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Influence of aqueous extracts of urban airborne particulate matter on the structure and function of human serum albumin☆

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ABSTRACT

Human exposure to particulate matter (PM) originating from air pollution is inevitable since more and more population is present in large cities that are characterized by poor air quality. The impact on human health is evident and we need to intensify research regarding this problem to get molecular insight into versatile effects of chronic exposure to PM inducing organism responses and initiating the development of selected disorders. Herein, the impact of standard PM representing urban pollution on the structure and function of human serum albumin (HSA) was evaluated by the application of various analytical techniques. HSA was selected due to its high likeliness of being exposed to PM because of the abundance of this protein in blood. The studies were focused mainly on the inorganic residue of PM resulting from removing organic components by a low-temperature plasma. To mimic physiological conditions, dialysis technique was used to simulate the release of nanoparticles and ions from PM to aqueous environment under, which in turn may interact with biomolecules inside the living system. Capture of metals from the bulk suspension was found for many metals like Al, Fe, Zn and Pb in quantities of more than 1 mol of metal ions per mole of HSA. No significant structural changes of the protein upon dialysis with PM were observed, however, an increase in the thermal stabilization of the HSA structure was observed. Moreover, the interaction of HSA dialyzed in the presence of PM with selected drugs (warfarin, aspirin) was negatively affected, indicating a lower affinity of drugs towards the protein, even though only small conformational changes of the PM exposed protein were observed. Our findings point to a possible interference of air pollutants with the drugs taken by patients living in highly polluted areas.

1. Introduction

Particulate matter (PM) in the nano-, submicron- and micron-size range, originating from air pollution, is receiving increasing attention from researchers due to the role that PM plays in environmental health issues. Numerous studies have demonstrated that air pollution adversely affects the outcome of many health-related problems (Al-Hemoud et al., 2019; Brook et al., 2010; Kampa and Castanas, 2008; Lu et al., 2020; Maesano et al., 2020; Pope et al., 2015; Sinharay et al., 2018; Yang et al., 2020) and results in negative economic and social effects (Caplin et al., 2019; Lu, 2020). Among others, epidemiological studies have revealed that there is a connection between air pollution (e.g. elevated PM content) and cardiovascular diseases such as heart failure and arrhythmias, heart attack or ischemic heart disease (Franchini and Mannucci, 2012; Lee et al., 2014a). The impact of PM on human health depends on their diameter, which determines their final deposition. It was shown that large particles (>2.5 μm) are mainly deposited in the tracheobronchial region, whereas fine particles (<2.5 μm) are mainly deposited in the pulmonary region (Deng et al., 2019). In consequence, particles with smaller diameter have a greater potential for penetrating the respiratory system. Further diffusion to the alveolar duct is possible, and part of these fine particles are allowed to enter the blood circulation where exposure of blood components to PM is evident, as well as they can go into

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the heart, kidneys and other organs (Deng et al., 2019; Jin et al., 2016; Nemmar et al., 2002). However, the exact mechanism leading to health problems is still largely unknown. Among the postulated processes, the greatest emphasis is placed on induction of oxidative stress, inflammation, enzyme dysfunction, and lipid peroxidation (Baulig et al., 2009; Billet et al., 2007; Gawda et al., 2018; Signorelli et al., 2019). Identifying the mechanism by which air pollutants affect individuals is difficult due to the complex composition of pollutants, personal biological differences and various adaptation processes that can take place. Furthermore, the effects of acute and chronic exposure often differ.

Unveiling the impact of PM on proteins would be helpful in evaluating the hazards of air pollution exposure and can contribute to the elucidation of the mechanism behind such interactions. Binding with exogenous materials often leads to alteration of the protein's structure. Conformational changes induced upon interaction with nano- and micro-particles can significantly change the protein's stability, binding capability and physiological functions. Efforts have been dedicated to clarify the interaction between proteins and nanoparticles, including carbon nanomaterials (Wu et al., 2018), titanium dioxide (Ranjan et al., 2016), silver (Gorshkov et al., 2019) and iron nanoparticles (Abbaszadeh Asl et al., 2018). However, the majority of the studies were focused on the formation of surface protein layers, known as the protein corona that is crucial for the interaction of nanoparticles with living cells. A few available reports provide information on protein modification upon exposure to particulate matter, but they concentrated only on protein oxidation (Lai et al., 2016; Lee et al., 2014b).

