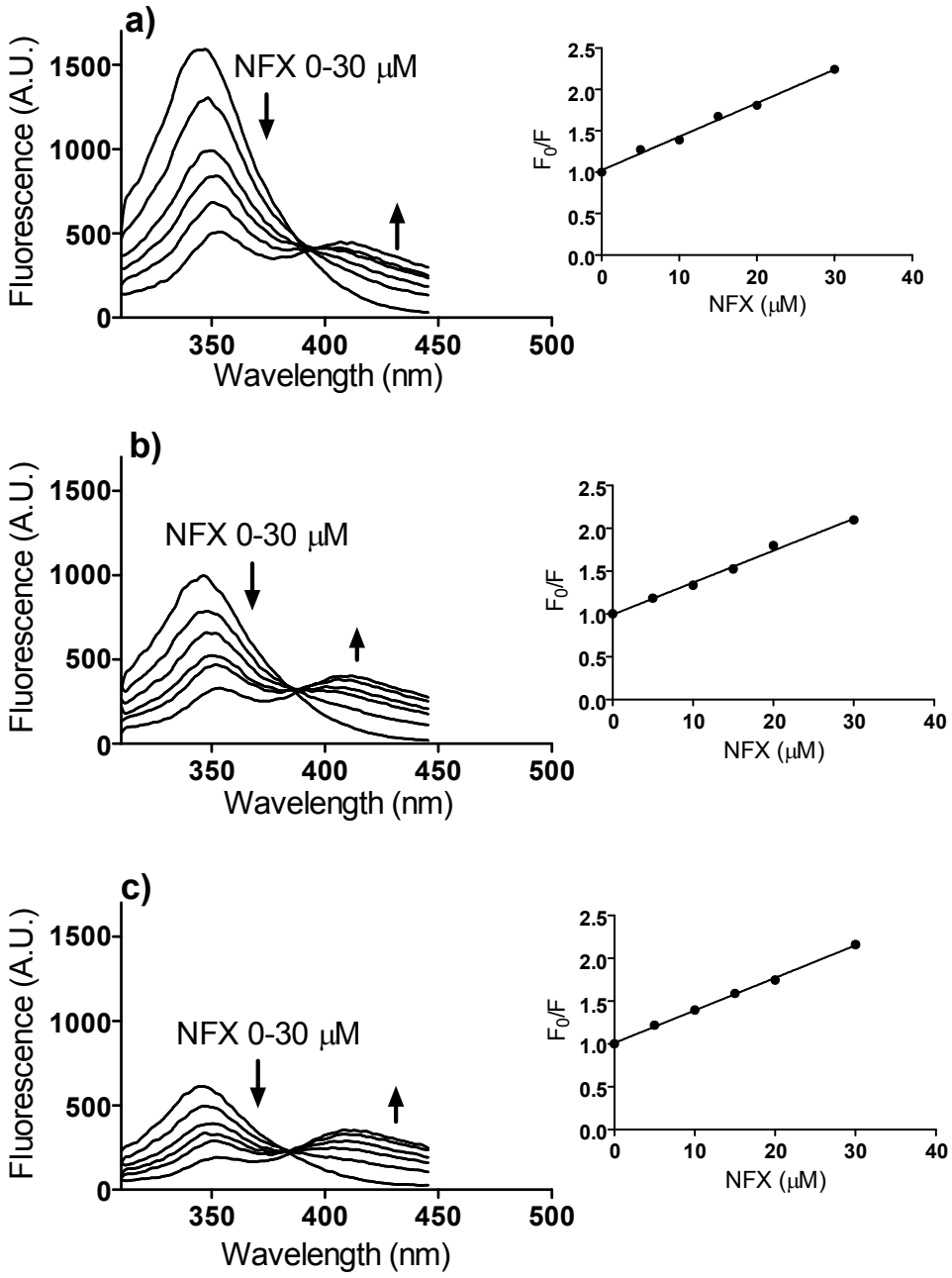

Fig. 6. Fluorescence quenching spectra of BSA by norfloxacin and Stern-Volmer plot
a) BSA, b) BSA + HOCl, c) BSA + HOBr. The reaction mixtures consisted of 10 μM BSA and 200 μM oxidant in 0.05 M phosphate buffer, pH 7.0 at 25°C. After oxidation, the excess oxidant was scavenged by adding 500 μM methionine before the addition of norfloxacin (NFX).

