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Mechanisms of Oxidative Stress and Vessels Sclerotic Transformation Initiated by Uremic Toxin Indoxyl Sulfate

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Abstract

The microspherical carbonaceous adsorbents SCN and AST-120 for oral use prevent chronic disease progression, delay dialysis initiation, lessen atherosclerosis development in kidney and decreased of overall and cardiovascular mortality. This therapeutic effect is due to the binding of indole by sorbents in large intestine, which is a precursor of indoxyl sulfate (IS). It is considered that IS accelerates the progression of chronical kidney disease (CKD) by inducing a formation of reactive oxygen species (ROS) and promotes aortic calcification (mineralization). Molecular mechanisms of IS action is unknown. Using density functional theory calculations in the frames of B3LYP exchange and correlation functional (basis set 6-311G) and solvation accounting on the base of polarizable continuum model (PCM) we have studied some chemical transformations of IS and have shown a possibility of indoxyl sulfate and hydroperoxyl radicals formation through the reaction of IS with endogenous singlet oxygen. Due to the high activity indoxyl sulfate radicals initiate uncontrollable processes of oxidative stress (OS) in kidney and vascular tissues that promote a development of CKD. We also proposed a hypothesis, which can explain the role of OS in the accelerated development of sclerosis (vessels mineralization) in patients with renal diseases. In particular it was hypothesized and then supported by B3LYP/6-311G(d) + PCM calculations that sulfonic groups (products of deep oxidation of thiol groups in tissue proteins under OS, induced by IS) can selectively bind of Ca^{2+} ions and, consequently, forming RSO_3Ca^+ groups which can fix HPO_4^{2-} and CO_3^{2-} anions. The products of anions fixation can then bind of Ca^{2+} ions, etc. Notably, these processes are, probably, primary starting point in case of sclerotic vessel changes. The beginning of this starting mineralization process most likely is possible with proteins carboxyl groups forming under OS that also can bind Ca^{2+}.

1. Introduction

Traditionally to prevent of CKD progression on the early stage is used a control of dietary factors, lipids and mineral balance, administration of hypotensive drugs and others. At the same time, clinical studies were made by one of the article authors in the early 80s of last century [1, 2] had showed, for the first time, a high therapeutic efficiency of spherically granular highly porous activated carbons with trade mark SCN (Fig. 1), developed by us [3–5] for therapy of kidney diseases (including a prevention of CKD progression). These medical sorbents were used for extra corporeal blood purification [1, 3] and as oral sorbents (enterosorbents) [2, 5]. Particularly, in [2, 5] was shown that the clinical usage of this spherical carbons orally decreases the main symptoms of uremic intoxication, namely: significantly softens (offset) the uremia symptoms, effectively reduces the level of uremic toxins (creatinine, uric acid, urea), olygopeptides, cholesterol and lipids, and also lipid peroxides, delays the dialysis initiation and increases actuarial life expectancy (actuarial survival) of patients [5].

Analogues of such spherical activated carbon AST-120 (Fig. 1) had also been developed in Japan (Kureha Corporation Tokio, Japan) [6] but their clinical usage as oral sorbents for control of body uremic toxins were started only in 1991 [6]. The Japanese colleagues obtained almost the same positive therapeutic effects of such carbons, information about which we had published previously for carbons SCN [1].

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(a)

(b)

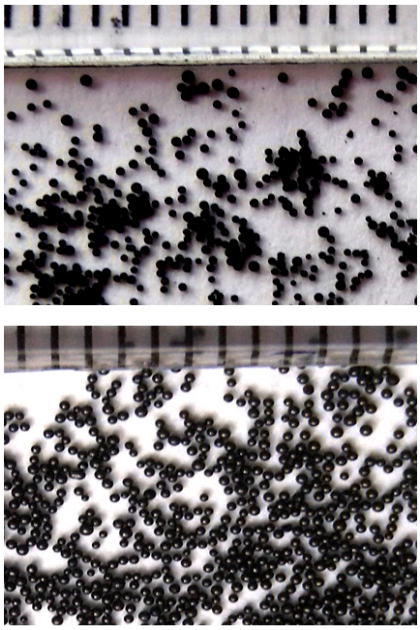


Fig. 1. Microspherical carboneseous oral sorbents SCN (a) and AST-120 (b).