The main aim of this work was to investigate the influence of particulate matter and their inorganic components obtained by removing organic components by treatment with a low-temperature plasma on the structure and function of proteins using human serum albumin as a model protein. Since organic and inorganic fractions of PM can induce a pathophysiological response via different mechanisms, samples of PM without organic components were used to evaluate the role of inorganic components in possible protein modification. Human serum albumin was selected due to its predominant composition in blood plasma (He and Carter, 1992). It performs a variety of physiological functions, such as control of osmotic pressure, transport of different biomolecules or metabolism of certain drugs (Kumar and Banerjee, 2017). The long half-life of albumin (approximately three weeks) allows to collect information about PM exposure and to induce a potential biological response. Several recent studies tried to identify HSA adducts as potential markers of exposure to air pollutants (Liu et al., 2018; Lu et al., 2017; Preston et al., 2020). Synergetic effects of PM2.5 and human albumin on allergic airway inflammation were reported (Nagaoka et al., 2019).

In order to follow possible protein modifications, dialysis was used as an exposure technique to mimic the natural risk conditions. After inhalation of air pollutants, their soluble parts and the finest fractions pose the greatest threat to human health (Heyder, 2004; Pinkerton et al., 2000; Salvi and Holgate, 1999; Williams et al., 2011). Since both electrostatic and hydrophobic interactions are critical for molecules binding to HSA (Kumar and Banerjee, 2017), accumulation of PM on the protein can have a tremendous impact on its function. The structural and functional changes of the proteins were monitored by ICP-OES, circular dichroism, fluorescence emission studies, thermal shift assay as well as electrophoretic and chromatographic techniques. The collected data pointed to the impact of PM on the binding properties of albumin for commonly used drugs, whereas minor conformational changes in the secondary structure of biomolecules were identified.

In this study, we would like to draw the attention of the scientific community to the potential interference of urban air pollutants on drugs taken by patients living in highly polluted areas. This hypothesis needs to be further verified by detailed clinical studies.

2. Experimental part

2.1. Materials and solutions

Proteins such as human serum albumin (HSA) was purchased from Sigma-Aldrich. Standard Reference Material 1648a (encoded as SRM) was supplied by the National Institute of Standards and Technology (USA). PM samples with removed organic content (<2% of organic carbon, <1% of nitrogen, encoded as ΔC-SRM) were obtained by the treatment of SRM with a low-temperature plasma for 2 h according to the published procedure (Mikrut et al., 2018). Cold plasma treatment resulted in a ca. 33% decrease in mass of the sample. Phosphate buffered saline (PBS, pH 7.4) was purchased from Corning.

2.2. Exposure of human serum albumin to particulate matter by dialysis

In a typical experiment 0.9 mL of HSA dissolved in PBS at 5 mg/mL concentration (75 μM) was placed in Pur-A-Lyzer Midi Dialysis Kit (Sigma-Aldrich) device with a cut off at 3.5 kDa. The dialysis was carried out for 12 h in 15 mL of PBS solution alone or in PBS suspensions containing various amounts of either SRM or ΔC-SRM (0.125–2 mg/mL). It was followed by dialysis in a fresh portion of PBS for another 12 h. The obtained protein was collected by reversed centrifugation, frozen in small aliquots and used for further analysis. The concentration of protein after dialysis was measured spectrophotometrically using the absorption coefficients at 280 nm of 42,000 M^{-1} cm^{-1} (Sanna et al., 2009) and typically was in the range of 65 ± 10 μM. The metal content in dialyzed protein and in particulate matter suspension after dialysis (see Scheme 1) was determined in the mineralized samples by inductively coupled plasma optical emission spectrometry (ICP-OES). Mineralization was conducted using ultrapure concentrated nitric acid (Sigma-Aldrich). The obtained metal concentrations are presented as mean values from a minimum of three independent experiments. The metal content of PBS is shown in Fig. S1 (Supplementary Information).

