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Metal and Ligand Control on Structure and Functions of Ni(Fe)ARD: Role of Supramolecular Structures

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Abstract: Role of Ni(Fe)-macrostructures due to H-bonds in mechanisms of Ni(Fe)ARD action in methionine salvage pathway is discussed. The AFM method was used to research the possibility of the formation of stable supramolecular nanostructures based on Ni(Fe)ARD model systems {Ni(acac)$_2$ + L^2 + Tyr} (L^2 = NMP (NMP = N-Methyl-2-pirrolidone), His (His = L-Histidine), Tyr (Tyr = L-Tyrosine)—with the assistance of intermolecular H-bonds. In the course of scanning of investigated samples, it has been found that the structures based on model systems are fixed on a surface strongly enough due to H-bonding. The self-assembly-driven growth of the supramolecular structures on modified Silicone surface based on researched complexes, due to H-bonds and perhaps the other non-covalent interactions was observed.

Key words: Systems {Ni(acac)$_2$ + NMP(or His) + Tyr}, models of Ni(Fe)ARD, nanostructures, dioxygen, AFM.

1. Introduction

The MSP (methionine salvage pathway) (fig. 1) plays a critical role in regulating a number of important metabolites in prokaryotes and eukaryotes. MSP is a ubiquitous pathway found in plants, animals, and bacteria. MTA (methylthioadenosine) is the first intermediate in this pathway and is formed from SAM (S-adenosyl methionine) during polyamine synthesis in animals and ethylene synthesis in plants. Polyamine is required for cell growth and proliferation, and ethylene is required for ripening of fruits and vegetables. MTA is an inhibitor of both polyamine synthesis and reactions of transmethylation. Inhibition of polyamine synthesis arrests DNA replication, and elevated polyamine is associated with tumor formation. Hence, the concentration of MTA in cells must be tightly regulated. The MSP controls the concentration of MTA by returning it through a series of reactions to methionine, thereby “salvaging” the thiomethyl group of SAM. Acireductone Dioxygenases Ni(Fe)ARD (both of these enzymes were discovered in the laboratory of Prof. R. Abeles [1]) are enzymes involved in the methionine recycle pathway [1]. Unusual is that regioselectivity of the oxidative carbon-carbon bond cleavage of substrate Acireductone (1, 2-dihydoxy-3-keto-5-(thiomethyl)pent-1-ene), catalyzed with Ni(Fe)ARD is determined by the nature of the metal ion. Fe-ARD catalyzes the 1,2-oxidative decomposition of Acireductone to formate and 2-keto-4-(thiomethyl) butyrate (KMTB), the keto-acid precursor of methionine. Ni-ARD catalyzes a 1,3-oxygenolytic reaction, yielding formate, carbon monoxide, and 3-methylthiopropionate, an off-pathway transformation of the Acireductone. The purpose of the off-pathway reaction catalyzed by Ni-ARD is unknown [1].

Both enzymes Ni(Fe)ARD are members of the structural super family, known as cupins, which also include Fe-Acetyl acetone Dioxygenase (Dke1) [2, 3] and Cysteine Dioxygenase, Quercetinases. These family of cupins use a triad of histidine-ligands (His),

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and also one or two oxygens from water and a carboxylate oxygen (Glu) for binding with Fe(Ni)-center [2].

We have offered the new approach to the research of mechanism of homogenous catalysis, and also the mechanism of action of enzymes [4, 5]. The first time we have successfully used the method of AFM (atomic force microscopy) to study the possibility of formation of supramolecular nanostructures, based on heteroligand nickel and iron complexes, due to the intermolecular hydrogen bonds [4, 5]. So, the complexes $Ni_2(acac)(OAc)_3 \cdot NMP \cdot 2H_2O$ ("A"), $Fe^{III}_x(acac)_y 18C6_m(H_2O)_n$ ("B"), $\{Ni(acac)_2 \cdot L^2 \cdot PhOH\}$ ("C") are effective catalysts of selective ethyl benzene oxidation to α-phenyl ethyl hydro peroxide and also are structure and functional models of Dioxygenases Ni(Fe)ARD (A-C) and Fe^{II}-Dke1 (B) [6]. We assumed that the stability of the complexes A-C as the alkylarenes oxidation catalysts might be due to formation of the stable supramolecular structures by the intermolecular hydrogen bonds [6]. And specific activities of Ni(Fe)ARD towards common substrates (Acireductone and dioxygen) in synthesis and reproduction of methionine is one of the reasons—with self-organization into various macrostructures due to intermolecular hydrogen bonds. We confirmed these assumptions by our AFM-research outlined in this article [4, 5].