It is well known that uremic toxins accumulate in blood and tissues of renal patients and disturb some biological functions of organism. The most dangerous uremic toxin is organic sulfonic acid – indoxyl sulfate (IS) accelerates chronical kidney disease (CKD) progression by inducing a formation of reactive oxygen species (ROS) up to oxidative stress (OS) and promotes aortic calcification [7, 8]. Overall in detail about oxidative stress, then IS deregulate oxygen metabolism by increasing oxygen consumption in proximal renal tubules, decreasing renal oxygenation and consequently cause a hypoxia in kidneys [9]. A precursor of indoxyl sulfate is indole, which, in turn, is produced by the action of colonic bacterias *E. coli* on dietary amino acid tryptophan, originated from food proteins (especially meat) [7, 8].

However, despite the attractiveness of the theory of uremic and cardiovascular IS toxicity, proposed by T. Niwa [7, 8], still remain the doubts about the adequacy of understanding and confirmation of the possible molecular mechanisms of IS pathogenic influence. On the one hand, it is established exactly that IS induces pathological processes in kidneys and blood vessels by initiation of OS. However, it is not clear whether the IS can play a role not only as initiator of formation of free radicals in balanced biological processes, but also as a specific reagent, capable to generate them by participation in specific biochemical reactions in which are produced ROS. One of the reasons for such doubts are the results of the work [10], in which was studied the effect of IS on a red-ox status of rat mesangial cells and a generation of ROS. The investigations had showed by using fluorescence and EPR spectroscopy [10] that there was a symbate dependency between ROS accumulation inside cells and a concentration of the input IS.

It is established also that the ROS generation in life processes is carried out with the participation of NADPH-oxidaze. However, as it was shown in [10], an inhibitor of this enzyme (diphenylene iodinium) suppressed the production of superoxide radicals only at low or moderate concentrations of IS and had no significant effect when large quantities of IS were administered, that indicates another way of $O_2^{\cdot -}$ generation, without NADPH. The authors [10], using EPR method with intra- and extracellular spin trapping, had shown that IS increased production of $O_2^{\cdot -}$ radicals outside the cells and $HO^{\cdot}$ radicals within cells. Taking into account this data it is logical, in our view, to suggest that IS directly (besides NADPH-oxidaze) involves in biochemical processes (in the case of kidney failure), which lead to the formation of ROS in cells and extracellular fluid. Taking into account this data it is logical, in our view, to suggest that IS directly involves in biochemical processes and can react with active endogenous molecules. Interaction IS with active acceptors of hydrogen atom may be lead to the formation of free radicals in cells and extracellular fluid.

The reality of this hypothesis is possible to confirm in some ways by screening the heat effects (difference of total energies reagents molecules and reaction products) of the most probable reactions of radical formation and their following transformations (including IS) through simulation of various processes by using modern *ab initio* methods of quantum chemistry.

2. Methods of quantum chemical calculations

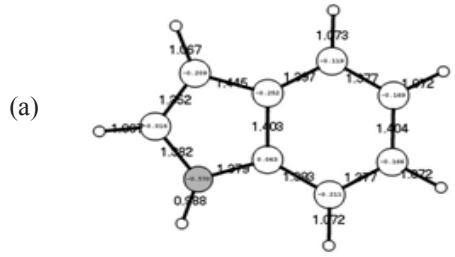
In this paper calculations of energies were performed by methods of density functional theory using well known effective B3LYP exchange and correlation functional with extended split valence basis set 6-311G (for modeling of vascular mineralization reactions we used 6-311G(d) basis set). All calculations were performed by using GAMESS software package [11] (version Firefly8, worked by A. Granovsky (Moscow University, Russia)) on a dual-core computer YI PC (2.0 GHz) with full geometry optimization of studied molecules, radicals, ions and solvation accounting in the frames of polarizable continuum model [12].

Total energies of small negative ions ($O_2^{-\cdot}$, HOO, HO^-) were calculated by adding of experimental electron affinity values of related compounds (O_2, $HO^\cdot$, $HOO^\cdot$) to its total energies.