2.3. Circular dichroism (CD)

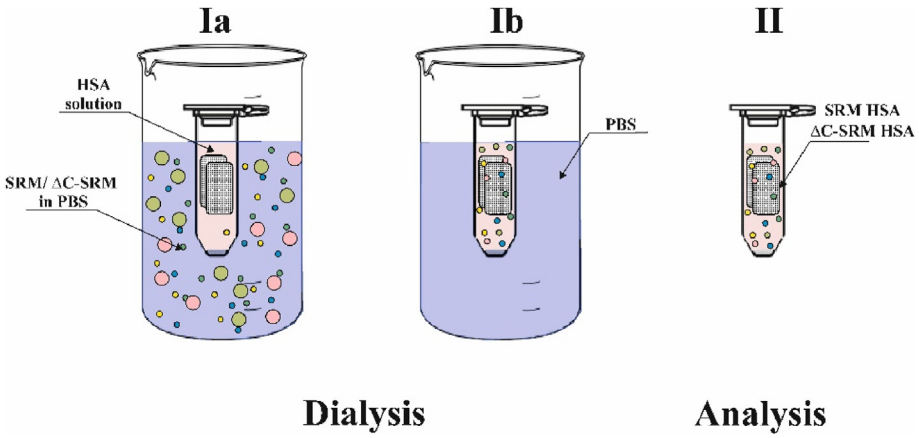
Circular dichroism measurements were performed using a Jasco J-710 spectropolarimeter (Jasco Analytical Instruments, Easton, MD, USA). The spectra were recorded in the range of 195–250 nm with a 1 nm data pitch, 10 nm min^{-1} scan speed, 8 s response time and 2 nm bandwidth, averaged over 3 acquisitions. Protein samples were measured in a cuvette with a 0.1 mm light path (Hellma, Müllheim, Germany).

2.4. Thermal denaturation

In the thermal denaturation measurement, the protein samples (0.5 mg/mL) at pH 7.4 were heated from 20 to 95 °C with a heating rate of 2 °C min^{-1}. The dichroic activity at 222 nm was continuously monitored every 0.2 °C with an averaging time of 4 s.

2.5. Fluorescence spectroscopy

Fluorescence spectra were recorded on a PerkinElmer LS55 spectrofluorimeter equipped with a thermostat (Grant LTD6G)



Scheme 1. Human serum albumin (HSA) was dialyzed during 12 h in SRM or ΔC-SRM suspension prepared in PBS buffer at different concentrations (0–2 mg/mL), **Ia**. After that, the protein was dialyzed in a fresh portion of PBS for the next 12 h, **Ib**. The resulted SRM HSA or ΔC-SRM HSA were further analyzed, **II**.

(±0.1 °C) in a 1 cm quartz cuvette at 25 °C. Quantum yields of internal fluorescence (Q) of HSA and other proteins (apo-Tf, Fe_2Tf, apo-Lf, Fe_2Lf, Mn_2Lf) were determined by comparison with tryptophan as a standard with a known quantum yield of fluorescence ($Q_R = 0.13 \pm 0.01$)(Lakowicz, 2006). The following formula was used:

$$Q = Q_R \frac{I}{I_R} \frac{OD_R}{OD} \quad (1)$$

where I is the integrated intensity of fluorescence, OD is the optical density, the subscript R refers to the reference fluorophore with known quantum yield – tryptophan.

Binding constants of HSA dialyzed in PBS buffer in the absence and the presence of SRM or ΔC-SRM with selected drugs (warfarin or aspirin) were determined using fluorescence quenching spectroscopy. In a typical experiment an aqueous solution of an appropriate drug was added to 2 μM HSA dissolved in PBS buffer. The emission spectra were recorded in the wavelength range 305–500 nm upon excitation at 295 nm. The fluorescence spectra were corrected for self-absorbance and inner filter effects according to the equation:

$$F_{corr} = F_{obs} \times 10^{\frac{A_{ex}+A_{em}}{2}} \quad (2)$$

where F_{corr} and F_{obs} are the corrected and observed fluorescence intensity values, respectively, and A_{ex} and A_{em} the absorbance values at the excitation and the emission wavelengths, respectively (Lakowicz, 2006). UV–Vis absorption spectra were recorded on a PerkinElmer Lambda 35 UV–Vis spectrophotometer. The binding constant, K_b, as well as the interaction stoichiometry, n, were calculated using a linear equation fit:

$$log\frac{F_0 - F_{corr}}{F_{corr}} = logK_b + nlog[D_{tot}] \quad (3)$$

where F_0 is the initial albumin fluorescence, D_{tot} is the total drug concentration. The presented K_b is a calculated mean value from a minimum of three independent experiments.

2.6. Statistical analysis

Data are expressed as the mean ± standard error of the mean (SEM). Significant differences among tested samples were determined by one way analysis of variance (ANOVA) using OriginPro 2018 software. Probabilities of $p < 0.05$ were considered as statistically significant. The following notification is used * $p < 0.05$, **$p < 0.001$.

3. Results

3.1. Sample preparation

HSA was dialyzed in PBS buffer in the presence of various concentrations (0–2 mg/mL) of SRM or ΔC-SRM as shown in Scheme 1.