In this article we discuss the possible role of Tyr-fragment and histidine-ligands (His), in mechanism of Ni-ARD Dioxygenase actions, based on experience data that we received at the first time with AFM on model systems.

2. Materials and Metodology

AFM SOLVER P47/SMENA/ with Silicon Cantilevers NSG11S (NT MDT) with curvature radius 10 nm, tip height 10-15 μm and cone angle ≤ 22° in taping mode on resonant frequency 150 KHz was used [4, 5].

As substrate, the polished Silicone surface special chemically modified was used.

Waterproof modified Silicone surface was exploited for the self-assembly-driven growth due to H-bonding of complexes $Fe^{III}_x(acac)_y 18C6_m(H_2O)_n$, $Ni_xL^1_y(L^1_{ox})_z(L^2)_n(H_2O)_m$, $\{Ni(acac)_2 \cdot L^2 \cdot PhOH\}$ (L^2 = NMP), systems $\{Ni(acac)_2 + L^2 + Tyr\}$ and $\{Ni(acac)_2 + Tyr\}$ (L^2 = NMP, His, His = L-Histidine, Tyr = L-Tyrosine) with Silicone surface. The saturated water solutions of complexes was put on a surface, maintained some time, and then solvent was deleted from a surface by means of special method—spin-coating process.

It was found during the scan samples, that the structures are fixed on a surface strong enough due to H-bonding. The self-assembly-driven growth of the supramolecular structures on modified Silicone surface based on researched complexes, is due to H-bonds and perhaps the other non-covalent interactions was observed.

3. Results and Discussion

3.1 The Mechanism of Formation of High Effective Catalysts—Heteroligand Ni (or Fe)-complexes in the Hydrocarbon Oxidations with Dioxygen: The Role of H-Bonds

In our works we have modeled efficient catalytic systems $\{ML^1_n + L^2\}$ (M = Ni, Fe, $L^1 = acac^-$, and L^2 are crown ethers or quaternary ammonium salts, different electron-donating modifying extra-ligands) for selective ethylbenzene oxidation to α-phenyl ethyl hydro peroxide. This was based on the established (for Ni complexes) and hypothetical (for Fe complexes) mechanisms of formation of catalytically active species and their operation [6]. The high activity of systems $\{ML^1_n + L^2\}$ is associated with the fact that during the ethylbenzene oxidation, the active primary $(M^{II}L^1_2)_x(L^2)_y$ complexes and heteroligand $M^{II}_xL^1_y(L^1_{ox})_z(L^2)_n(H_2O)_m$ complexes are formed to be involved in the oxidation process [6].

We established mechanism of formation of high effective catalysts—heteroligand complexes

$M^{II}_xL^1_y(L^1_{ox})_z(L^2)_n(H_2O)_m$. The axially coordinated electron-donating ligand L^2 controls the formation of primary active complexes $ML^1_2{\cdot}L^2$ and the subsequent reactions of β-diketonate ligands in the outer coordination sphere of these complexes. The regioselectivity of outer-sphere reaction of O_2 incorporation into the chelate ring depends on the nature of the metal and the modifying ligand L^2 [6]. Thus formation of nickel complexes $Ni^{II}_xL^1_y(L^1_{ox})_z(L^2)_n$, as a result of the reaction of oxygenation of ligand L^1 = $acac^-$ in $Ni^{II}(acac)_2$, follows a mechanism analogous to those of Ni^{II}-containing ARD (Aci-reductone Dioxygenase) [1] or Cu- and Fe-containing Quercetin 2,3-Dioxygenases [7, 8]. Namely, incorporation of O_2 into the chelate acac-ring was accompanied by the proton transfer and the redistribution of bonds in the transition complex leading to the scission of the cyclic system to form a chelate ligand L^1_{ox} = OAc^-, acetaldehyde and CO (in the Criegee rearrangement).