3. Results and Discussion

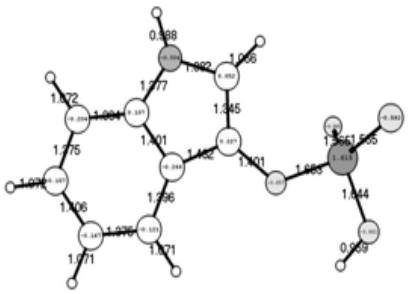
3.1. Molecular mechanisms of oxidative stress inducing by indoxyl sulphate

The equilibrium structure of indoxyl sulfate molecule, lengths of valence bonds, charge distribution, energy of frontier molecular orbitals, and ionization potential are shown in Fig. 2. On the same Fig. 2, analogues information for indole is presented for comparison.



(a)

E_{HOMO}= -7.86 eV E_{LUMO}= 3.18 eV IP = 6.86 eV



(b)

E_{HOMO}= -8.44 eV E_{LUMO}= 2.37 eV IP = 7.29 eV

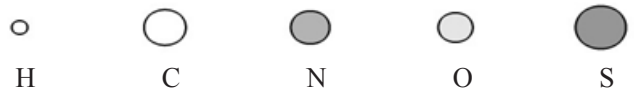


Fig. 2. Equilibrium structure and characteristics of indole (a) and indoxyl sulfate (b) molecules: valence bond distances (Å), Mulliken charges on atoms (a.u.), energy of higher occupied (E_{HOMO}) and lower unoccupied (E_{LUMO}) molecular orbitals and ionization potentials (eV), calculated by restricted Hartree-Fock method (basis set 6-311G).

Analysis of data presented in Fig. 2 does not reveal any specific features about electronic structure of the molecule IS, that can be clarified the special reactivity in the transformation to radicals. It would seem rather indole, which has a lower ionization potential (i.e. greater ability to electron transfer) can play some role in free radical generation, moreover, indole is easy oxidized by molecular oxygen [13]. Without going into details of all our quantum chemical calculations in order to analyze the possible mechanisms of indole (IndH) oxidation, we note only that the greatest exothermic effect (the highest probability) makes the process, going through the intermediate formation of radicals $Ind^\cdot$ and $HO_2^\cdot$ and leading to formation of indole-3-hydroperoxide [13]. The further products of indol-3-hydroperoxide interaction with water molecule according to [13] are indoxyl (in a keto-form) and H_2O_2. Therefore it is logical to assume that indole is not able to play a significant role in the real processes of $O_2^{\cdot-}$ and other free radicals generation in a human body.

To determine the potential IS ability of radical forming, it is reasonable to consider the energy of some chemical reactions of IS which can generate the expectable products such as ROS, including a detectable in the oxidative stress – superoxide radical $O_2^{\cdot-}$.

Due to the mentioned above our hypothesis, any exothermic reaction of IS (or the products of its transformations) with substances really present in biological media, that lead to the formation of radicals can be potentially promising. It is reasonable and enough interesting for this purpose to consider a possibility of reaction between IS and an enzymatically formed singlet oxygen $^1O_2$, that in this electronic state have a significantly higher reactivity than normal triplet oxygen $^3O_2$ [14]. The molecule transition from a triplet $^3O_2$ to singlet state is associated with the spin transposition of one of the π*-electrons, which associate with the excitation energy expenditure. In this case, the electrons with opposite spins may be located on the same orbitals π2px* and π2py*, similar in $^3O_2$ (short-lived state $^1\Sigma_g$ molecule [14]). In the case of the electron location on one orbital π2px* is realized chemically meaningful state $^1\Delta_g$ of singlet oxygen with a significantly greater lifetime [14].

It was found that singlet oxygen $^1O_2$ is formed in the body by myeloperoxidase – enzyme of activated phagocytes. The quantity of formed $^1O_2$ may reach up to 19% of all oxygen absorbed by phagocytic cells [15]. Additional sources of $^1O_2$ may be the reaction between superoxyl radical and H_2O or dismutation of $O_2^{-\cdot}$ induced by water [16, 17]. Due to the high reactivity of singlet oxygen, it causes a lipid peroxidation, oxidative damage of proteins, in particular, collagen, skin epithelium, catalase and superoxide dismutase [18, 19], DNA [20] and others, and thereby display its pathogenic functions [18, 20–22]. Taking into account all specific properties of singlet oxygen, primarily its high reactivity,