The content of metal ions in the protein gradually increased with increasing concentration of particulate matter (PM). For the sample dialyzed in ΔC-SRM it was always higher, which is understandable since this sample contains a higher content of the inorganic fraction (during cold plasma treatment a ca. 33% decrease in mass of the sample was observed). Changes of the iron content in HSA in relation to the iron present in suspension after 12 h of dialysis are shown in Fig. 1A (for other metals see Fig. S2, Supplementary Information). Since the protein was always dialyzed in a fresh portion of PBS containing very small amounts of metals (only zinc was present at higher concentration, see Fig. S1), one can assume that only the metal ions bound to albumin in a specific or nonspecific way can survive this procedure. It is well known that HSA is an important transporter of essential metal ions like Cu^{2+} and Zn^{2+}, but it can also bind many other transition metal ions (Fe^{3+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, etc.), which may contribute to pathological processes (Bal et al., 2013). Some of the detected metals may bind to HSA in forms different from free metal ions, for example as oxides or complexes with organic ligands, but also as nanoparticles. The amounts of selected metals calculated as mole of metal per mole of HSA for samples dialyzed in SRM and ΔC-SRM suspensions at a 2 mg/mL concentration level, are presented in Fig. 1B. ICP-OES analyses revealed an increase in the concentration of Al, Fe, Zn and Pb in the dialyzed protein samples when compared to the bulk suspension (Fig. 1C). Metal capture from the bulk suspension was found also for Cu, Mn and Ti at a level of one order of magnitude lower than for previously listed ions, but still clearly detectable (Fig. 1D). However, less than one equivalent of these metals was bound to HSA. Increased concentration of some metals bound to HSA after dialysis with ΔC-SRM vs. SRM (Ni, Pb) cannot be explained only by mass changes of the particulate matter sample due to the plasma treatment (Fig. S3). It is likely that removal of organic components, which might include also chelating agents, additionally increase the free metal ion concentrations available in suspensions.