In the effect of iron (II) acetylacetonate complexes $Fe^{II}_xL^1_y(L^1_{ox})_z(L^2)_n$, we have found [6] an analogy with the action of Fe^{II}-ARD [1] or Fe^{II}-acetyl acetone Dioxygenase (Dke1) [3]. For iron complexes oxygen adds to C−C bond (rather than inserts into the C=C bond as in the case of catalysis with nickel (II) complexes) to afford intermediate, i.e., a Fe complex with a chelate ligand containing 1,2-dioxetane fragment. The process is completed with the formation of the $(OAc)^-$ chelate ligand and methylglyoxal as the second decomposition product of a modified acac-ring (as it has been shown in [3]).

One of the most effective catalytic systems of the ethylbenzene oxidation to the α-phenyl ethyl hydroperoxide is the triple systems [6, 9]. Namely, the phenomenon of a substantial increase in the selectivity (***S***) and conversion (***C***) of the ethylbenzene oxidation to the α-phenyl ethyl hydro peroxide upon addition of PhOH together with ligands N-methyl-2-pyrrolidone (NMP), HMPA (hexamethylphosphorotriamide) or alkali metal stearate MSt (M = Li, Na) to metal complex $Ni^{II}(acac)_2$ was discovered in works of Matienko and Mosolova [6, 9]. The role of intramolecular H-bonds was established by us in mechanism of formation of triple catalytic complexes $\{Ni(acac)_2{\cdot}L^2{\cdot}PhOH\}$ (L^2 = NMP) in the process of ethylbenzene oxidation with molecular oxygen [6, 9]. The formation of triple complexes $Ni(acac)_2{\cdot}L^2{\cdot}PhOH$ from the earliest stages of oxidation was established with kinetic methods [6, 9]. We assumed that the stability of complexes $Ni(acac)_2{\cdot}L^2{\cdot}PhOH$ in the process of ethyl benzene oxidation can be considered as one of reasons with formation of supramolecular structures due to intermolecular H-bonds (phenol–carboxylate) and, possible, the other non-covalent interactions:

$$\{Ni(acac)_2+L^2+PhOH\}\rightarrow Ni(acac)_2{\cdot}L^2{\cdot}PhOH \rightarrow \{Ni(acac)_2{\cdot}L^2{\cdot}PhOH\}_n$$

In favor of formation of supramolecular macrostructures based on the triple complexes $\{Ni(acac)_2{\cdot}L^2{\cdot}PhOH\}$ (and $Ni(acac)_2{\cdot}L^2{\cdot}Tyr$ (L^2 = NMP, L-Histidine, Tyr = L-Tyrosine)) in the real systems of homogeneous (and enzymatic catalysis) show data of AFM.

3.2 Role of Supramolecular Nanostructures Formed Due to H-Bonding in Mechanisms of Catalysis. Models of Ni(Fe)ARD Dioxygenases: Role of Tyr-Fragment

Hydrogen bonds vary enormously in bond energy from 15-40 kcal/mol for the strongest interactions to less than 4 kcal/mol for the weakest. Hydrogen bonds are important in non-covalent aromatic interactions, where π- electrons play the role of the proton acceptor, which is a very common phenomenon in chemistry and biology. They play an important role in the structures of proteins and DNA, as well as in drug receptor binding and catalysis [10].

Pochapsky et al. [11] showed that this dual chemistry of Ni(Fe)ARD can also occur in mammals (MmARD). (before Ni(Fe)ARD activity was discovered by Pochapsky et al. [1, 12] for bacteria and

plants). Interestingly, in the case of KoARD (*Klebsiella oxytoca* ARD, bacterial enzyme ARD) unlike MmARD, Ni-bound form has higer activity than Fe-KoARD [1]. All forms remain monomeric regardless of bound metal ion. While both Fe- and Ni-ARD from *Klebsiella oxytoca* are monomers [11], Fe-ARD from *Oryza sativa L* (OsARD) is a trimer, and Ni-bound OsARD is a polymer consisting of several types of oligomers [11].