it is logical to suggest a possibility of reaction of a hydrogen atom transfer from IS on $^1O_2$ with formation of a radical of indoxyl sulfate. The argument for the possibility of such transfer are data of the articles [23, 24] about antioxidant properties of IS in normal physiological conditions that illustrated the ability of IS to neutralize of hydroxide radicals. It is showed that indoxyl sulfate is able to neutralize super oxide anion radicals, i.e. IS has also prooxidant properties. The interaction of IS with $HO^\cdot$ and $O_2^{\cdot-}$ proceed apparently in accordance with reactions:

$$IndOSO_2OH + O_2^{\cdot-} \rightarrow IndOSO_2O^- + HOO^\cdot, \quad (1)$$

$$IndOSO_2OH + HO^\cdot \rightarrow IndOSO_2O^\cdot + H_2O. \quad (2)$$

The results of our DFT calculations confirm exothermic character of these reactions with heat effects 143.9 kJ/mol and 228.7 kJ/mol accordingly.

Indeed, our calculations show also that the interaction between molecule of indoxyl sulfate and $^1O_2$ ($^1\Delta_g$) that simulates one of the possible non-physiological processes of radical form of IS generation is an exothermic reaction:

$$IndOSO_2OH + {}^1O_2 \rightarrow IndOSO_2O^\cdot + HOO^\cdot + 176.9 \text{ kJ/mol}, \quad (3)$$

By act of Bell-Evans-Polanyi principle it is logically to assume that this reaction can be realized without a significant activation barrier, producing highly reactive radicals $HOO^\cdot$ and $IndOSO_2O^\cdot$. Scan linear cross section of the total energy surface in the frame of DFT/6-311G(d) approximation along the line of drawing nearer ($IndOSO_2O$) H – $^1O_2$ (reaction coordinate) revealed absence of energy maxima above ~22 kJ/mol concerning of reagents total energies sum. This result confirms our assumption about comparatively low activation barrier of reaction (3) in spite of failure in location of its transition state, due to small curvature of energy surface near zone of formation and break of hydrogen bonds.

Hydroperoxyl radical $HOO^\cdot$ can easily penetrate the lipid fragments of cell membranes, and initiate the oxidation of unsaturated fatty acids to peroxides, with forming of H_2O_2 [18, 25], which may be one of the demonstration factors of oxidative stress caused by IS.

Calculated by us the value of the electron affinity for radical $IndOSO_2O^\cdot$ ($A_{IndOSO_2O^\cdot} = 3.25$ eV) significantly higher than the experimental value of the electron affinity even for such superactive radical as $HO^\cdot$ with $A_{HO^\cdot} = 1.828$ eV. For comparison, the experimental values of electron affinity for $CN^\cdot$, $NCO^\cdot$, $NCS^\cdot$ radicals are 3.862, 3.609, 3.537 eV, respectively [26]. Therefore, it is possible to suggest that even the interaction between $IndOSO_2O^\cdot$ and HO^- anion can lead to the formation of hydroxide radicals $HO^\cdot$. The argument for the possibility of such non-physiological generation of radicals $HO^\cdot$, with conditional presence of IS in biological media, are the results of heat effect calculation of the corresponding model reaction which is, as expected, really exothermic:

$$IndOSO_2O^\cdot + OH^- \rightarrow IndOSO_2O^- + HO^\cdot + 395.8 \text{ kJ/mol}, \quad (4)$$

Thus, the exothermic nature of the processes (1) and (2) makes the reasons with high assurance to suggest that IS participate in reactions that lead to ROS formation in biological media, namely radicals $HOO^\cdot$ and $HO^\cdot$. Concerning the possibility of involving IS in the reactions, which directly lead to the formation of $O_2^{-\cdot}$ [10], the calculated data show such possibility only when IS interaction with singlet oxygen in a reaction medium which contains enough strong proton acceptors, for example, the anion HPO_4^{2-}:

$$IndOSO_2OH + {}^1O_2 + HPO_4^{2-} \rightarrow IndOSO_2O^\cdot + O_2^{-\cdot} + H_2PO_4^- + 192.0 \text{ kJ/mol}. \quad (5)$$

The reaction (5) is exothermic and in principle can correspond to the actual generation process of oxygen anion-radicals due to the IS accumulation in kidneys and other organ and tissues.