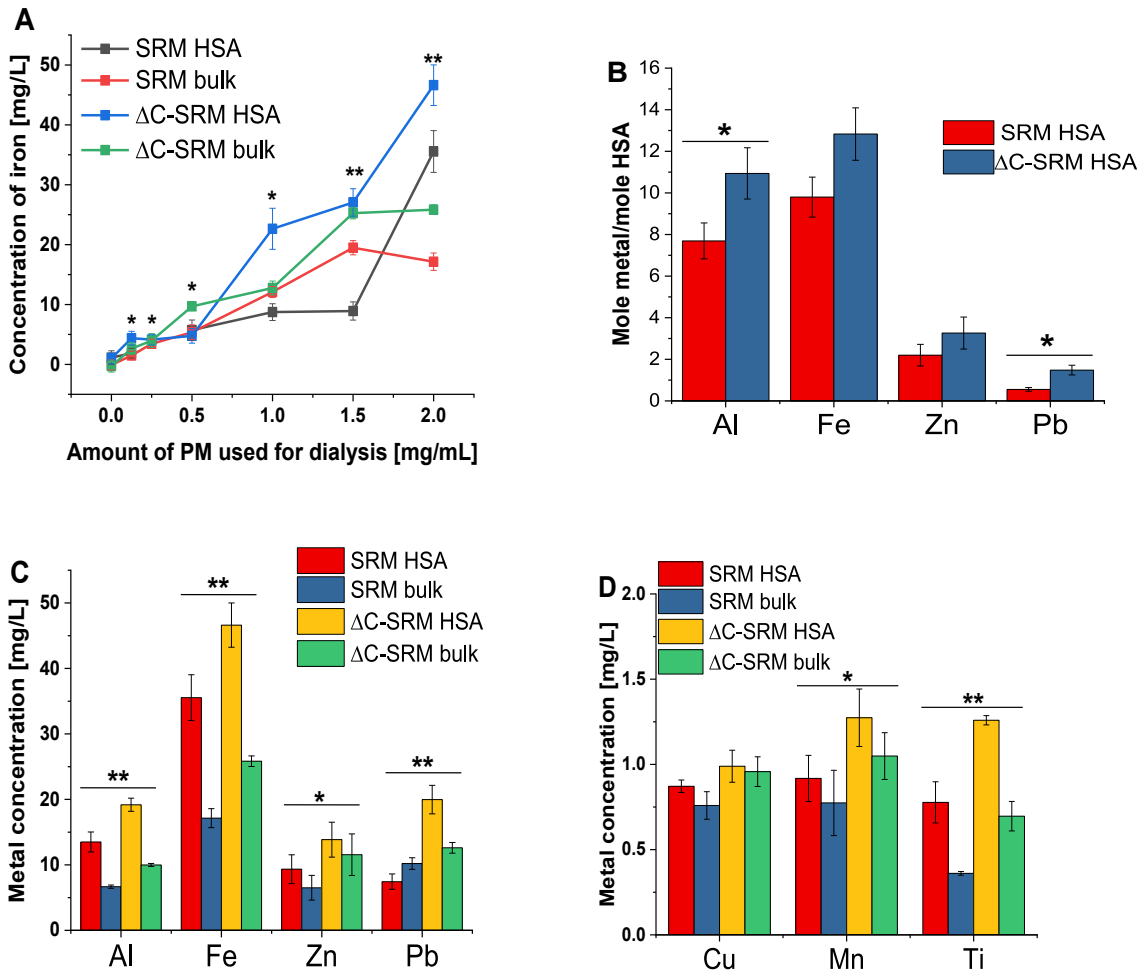


Fig. 1. SRM HSA and ΔC-SRM HSA denote samples of protein dialyzed for 12 h in PM suspension in PBS, followed by 12 h dialysis in PBS. SRM bulk and ΔC-SRM bulk denote PM suspension in PBS, in which the protein was dialyzed for 12 h. **A)** Changes in the iron concentration in HSA and bulk suspension depending on the amount of PM used. **B)** The metal content in HSA dialyzed in 2 mg/mL SRM or ΔC-SRM shown as mole of metal per mole of HSA. **C)** and **D)** The metal content in HSA and in the suspension of PM in PBS, in which the protein was dialyzed when 2 mg/mL SRM or ΔC-SRM was used. *p < 0.05, **p < 0.001.

Such significantly high concentrations of iron and aluminum found in the protein may arise from the interaction of HSA with nanoparticles, e.g. their oxides, which were able to cross the dialysis membrane or were being formed inside the membrane (Sekar et al., 2017; Zolghadri et al., 2010).

3.2. Quantum yield of HSA fluorescence after dialysis

HSA possesses one tryptophan residue (Trp-214), which is responsible for fluorescent properties of this protein. The variations in the fluorescence quantum yield (Q) may be used to monitor the changes in the local microenvironment of the Trp residue. This phenomenon is routinely exploited to follow a variety of protein changes such as ligand/substrate binding or structural changes having different origin (Lakowicz, 2006). As can be seen in Fig. 2, dialysis of HSA in the presence of PM resulted in a decrease in Q, slightly more pronounced for unmodified material (SRM). Since only minor structural changes of the protein occur (see ***Structural and stability changes of protein after dialysis)***, the observed decrease in Q most likely arises from the presence of metal ions bound to the protein (mainly iron, zinc, copper, manganese, etc.), as shown by the ICP-OES studies. It is well known that heavy metal

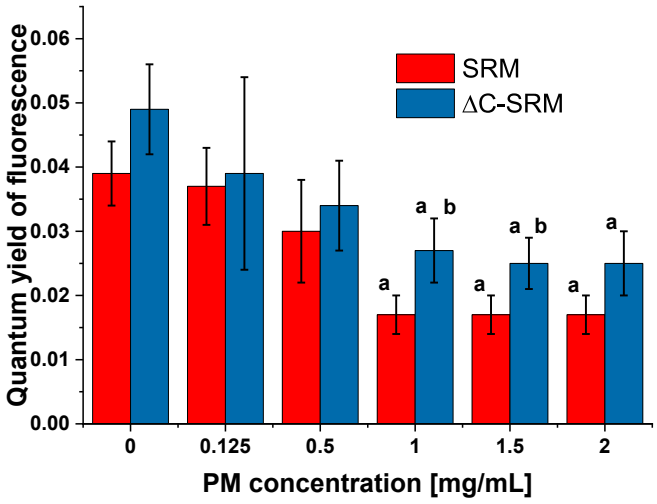


Fig. 2. Changes in quantum yield of fluorescence for human serum albumin dialyzed for 12 h in PBS buffer in the absence and presence of various concentrations of PM (SRM and ΔC-SRM), followed by 12 h dialysis in water. (a) Statistically significant in relation to the control, (b) statistically significant in relation to SRM (p < 0.05).

ions have a quenching effect on fluorescence due to strong spin-orbital coupling. For example, metalloproteins have much lower Q values than the corresponding apo forms (see Table S1). Binding of organic components of air pollutants upon dialysis can account for the stronger quenching of fluorescence in the case of HSA/SRM interaction than in HSA/ΔC-SRM.