As mentioned before, both enzymes Ni(Fe)-ARD are members of the structural super family, known as cupins. Metal-dependent Dioxygenases of the cupin superfamily, fungal Quercetinases, are copper proteins. However, recombinant *Streptomyces* Quercetinase (QueD) was previously described to be capable of incorporating Ni^{2+} with some other divalent metal ions. The role of metal and ligands was examined. It was established that metal occupancies of heterologously produced QueD proteins followed the order Ni > Co > Fe > Mn. Iron, in contrast to the other metals, does not support catalytic activity. Replacement of individual amino acids of the 3His/1Glu metal binding motif by alanine drastically reduced or abolished Quercetinase activity and affected its structural integrity. Only substitution of the glutamate ligand (E76) by histidine resulted in Ni- and Co-QueD variants that retained the native fold and showed residual catalytic activity [13].

We assumed that one of the reasons for the different activity of $Ni^{II}(Fe^{II})$-ARD in the functioning of enzymes in relation to the common substrates (Acireductone and O_2, fig. 1) may be the association of catalyst in various macrostructure due to intermolecular H-bonds.

Earlier we demonstrated a specific structural organization of functional models of iron (nickel) enzymes $Ni^{II}(Fe^{II})$ARD. The possibility of the formation of stable supramolecular nanostructures is based on iron (nickel) heteroligand complexes due to intermolecular H-bonds we researched with the AFM method [4, 5].

We assumed that the Fe^{II}ARD operation comprises the step of oxygen activation ($Fe^{II} + O_2 \rightarrow Fe^{III} - O_2^-$) (by analogy with Dke1 action [3]). Specific structural organization of iron complexes may facilitate the following regioselective addition of activated oxygen to Acireductone ligand and the reactions leading to formation of methionine (and ethylene also). So, in the