Analyzing the entire set of potentially interesting formation processes of ROS in biological media, it is necessary to consider the possibility of recombination of radical pairs $HO^\cdot$ – $HO^\cdot$, $HOO^\cdot$ – $HOO^\cdot$ and $HOO^\cdot$ – $HO^\cdot$, that can lead to the formation of hydrogen peroxide and relatively unstable dihydrogen polyoxides (HOOOOH and HOOOH) [27–29], further transformations of which are accompanied by the formation of ROS such as singlet oxygen and ozone. It is established that in biological fluids even possible the process of water oxidation by singlet oxygen with forming H_2O_2 and O_3 [30]. It is possible to assume that HOOOH is an intermediate of this transformation. It is also reasonable to mention that even trace amounts of Fe^{2+} or Cu^+ ions can catalyze Fenton reaction, namely decomposition of H_2O_2 to $HO^\cdot$ and HO^- [14]. The product of reaction H_2O_2 with HO^- – anion of hydrogen peroxide HOO^- can efficiently interact with active reagents such as $^1O_2$ and $HOO^\cdot$, that con-

firms by modeling transformations:

$$HOO^- + {}^1O_2 \rightarrow HOO^{\cdot} + O_2^{-\cdot} + 189.1 \text{ kJ/mol}, \quad (6)$$

$$HOO^- + HOO^{\cdot} \rightarrow O_2^{-\cdot} + H_2O_2 + 149.0 \text{ kJ/mol}. \quad (7)$$

It is suggest that all of these reactions with the formation of ROS and the typical active intermediates generally determine the origin and development of oxidative stress, initiated by indoxyl sulfate, but, apparently, do not exhaust variety of actually occurring redox transformations in organs and tissues in the created IS adverse conditions.

Logically, it is also possible to expect enough easy regeneration of IS, due to high reactivity of radical $IndOSO_2O^{\cdot}$ and significant proton affinity of anion $IndOSO_2O^-$ and further procession with generation of free radicals. Proportionally to accumulation of IS in kidneys, the negative influence of the number free radicals will increase up to the oxidative stress in renal cells with progressive renal dysfunction.

To summarize all mentioned above, should be emphasized the fact that the IS induces the generation of free radicals in kidneys and nephrotoxicity of IS may be caused by impairing the kidney's antioxidative systems [7]. The data of our quantum chemical calculations demonstrate the possible mechanisms on molecular levels and causes of this phenomenon.

3.2. A possible mechanism of aortic calcification, induced by IS

At the same time, very interesting is the fact that according to a number of studies [7, 8] IS promotes vascular (aortic) calcification (mineralization) and stimulates glomerular sclerosis, but its mechanism remains poorly understood. Meanwhile, the investigation of indoxyl sulfate influence mechanism on vascular calcification is highly relevant: in fact cardiovascular diseases accounts from 40 to 60% death among dialysis patients, and its mortality among haemo- and peritoneal patients is much higher [8].

From our point of view, mentioned cardiovascular effect of the IS is also associated with its ability to promote ROS generation and to cause an oxidative stress. In this case, the role of IS is add up to the fact that in a zone of oxidative stress (vascular proteins and glomerular tissues) is occurred intensive oxidation of a large group of biomolecules, including nucleic acids and lipids. However, due to their higher content in tissues, proteins are preferential targets [31]. Detailed consideration about the possible influence of oxidative stress on proteins and the role of oxidative transformations on the cardiovascular morbidity of CKD patients, in our opinion, lead to the conclusion that the most significant is an oxidative conversion of protein thiol groups (R-SH) to irreversible higher oxidation states, namely to sulfonic groups ($R\text{-}SO_3H$ functionality). In fact, namely thiol groups provide antioxidant protection of tissues, and therefore ROS have to react with them primarily in the oxidative stress conditions. The evidences about possibility of such processes are shown in [32], where was studied the oxidative conversion of thiol groups of albumin into corresponding sulfur-containing acidic groups. The obtained in this case oxidized albumin can be considered, in our opinion, as a protein, that in some way simulate the transformations of polypeptides in tissues in oxidative stress conditions. It is well known that polypeptide fragments of proteins, in particularly bind by disulfide bonds –S–S– which may also can be oxidized in sulfonic groups [32]. This means that an additional source of sulfonic groups in proteins under oxidative stress conditions also can be oxidized –S–S– groups.