3.3. Structural and stability changes of protein after dialysis

Several methods were applied to inspect the influence of SRM and ΔC-SRM on the changes in the structure of HSA that might result either in stabilization or destabilization of the protein. CD spectroscopy was employed to reveal the secondary and tertiary structural modifications. The obtained CD spectra are presented in Fig. 3A. They reveal that dialysis of the protein in PM suspensions did not induce secondary structural changes of HSA since only slight differences are observed in the CD spectra. These suggest that the native structure of the protein was retained. However, the protein upon dialysis in PM suspensions underwent some conformational changes, in particular for the protein dialyzed in the presence of SRM, as was evidenced by an increase in the observed melting temperature (Fig. 3B), which leads to the conclusion that the composition of inorganic components together with organic compounds dissolved in PBS, have a stabilizing effect on HSA.

Furthermore, the impact on protein stability upon dialysis with PM was examined using thermal shift assay (TSA) with fluorescence detection using SYPRO-orange dye. The fluorescence of the solution upon heating increased gradually due to the interaction of the dye with the exposed hydrophobic core of the protein until the protein was completely denatured. The evaluation of the temperature of the hydrophobic exposure, defined as the temperature at which half of the protein population is unfolded, revealed that the dialysis of the protein in the PM suspension slightly stabilized the protein structure (see Fig. S4, see Supplementary Information).

Proteins dialyzed in the presence of PM were also examined for the formation of aggregates. Native gel electrophoresis studies (Fig. S5, see Supplementary Information) and application of size exclusion chromatography (Fig. S6, see Supplementary Information), confirmed that the aggregation of HSA did not occur.

3.4. Inspection of protein changes by evaluation of its affinity towards selected drugs

To inspect how functional properties of HSA can be altered by dialysis in the presence of PM, the binding constants between HSA and several model drugs like warfarin and aspirin were evaluated. Warfarin is an anticoagulant drug commonly used in the prevention of thrombosis and thromboembolism, helping the blood to flow freely through the body by stopping the formation of clots (Ni et al., 2011). Aspirin (acetylsalicylic acid) is a non-steroidal anti-inflammatory drug and it has a wide application due to its analgesic, antipyretic and anti-inflammatory properties (Ni et al., 2011). Warfarin and aspirin are generally regarded as stereotypical marker ligands for site I (Li et al., 2011; Ni et al., 2011). The binding constants were determined using fluorescence quenching by examining the change in the fluorescent intensity of HSA at the selected emission wavelength upon excitation at 295 nm, before and after addition of the drug. This procedure is well established for all studied drugs (Ni et al., 2011; Ràfolsa et al., 2018). A decrease in fluorescence intensity of HSA upon interaction with metal ions (observed in dialyzed samples) does not prevent the determination of the binding constant between drugs and proteins, as was tested by studying the interaction between HSA and warfarin in the presence of different amounts of iron(III) chloride (Fig. S7, see Supplementary Information).

Typical titration spectra and resulting Stern-Volmer dependences for various concentrations of SRM PM present during protein dialysis, are shown in Fig. 4A and B, respectively. Increasing amounts of warfarin decreased the fluorescent intensity of the protein solution at 345 nm while the simultaneous increase of the 400 nm band originates from warfarin (Fig. 4A). The influence of PM on the HSA binding capacity was evaluated by comparison of the binding constant K_b calculated according to eq. (3) at 339 nm to minimize the influence of warfarin fluorescence. The overall results obtained for SRM and ΔC-SRM are presented in Fig. 4C and D, respectively.

Dialyzed samples of HSA with PM have lower HSA-warfarin binding constants than the native dialyzed protein (Fig. 4B). The observed effect is initially concentration dependent, however, increases in the PM concentration range higher than 0.5 mg/mL (ΔC-SRM) and 1 mg/mL (SRM) did not induce a further decrease in the value of the binding constant. The detected changes are more profound for SRM samples. After dialysis with 1 mg/mL SRM sample, K_b dropped to 5% of the initial value, while dialysis with 1 mg/mL of ΔC-SRM caused a 40% decrease of its value. The number of binding sites also slightly decreased in case of both ΔC-SRM and SRM.

Similar studies were performed for aspirin (compare Fig. S8, see Supplementary Information). The calculated aspirin binding