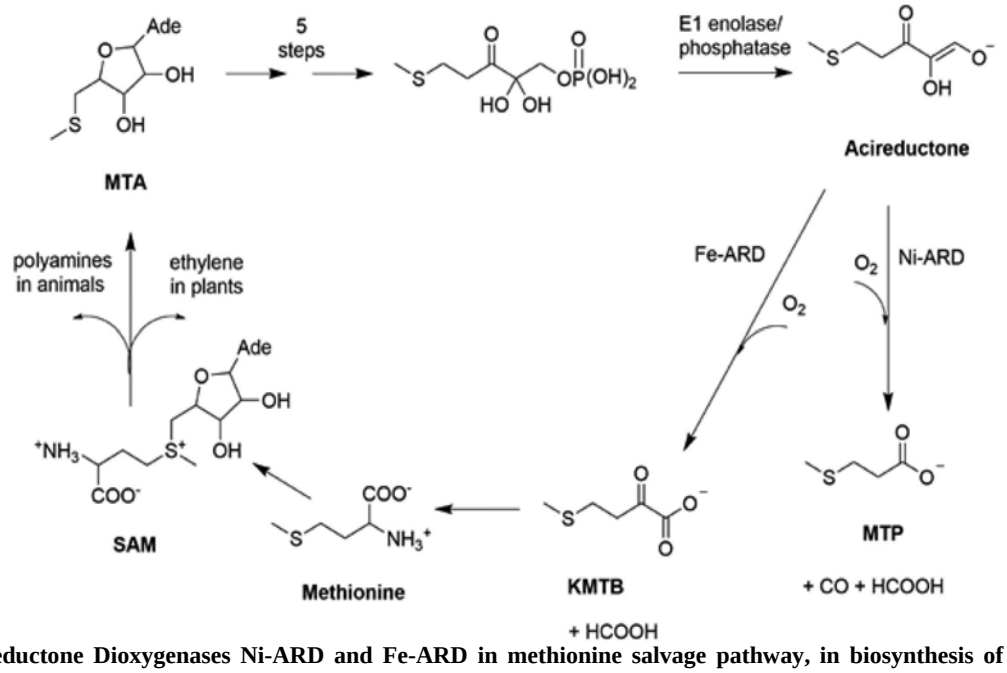
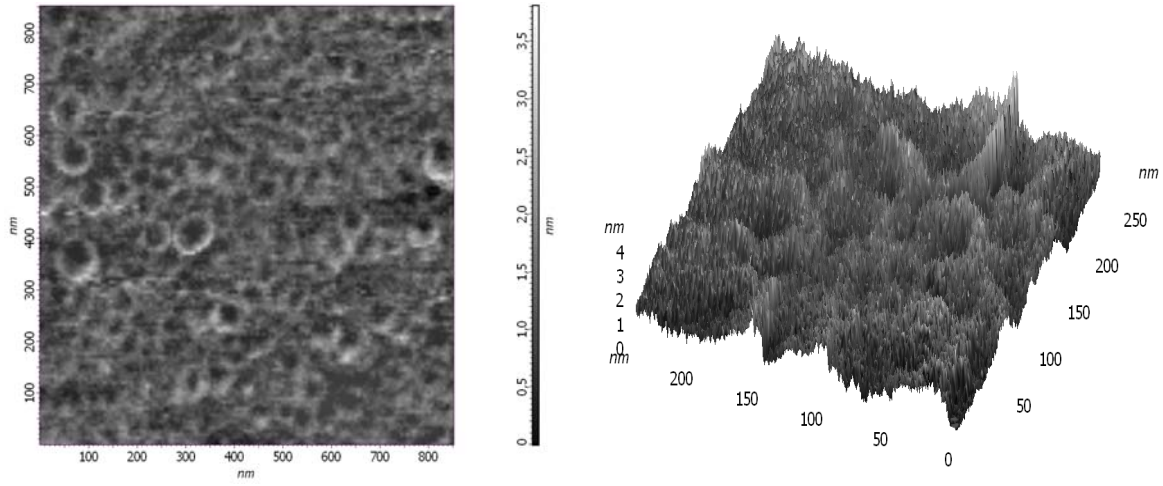
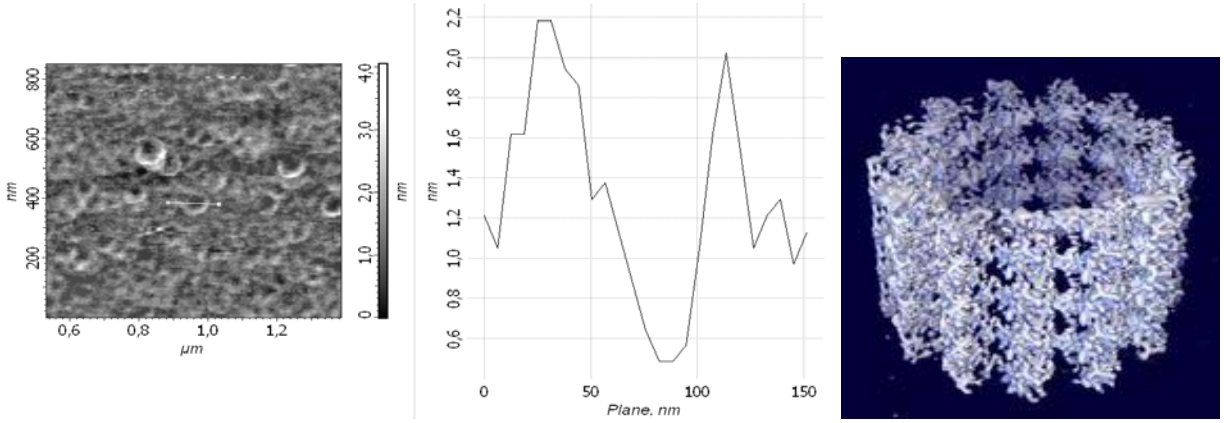


Fig. 1 Acireductone Dioxygenases Ni-ARD and Fe-ARD in methionine salvage pathway, in biosynthesis of ethylene and polyamines [11, 12].



(a) (b)

Fig. 2 The AFM two-(a) and three-dimensional (b) image of nanoparticles based on $Fe^{III}_x(acac)_y18C6_m(H_2O)_n$ formed on the surface of modified silicone.