Taking into account the known high affinity of sulfonic groups towards Ca^{2+} cations it is possible to suggest that the origin of calcification in the oxidative stress areas may be as result of initial strong binding of calcium ions by sulfonic groups. For quantum chemical estimation of this suggestion realizability it is essential to choice a representative models of sulfur containing proteins. If as a model protein with sulfonic groups we used an oxidized cysteine-containing fragment of albumin, simulated by a minimal cluster $CH_3NHCOCH(NH_2)CH_2SO_2OH$ (for short $R_{alb}SO_2OH$), then the initial binding of Ca^{2+} can be presented as a simple reaction:

$$R_{alb}SO_2O^- + Ca^{2+} \rightarrow R_{alb}SO_2OCa^+ + 109.2 \text{ kJ/mol}, \quad (8)$$

Positive heat effect of the reaction (8) indicates the possibility for realization of such process of the Ca^{2+} binding.

However, the excess of phosphate anions in biological fluids of the renal patients inevitably promote their interaction with RSO_3Ca^+ groups with a strong fixation of phosphate groups according to scheme (9), due to the fact that calcium phosphates are insoluble:

$$R_{alb}SO_2OCa^+ + HPO_4^{2-} \rightarrow R_{alb}SO_2OCaOPO(OH)O^- + \\ +134.3 \text{ kJ/mol}. \quad (9)$$

Naturally, that the terminal $–OPO(OH)O^-$ group

in turn can selectively bind calcium, that proves the exothermicity of the reaction :

$$R_{alb}SO_2OCaOPO(OH)O^- + Ca^{2+} \rightarrow R_{alb}SO_2OCaOPO(OH)OCa^+ + 189.1 \text{ kJ/mol.} \quad (10)$$

It is clear that in the continuation of the process (10) can be realized the further binding of hydrophosphate groups according to a reaction:

$$R_{alb}SO_2OCaOPO(OH)OCa^+ + HPO_4^{2-} \rightarrow R_{alb}SO_2OCaOPO(OH)OCaOPO(OH)O + 213.8 \text{ kJ/mol,} \quad (11)$$

which then can fix Ca^{2+}, etc.

The forming calcium phosphate chain may be broken off in case of availability of sterically favourable contact with an anion center $–RO^-$ on one of the polypeptide fragments. It is arguable that the chain breaking on such anionic centers cause, in fact, cross-linking of peptide fragments located at different distances:

$$\begin{array}{c} O \\ \| \\ –(OCaOPO)_nCa^+ \\ | \\ OH \end{array} + O^-–R– \rightarrow \begin{array}{c} O \\ \| \\ –(OCaOPO)_nOR– \\ | \\ OH \end{array} . \quad (12)$$

It is undoubtedly that such cross-linking will reduce a flexibility of polypeptide chains and thus reduce the elasticity of blood vessels (increasing their stiffness), as observed in their sclerotic transformations. As for the possible presence of anionic centers of $R\text{-}O^-$ in adjacent fragments of polypeptides, the anionic oxygen can be not only in the sulfonic, but also in others, most likely in carboxyl groups, which also have the increased affinity to Ca^{2+} ions. This is possible, if we take into account the data [32], according to which the molecule of native albumin (a model protein) at pH 7.4 has ~ 19 negative charges in polypeptide chains. It seems that fragments with type (11) and (12) occurring in oxidative stress conditions on the intravascular surface of vascular proteins may also play role as formation centers for calcium sclerotic plaques, because the presence of anions ($R_1–O^-$) and cations ($R_2–Ca^+$) groups in these mineral fragments opens also the way to three-dimensional growth of these mineral-organic formations. On the base of data of work [33] in which was established the formation of amorphous calcium phosphate in the initial stages of mineralization we supposed that the reactions (8) – (11) reflect, obviously, only initial (starting) elementary acts of this process. It is logical to suggest also a possibility of oxidized cholesterol participation in the formation process of cholesteric plaques [33, 34]. This compound, apparently, is capable to enter into esterification reactions with PO_3OH groups calcium-phosphate chains.