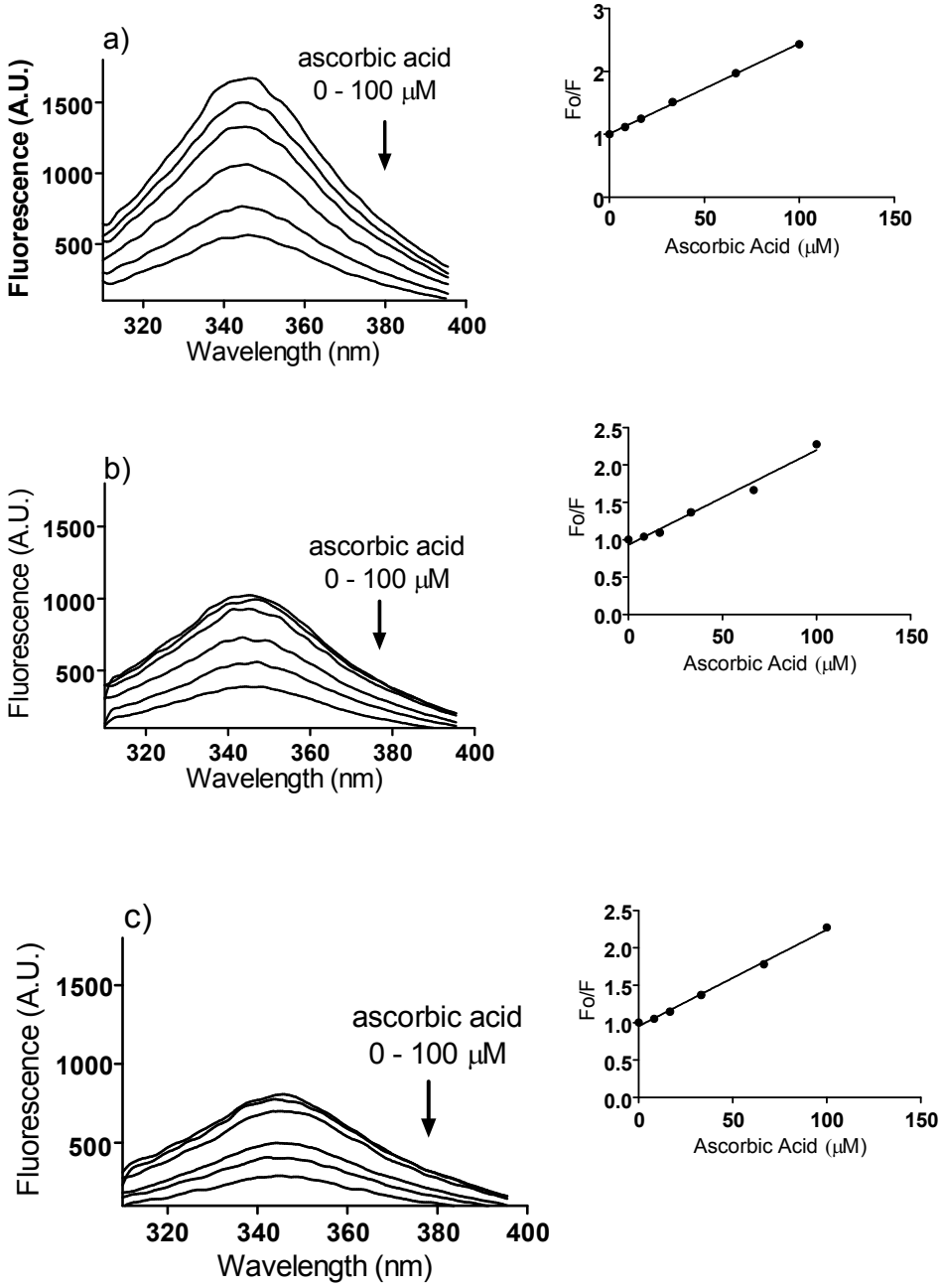

Fig. 7. Fluorescence quenching spectra of BSA by ascorbic acid and Stern-Volmer plot

a) BSA, b) BSA + HOCl, c) BSA + HOBr. The reaction mixtures consisted of 10 μM BSA and 200 μM oxidant in 0.05 M phosphate buffer, pH 7.0 at 25ºC. After oxidation, the excess oxidant was scavenged by adding 500 μM methionine and the reaction mixtures were diluted 10-fold before the addition of ascorbic acid.