(a) (b) (c)

Fig. 3 The AFM two-dimensional image (a) of nanoparticles based on $Fe^{III}_x(acac)_y18C6_m(H2O)_n$ formed on the hydrophobic surface of modified silicone. The section of a circular shape with fixed length and orientation is about 50-80 nm (b). The structure of the cell microtubules (c).

fig. 2-3 three-dimensional and two-dimensional AFM image of the structures on the basis of iron complex with 18-crown-6 (18C6), $Fe^{III}_x(acac)_y18C6_m(H_2O)_n$**,** formed at putting a uterine solution on a hydrophobic surface of modified silicone are presented. It is visible that the generated structures are organized in certain way forming structures resembling the shape of tubule micro fiber cavity (figure 3c). The heights of particles are about 3-4 nm. In control experiments it was shown that for similar complexes of nickel $Ni^{II}(acac)_2{\cdot}18C6{\cdot}(H_2O)_n$ (as well as complexes $Ni_2(OAc)_3(acac){\cdot}NMP{\cdot}2H_2O$) this structures organization is not observed. We did not observe these iron constructions in the absence of the aqueous environment.

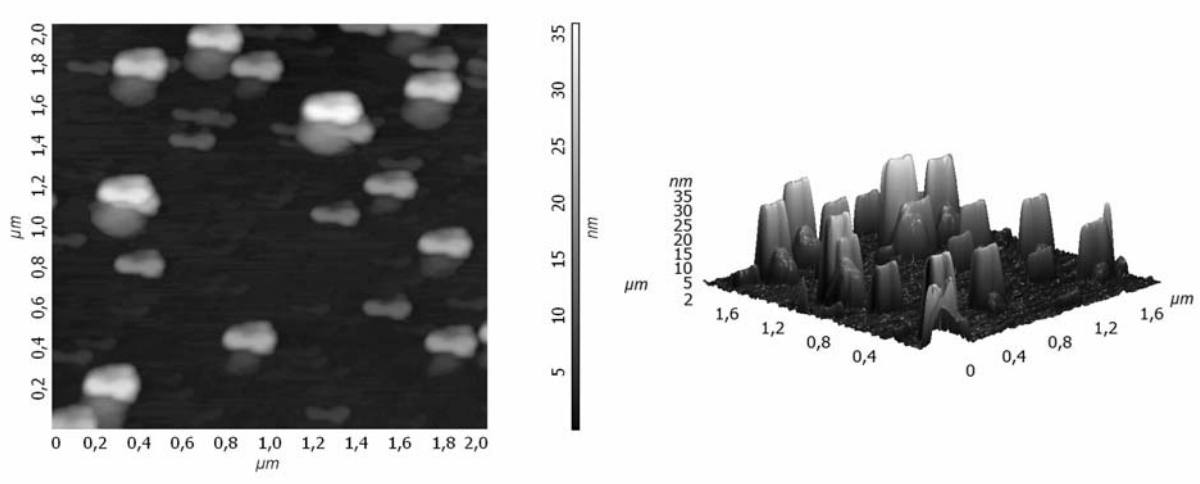
Unlike catalysis with Fe-ARD, mechanism of catalysis by Ni-ARD does not include O_2-activation, and oxygenation of Acireductone leads to the formation of products not being precursors of methionine [1, 11, 12]. In our previous works, we have shown that formation of multidimensional forms based on nickel complexes can be one of the ways of regulating the activity of two enzymes [4]. The association of complexes $Ni_2(AcO)_3(acac){\cdot}NMP{\cdot}2H_2O$, which are functional and structure models of Ni-ARD, to supramolecular nanostructure due to intermolecular H-bonds (H_2O–NMP, H_2O–(OAc^-)(or ($acac^-$)), is demonstrated on the next fig. 4. All structures (fig. 4)

are various on heights from the minimal 3-4 nm to ~20-25 nm for maximal values (in the form reminding three almost merged spheres) [5].

In case of binary complexes {$Ni(acac)_2$·NMP} (see fig. 6b in the text below) we also observed formation of nanostructures due to H-bonds. But these nanoparticles differ on form and are characterized with less height: h_{max}~8-12 nm as compared with nanostructures based on complexes $Ni_2(AcO)_3(acac)\cdot L^2\cdot 2H_2O$ (fig. 4).

We assume that it may be necessary to take into account the role of the second coordination sphere, including Tyr-fragment (see fig. 5 [11]). We first suggest the participation of Tyrosine moiety in mechanisms of action of Ni(Fe)ARD enzymes.