Taking these assumptions into account, as well as high affinity of Ca^{2+} to carbonate anions, it can be suggested that the groups $R_{alb}SO_2OCa^+$ can bind not only the phosphate anions, but also carbonate anions; as showed our quantum chemical calculations, the following model reaction can be a confirmation of such binding:

$$R_{alb}SO_2OCa^+ + CO_3^{2-} \rightarrow R_{alb}SO_2OCaCO_3^- + 2536 \text{ kJ/mol.} \quad (13)$$

By-turn, the development of a process may lead to the binding of Ca^{2+} cations in the same manner as in the case of calcium phosphate chains (8). Confirmation of this can be data [32], which noted the presence of certain amounts of Ca and Mg carbonates in products of tissue mineralization. Clearly that a formation of calcium phosphate or calcium carbonate cross-linking of the proteins of polypeptide chains under oxidative stress conditions will depend on the ratio of HPO_4^{2-} and CO_3^{2-} anions in biological fluids. It is also undoubtedly that these processes should affect the vessel mineralization. We also believe that the reactions (8–12) can be considered as the real processes of bioceramics bioactive fixation in living tissue and the hydroxycarbonate apatite formation at implant zone results from ion exchange binding of Ca^{2+} and HPO_4^{2-} ions in their surface layer [35].

However, the confirmation of the validity of our hypothesis about the role of IS at mineralization process and sclerotic changes in blood vessels and tissues requires the conducting of a complex of specialized in-depth investigations.

4. Conclusions

To summarize, it should be emphasized one more time that mentioned above our hypothesis about a possibility of direct indoxyl sulfate participation in the initiation uncontrollable by antioxidant systems development of free-radical oxidation processes in renal and vascular tissues was confirmed by our quantum chemical calculations.

Indeed, we have shown that a highly active singlet oxygen (produced *in vivo*) can react with indoxyl sulfate and form, respectively, very reactive radical $IndOSO_2O^{\cdot}$ and ROS: $HO^{\cdot}$, $HOO^{\cdot}$, and also

superoxide radical $O_2^{\cdot -}$ (Schemes 3-7). Our calculated data show that the radical $IndOSO_2O^{\cdot}$ with abnormally high electron affinity, can apparently not only react with any biological molecules, but also can oxidize even anion OH^-, transforming it into a radical $HO^{\cdot}$.

In the development of the mentioned hypothesis about the formation process of ROS with self-initiation by indoxyl sulfate, and, consequently, with its inducing of oxidative stress in renal and vascular tissues a mechanism of their mineralization was proposed. The essential point in the mechanism of sclerotic calcification, phosphatization and carbonization is the oxidation process under oxidative stress conditions of protein thiol groups into sulfonic groups that bind calcium. Associated with protein fragments of calcium sulfonate, in turn, can firmly fix phosphate and carbonate anions, which then in addition bind and retain Ca^{2+} cations, etc. Ultimately, proteins in renal and vascular tissue cross-links by calcium phosphate and carbonate bridges, which reduces the flexibility of neighboring polypeptide chains (reduces the number of possible conformational transformations) and thereby reduces the elasticity of blood vessels and, consequently, causes their growing sclerotic changes.

References

[1]. S.I. Ryabov, G.D. Shostka, B.G. Lukichev, and V.V. Strelko, Intern. Urology and Nephrology 16 (1984) 345–360.

[2]. G.D. Shostka, S.I. Ryabov, B.G. Lukichev, and V.V. Strelko, Therapeutic archive 56 (1984) 58–63 (in Russian).

[3]. V.G. Nikolaev, and V.V. Strelko, Hemosorption on activated carbons (Russ.), Kiev: Naukova dumka, (1979) 287 p.

[4]. V.V. Strelko. Intern. Conf. Carbon 90, France, Paris. Extend. Abstr. (1990) 16–17.

[5]. B.G. Lukichev, G.D. Shostka, V.V. Strelko, T.S. Azizova, Yu.R. Kavrayski, and I.Yu. Panina, Soviet Archives of Internal Medicine 64 (1992) 501–504.

[6]. T. Niwa, Y. Emoto, K. Maeda, Y. Uehara, M. Yamada, and M. Shibata, Nephrol. Dial. Transplant. 6 (1991) 105–109.

[7]. T. Niwa, Nagoya J. Med. Sci. 72 (1-2) (2010) 1–11.

[8]. T. Niwa, Uremic toxins (2012) Wiley&Suns.