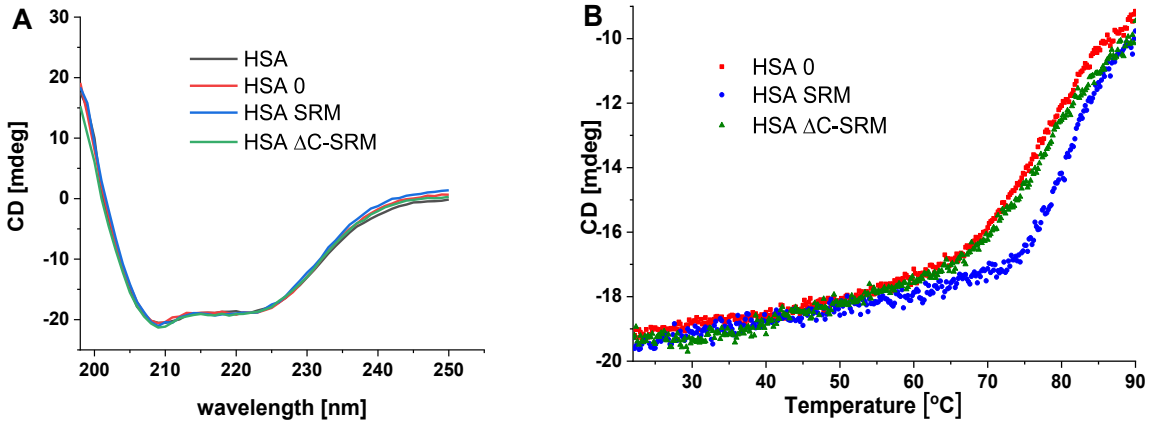


Fig. 3. A) CD spectra of HSA freshly prepared (HSA), dialyzed in PBS (HSA 0) and in PBS suspension of SRM (HSA SRM) or ΔC-SRM (HSA ΔC-SRM) at 2 mg/mL concentration. **B)** Thermal unfolding of protein monitored by CD at 222 nm.