Tyrosine can participate in different enzymatic reactions. Recently it has researched the role of Tyrosine residue in mechanism of Heme Oxygenase



(a) (b)

Fig. 4 The AFM two- (a) and three-dimensional (b) image of nanoparticles based on $Ni_2(AcO)_3(acac)\cdot NMP\cdot 2H_2O$ formed on the hydrophobic surface of modified silicone.

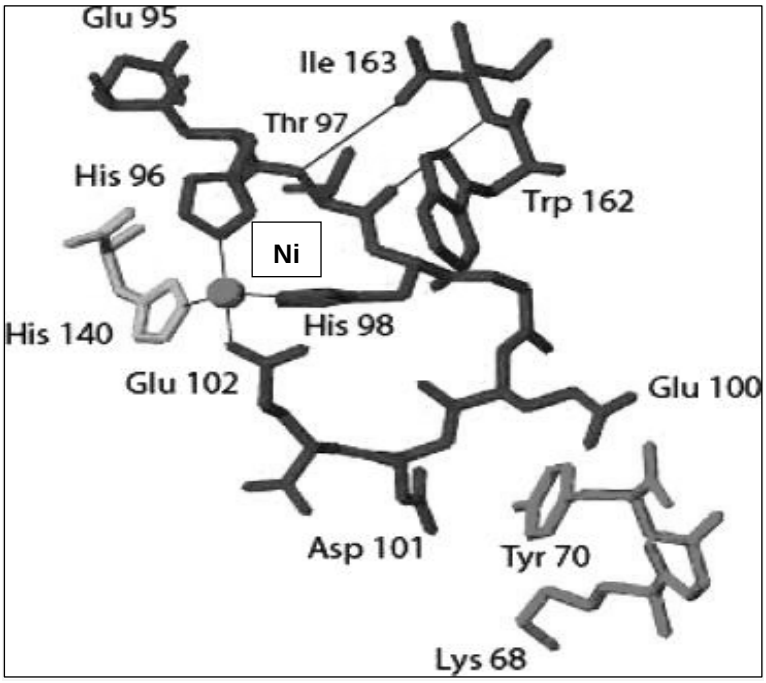


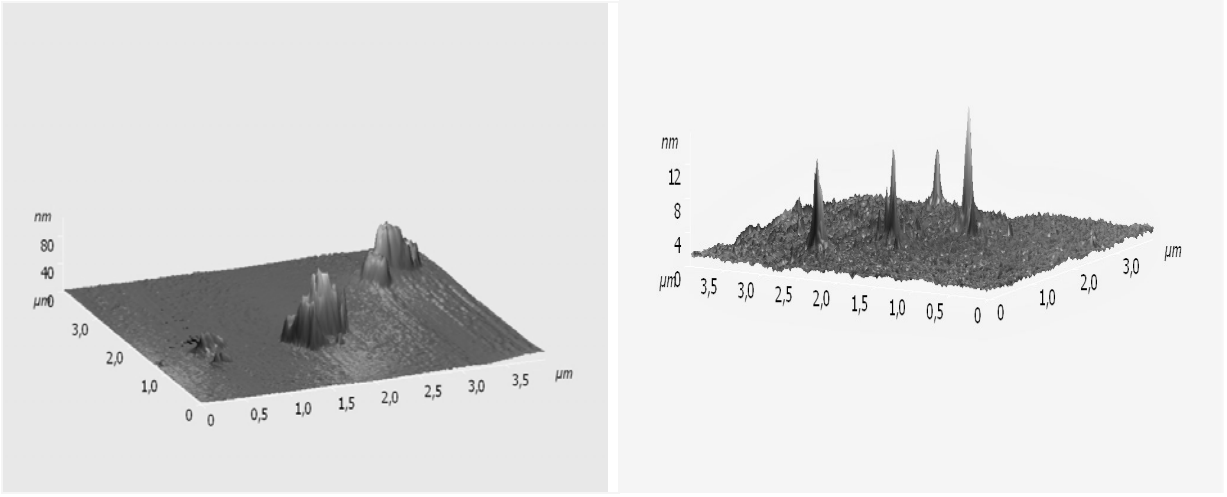
Fig. 5 The structure of Ni-ARD with Tyr residue in the second coordination sphere [11].