[9]. C.-K. Chiang, T. Tanaka, and M. Nangaku, J. Renal Nutr. 22 (1) (2012) 77–80.

[10]. A.K. Gelasco, and J.R. Raymond, Am. J. Physiol. Renal Physiol. 290 (6) (2006) F1551-F1558.

[11]. M.W. Schmidt, K.K. Baldridge, J.A. Boatz et al, J. Comput. Chem. 14 (11) (1993) 1347–1363.

[12]. J. Tomasi, B. Mennucci, and R. Cammi, Chem. Rev. 105 (8) (2005) 2999–3093.

[13]. T. Eicher, and S. Hauptmann, The chemistry of heterocycles. Structure, reactions, synthesis, and applications. Second ed., Wiley-VCH GmbH&Co. KGaA, Germany (2003) 556 p.

[14]. N.N. Greenwood, and A. Earnshaw, Chemistry of elements. Second ed., Butterworth-Heinemann, Oxford, England (1998) 1304 p.

[15]. M.J. Steinbeck, A.U. Khan, and M.J. Karnovsky, J. Biol. Chem. 267 (19) (1992) 13425–13433.

[16]. R.E. Lynch, and I. Fridovich, Biochim. Biophys. Acta 571 (2) (1979) 195–200.

[17]. E.J. Corey, M.M. Mehrotra, and A.U. Khan, Biochem. Biophys. Res. Commun. 145 (2) (1987) 842–846.

[18]. V.A. Kostiuk, and A.I. Potapovich, Bioradicals and Bioantioxidants (Russ.), Belarusian State University Publishing House, Minsk, Belarus, (2004) 174 p.

[19]. M.D. Carbonare, and M.A.Pathak, J. Photochem. Photobiol. B 30 (1-3) (1992) 105–124.

[20]. H. Sies, and C.F.M. Menck, Mutation Res. 275 (3-6) (1992) 367–376.

[21]. J.S. Zigler, J.D.Goosey, Photochem. Photobiol. 33 (6) (1981) 869–874.

[22]. R.C. Kukreja, M.L. Hess, Cardiovascular Res. 26 (6) (1992) 641–655.

[23]. Y. Miyamoto, Y. Iwao, Y. Tasaki, K. Sato, Y. Ishima, H. Watanabe, D. Kadawaki, and T. Maruyama, M. Otagiri, FEBS Letts. 584 (13) (2010) 2816–2820.

[24]. J.M. Gebicki, and H.J. Bielski, J. Am. Chem. Soc. 103 (23) (1981) 7020–7022.

[25]. Lange's Handbook of Chemistry, fifteenth ed. McGraw-Hill Inc., New York et al. (1999) 1561 p.

[26]. B. Plesničar, Acta Chim. Sloven. 52 (1) (2005) 1–12.

[27]. X. Xu, R.P. Muller, and W.A Goddard III, Proc. Natl. Acad. Sci. USA, 99 (6) (2002) 3376–3381.

[28]. X. Xu, and W.A. Goddard III, Proc. Natl. Acad. Sci. USA 99.(23) (2002) 15308–15312.

[29]. A.D. Wenworth, L.H. Jones, P. Wenworth, K.D. Jauda, and R.A. Lerner, Proc. Natl. Acad. Sci. USA 97 (20) (2002) 10930–10935.

[30]. M.J. Davies, Biochim. Biophys. Acta 1703 (2) (2005) 93–109.

[31]. L. Turell, S. Carballal, H. Botti, R. Radi, and B. Alvarez, Braz. J. Med. Biol. Res. 42 (4) (2009) 305–311.

[32]. N.B. Kavuçkuoglu, Q. Li, N. Pleshko, and J. Uitto, Matrix Biol. 31 (4) (2012) 246–252.

[33]. C.Y. Lin, and D.W.Morel, J. Lipid Res. 42 (13) (2003) 3949–3955.

[34]. J.X. Rong, S. Rangaswamy, S. Lijang, R. Dave, Y. Chtang, H. Peterson, H.N. Hodis, G.M. Chisolm, M. Guy, and A. Sevanian, Arterioscler. Thromb. Vascular Biol. 18 (12) (1998) 1885–1894.

[35]. A.J. Salinas, and M. Vallet-Regi, RSC Adv. 3 (28) (2013) 11116–11131.