Table 1. The Stern-Volmer quenching constants

	Norfloxacin			Ascorbic Acid		
	k_{sv} (10^4 L.mol^{-1})	R[a]	SD[b]	k_{sv} (10^4 L.mol^{-1})	R	SD
BSA	4.05	0.9930	0.16	1.43	0.9993	0.02
BSA + HOCl	3.74	0.9932	0.15	1.26	0.9899	0.07
BSA + HOBr	3.80	0.9985	0.07	1.29	0.9961	0.04

[a] *Correlation coefficient*
[b] *Standard deviation of the linear regression*

4. CONCLUSION

In summary, the oxidation of BSA by hypohalous acid induced alteration is its primary, secondary and tertiary structures. In general, these alterations were more effective using HOBr than HOCl and might be the result of a more specific oxidation of tryptophan residues. These alterations did not cause destabilization of BSA, as verified by thermal treatment, and caused only a minor effect in its capacity as a drug-transport protein. These findings are relevant, since BSA is an important antioxidant of the blood plasma and its oxidation by extracellular reactive oxygen species could impair its physiological function. However, we must emphasize that the concentration of the oxidants used here are higher than that found *in vivo*, hence the real effect of physiological concentrations of HOCl and HOBr on albumin might be irrelevant.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Prokopowicz Z, Marcinkiewicz J, Katz DR, Chain BM. Neutrophil myeloperoxidase: soldier and statesman. Arch Immunol Ther Exp (Warsz). 2012;60:43-54.
2. Winterbourn CC, Kettle AJ. Redox reactions and microbial killing in the neutrophil phagosome. Antioxid Redox Signal; 2012. In press.
3. Battistuzzi G, Bellei M, Vlasits J, Banerjee S, Furtmüller PG, Sola M, et al. Redox thermodynamics of lactoperoxidase and eosinophil peroxidase. Arch Biochem Biophys. 2010;494:72-7.

4. Hazen SL, Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. Clin Invest. 1997;99:2075-81.
5. Aldridge RE, Chan T, van Dalen CJ, Senthilmohan R, Winn M, Venge P, et al. Eosinophil peroxidase produces hypobromous acid in the airways of stable asthmatics. Free Radic Biol Med. 2002;33:847-56.
6. Stamp LK, Khalilova I, Tarr JM, Senthilmohan R, Turner R, Haigh RC, et al. Myeloperoxidase and oxidative stress in rheumatoid arthritis. Rheumatology (Oxford). 2012;51:1796-1803.
7. Thomson E, Brennan S, Senthilmohan R, Gangell CL, Chapman AL, Sly PD, Kettle AJ, et al. Identifying peroxidases and their oxidants in the early pathology of cystic fibrosis. Free Radic Biol Med. 2010;49:1354-60.
8. Fanali G, di Masi A, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: from bench to bedside, Mol Aspects Med. 2012;33:209-290.
9. Colombo G, Clerici M, Giustarini D, Rossi R, Milzani A, Dalle-Donne I. Redox albuminomics: oxidized albumin in human diseases. Antioxid Redox Signal. 2012;17:1515-27.
10. Nakayama A, Odake J, Kanke A, Sakatsume M, Kasama T, Shiba K. Redox state of urinary albumin in patients with IgA nephropathy. Rinsho Byori. 2011;59:1013-18.
11. Awadallah SM, Atoum MF, Nimer NA, Saleh SA. Ischemia modified albumin: an oxidative stress marker in β-thalassemia major. Clin Chim Acta. 2012;413:907-10.
12. Moon GJ, Shin DH, Im DS, Bang OY, Nam HS, Lee JH, et al. Identification of oxidized serum albumin in the cerebrospinal fluid of ischaemic stroke patients. Eur J Neurol. 2011;18:1151-8.
13. Hackett TL, Scarci M, Zheng L, Tan W, Treasure T, Warner JA. Oxidative modification of albumin in the parenchymal lung tissue of current smokers with chronic obstructive pulmonary disease. Respir Res. 2010;11:180-90.
14. Terawaki H, Takada Y, Era S, Funakoshi Y, Nakayama K, Nakayama M, et al. The redox state of albumin and serious cardiovascular incidence in hemodialysis patients. Ther Apher Dial. 2010;14:465-71.
15. Rasheed Z, Ali R. Reactive oxygen species damaged human serum albumin in patients with type 1 diabetes mellitus: biochemical and immunological studies. Life Sci. 2006;79:2320-8.
16. Van der Veen BS, de Winther MP, Heeringa P, Myeloperoxidase: molecular mechanisms of action and their relevance to human health and disease. Antioxid Redox Signal. 2009;11:2899-2937.
17. Winterbourn CC, Kettle AJ. Biomarkers of myeloperoxidase-derived hypochlorous acid. Free Radic Biol Med. 2000;29:403-9.
18. Gunn AP, Roberts BR, Bush AL. Rapid generation of dityrosine cross-linked Aβ oligomers via Cu-redox cycling. Methods Mol Biol. 2012;849:3-10.
19. Guerin-Dubourg A, Catan A, Bourdon E, Rondeau P. Structural modifications of human albumin in diabetes. Diabetes Metab. 2012;38:171-8.
20. Ximenes VF, da Fonseca LM, de Almeida AC. Taurine bromamine: a potent oxidant of tryptophan residues in albumin. Arch Biochem Biophys. 2011;507:315-22.
21. Tokunaga S, Kanayama A, Miyamoto Y. Modification of IκBα by taurine bromamine inhibits tumor necrosis factor α-induced NF-κB activation. Inflamm Res. 2007;56:479-86.
22. Pace CN, Vajdos F, Fee L, Grimsley G, Gray T. How to measure and predict the molar absorption coefficient of a protein. Protein Sci. 1995;4:2411-23.
23. Militello V, Vetri V, Leone M. Conformational changes involved in thermal aggregation processes of bovine serum albumin. Biophys Chem. 2003;105:133–41.