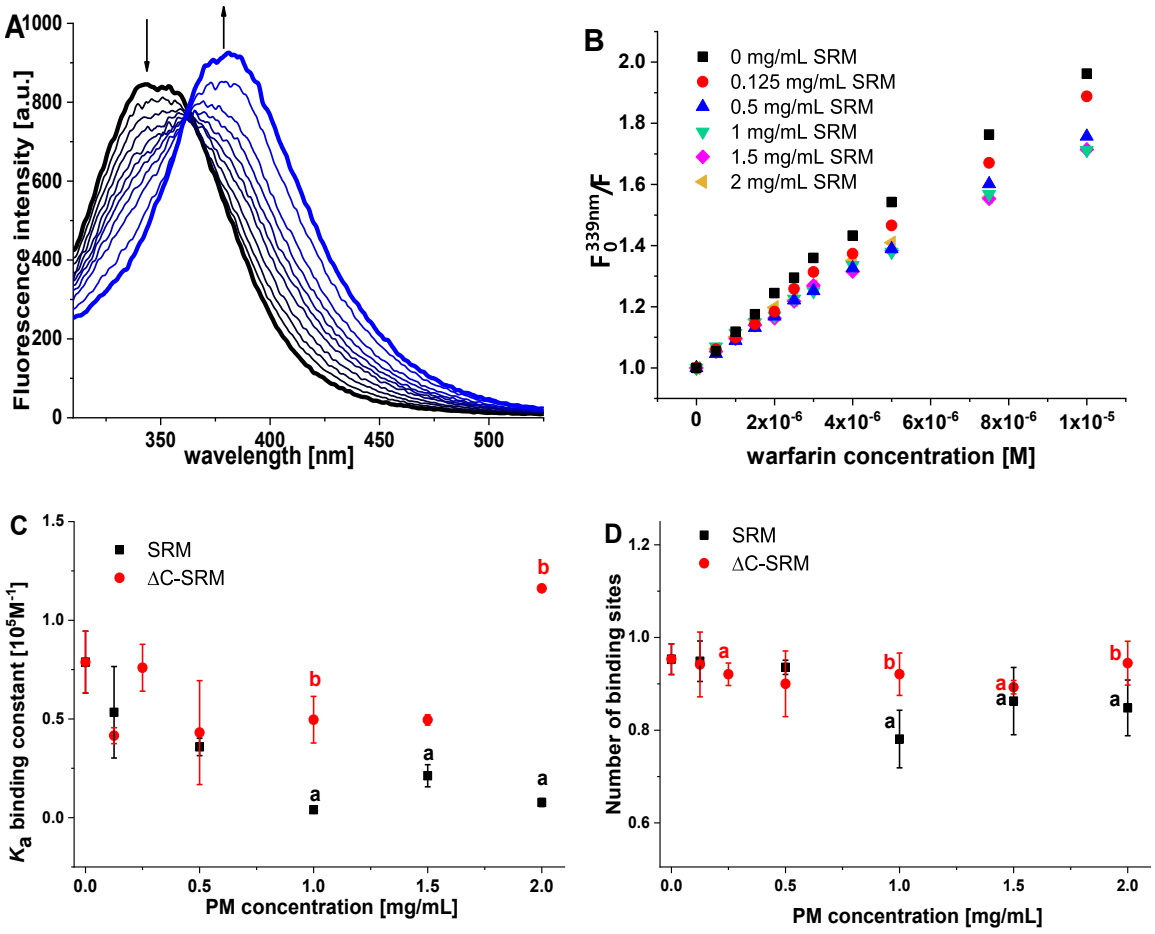


Fig. 4. A) Representative plot of the changes in emission spectra of dialyzed HSA (black bold line) upon titration with warfarin (1 μM) up to a final concentration of 10 μM (blue bold line); **B)** Stern-Volmer plot showing the influence of the increasing concentration of warfarin on the fluorescence intensity of HSA (0 mg/mL of SRM) and HSA-SRM dialyzed samples (0.125−2 mg/mL of SRM); **C**) Changes in the binding constants for HSA-warfarin adducts after dialysis of the protein in PM samples; **D)** The changes in the number of binding sites for HSA-warfarin adducts after dialysis of the protein in PM samples. (a) Statistically significant in relation to the control, (b) statistically significant in relation to SRM ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

constants for dialyzed HSA (Fig. S8C), showed a concentration-independent decrease in value. After the dialysis with PM, K_b dropped up to 20% of its initial value for both SRM and ΔC-SRM samples. The calculated number of binding sites (Fig. S8D) did not change significantly.

3.5. Critical implications of results

One of the most difficult aspects of research concerning the effects of pollution on human health is the fact of chronic exposure to harmful substances that is difficult to imitate under laboratory conditions. The observed changes induced by exposure to urban airborne particulate matter at quantities expected from the known content of PM in air, are usually at a very low level or negligibly small irrespective of whether the research is carried out on model systems (e.g. proteins), *in vitro* or *in vivo*. Therefore, to get any information about impact of air pollution on organism we need to create models, which enables us to detect small differences induced by PM but these models have their limitations. In this study we focused on the soluble part of PM presuming that it is more hazardous one. Our data showed that albumin upon exposure to aqueous extracts of urban airborne particulate matter does not aggregate, its structure is only slightly changed, but it is loaded with a variety of metal ions. In this form albumin might remain in the circulatory system and interfere with other proteins by delivery of accumulated metal ions in an uncontrolled way. We also pointed out that the interaction of albumin modified by PM aqueous extracts with the selected commonly used drugs, is slightly weaker and may interfere with their metabolism. This is our overall hypothesis, especially since up to now no research has been conducted in this area. There is an urgent need for epidemiological studies that will examine the impact of air pollution on the risk of lowering drug efficiency. Recently it has been suggested that the exposure to ambient air pollution was associated with increased risk of drug-resistant tuberculosis, but no mechanism of such resistance was evaluated (Yao et al., 2019). Evidences also showed that environmental pollution may influence insulin sensitivity, and future complications might be prevented by detailed control of exogenous factors and use selectively effective drugs (Latini et al., 2009).

4. Conclusions

In this work, the influence of chemical compounds released from the standard particulate matter (SRM) or their inorganic part (ΔC-SRM) on HSA structure, stability and function was investigated. The dialysis of HSA in the presence of SRM or ΔC-SRM resulted in increased metal concentrations in the treated protein. The

concentrations of some metals in the protein (Al, Fe, Zn, Pb) were higher than in the bulk suspensions, and such ion capturing confirms detoxifying functions of albumin. No significant structural changes of the protein upon dialysis with PM were observed, however increase in the thermal stability of the HSA structure was found.

A significant impact of PM was detected on one of the albumin key functions, which is the binding of drugs. The interaction of HSA dialyzed in the presence of SRM or ΔC-SRM with selected drugs (warfarin, aspirin), negatively affected the functional capability of this model protein. The binding constants decreased, indicating a smaller affinity of the drugs towards the protein modified by PM. Higher quantitative changes in the affinity constants were observed for warfarin than aspirin binding, indicating that this effect depends on the structure of the drug.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Olga Mazuryk: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Visualization, Writing - original draft, Writing - review & editing, Supervision. **Przemysław Gajda-Morszewski:** Methodology, Investigation, Writing - original draft. **Monika Flejszar:** Investigation. **Przemysław Łabuz:** Resources, Investigation. **Rudi van Eldik:** Writing - original draft, Writing - review & editing. **Grażyna Stochel:** Funding acquisition, Supervision. **Małgorzata Brindell:** Conceptualization, Methodology, Validation, Writing - original draft, Writing - review & editing, Supervision.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.114667.

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