24. Fagan JM, Sleczka BG, Sohar I. Quantitation of oxidative damage to tissue proteins. Int J Biochem Cell Biol. 1999;31:751-7.
25. Roy S. Fluorescence quenching methods to study protein-nucleic acid interactions. Methods Enzymol. 2004;379:175-87.
26. Muzammil S, Kumar Y, Tayyab S. Molten globule-like state of human serum albumin at low pH. Eur J Biochem. 1999;266:26-32.
27. Lakowicz JR. Principles of Fluorescence Spectroscopy, 3th ed. New York: Springer; 2006.
28. McMullen RL, Chen S, Moore DJ. Spectrofluorescent characterization of changes in hair chemistry induced by environmental stresses. J Cosmet Sci. 2011;62:191-202.
29. Lissi E, Biasutt MA, Abuin E, León L. A fluorescence study of human serum albumin binding sites modification by hypochlorite. J Photochem Photobiol B. 2009;94:77-81.
30. Barcelos RP, Portella RL, da Rosa EJ, Fonseca AS, Bresolin L, Carratu V, et al. Thiosemicarbazone derivate protects from AAPH and Cu2+ -induced LDL oxidation. Life Sci. 2011;89:20-8.
31. Petrônio MS, Ximenes VF. Light emission from tryptophan oxidation by hypobromous acid. Luminescence; 2012. in Press. doi: 10.1002/bio.2445.
32. Burstein EA, Vedenkina NS, Ivkova MN. Fluorescence and the location of tryptophan residues in protein molecules. Photochem Photobiol 1973;18:263-79.
33. Petrônio MS, Ximenes VF. Effects of oxidation of lysozyme by hypohalous acids and haloamines on enzymatic activity and aggregation. Biochim Biophys Acta. 2012;1824: 1090-6.
34. Kelly SM, Price NC. The use of circular dichroism in the investigation of protein structure and function. Curr Protein Pept Sci. 2000;1:349-84.]
35. Sun C, Yang J, Wu X, Huang X, Wang F, Liu S. Unfolding and refolding of bovine serum albumin induced by cetylpyridinium bromide. Biophys J. 2005;88:3518–24.
36. Seetharamappa J, Kamat PB. Study of the interaction between fluoroquinolones and bovine serum albumin. J Pharm Biomed Anal. 2005;39:1046-50.
37. Xu H, Liu Q, Zuo Y, Bi Y, Gao S. Spectroscopic Studies on the Interaction of Vitamin C with Bovine Serum Albumin. J of Solution Chem. 2012;38:15-25.

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