(HO) action. [14]. It assumed that Tyr-fragment may be involved in substrate H-binding in step of O_2-activation by iron catalyst, and this can decrease the oxygenation rate of substrate in the case of Homoprotocatechuate 2,3-Dioxygenase action [15]. Tyr-fragment is discussed as important in methyl group transferred from S-adenosylmethionine (AdoMet) to dopamine [16]. The experimental findings with the model of Methyltransferase and structure survey imply that methyl CH···O hydrogen bonding (with participation of Tyr-fragment) represents a convergent evolutionary feature of AdoMet-dependent Methyltransferases, mediating a universal mechanism for methyl transfer [17].

In the case of Ni-ARD, Tyr-fragment, involved in mechanism, can reduce the Ni-ARD activity.

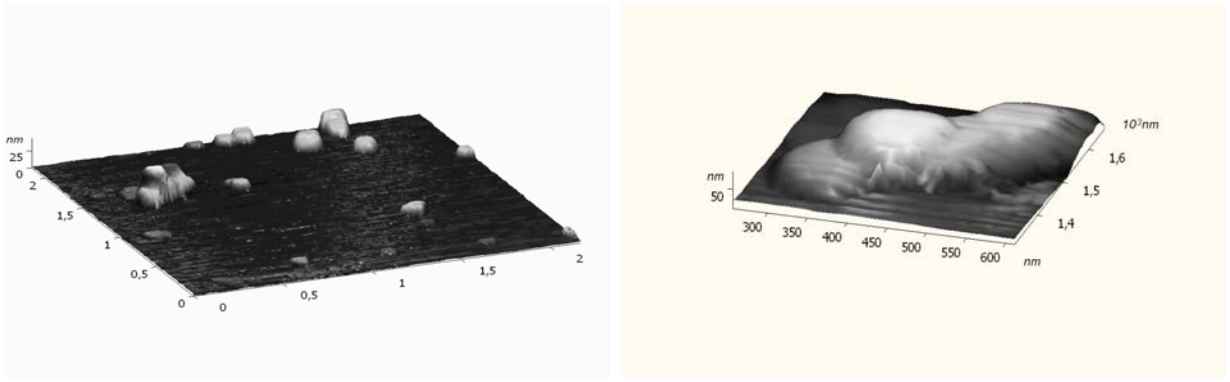
Really, as mentioned above, we have found [6] that the inclusion of PhOH in complex $Ni(acac)_2{\cdot}L^2$ (L^2=MP), which is the primary model of Ni^{II}ARD, leads to the stabilization of formed triple complex $Ni(acac)_2{\cdot}L^2{\cdot}PhOH$. In this case, as we have emphasized above, ligand $(acac)^-$ is not oxygenated with molecular O_2. Also the stability of triple complexes $Ni(acac)_2{\cdot}L^2{\cdot}PhOH$ seems to be due to the formation of supramolecular macrostructures that are stable to oxidation with dioxygen. Formation of supramolecular macrostructures due to intermolecular (phenol–carboxylate) H-bonds and, possible, the other non-covalent interactions, based on the triple complexes $Ni(acac)_2{\cdot}L^2{\cdot}PhOH$, that we have established with the AFM-method (in the case of L^2 = NMP, and also L^2 = HMPA, NaSt, or LiSt [4, 5]), is in favor of this hypothesis. See fig. 6: L^2 = NMP for binary and triple systems.

Conclusive evidence in favor of the participation of tyrosine fragment in stabilizing primary Ni- complexes as one of regulatory factors in mechanism of action of Ni-ARD has been obtained by AFM. We observed first the formation of nanostructures based on Ni-systems $\{Ni(acac)_2 + L^2 + Tyr\}$ using L-Tyrosine (Tyr) and L^2 = NMP or L-Histidine (His) as extra-ligands. The growth of self-assembly of supramolecular macrostructures based on the triple systems $\{Ni(acac)_2 + L^2 + Tyr\}$, due to intermolecular (phenol–carboxylate) H-bonds and, possible, the other non-covalent interactions [18-22], we observed at the apartment of a uterine solution of triple system $\{Ni(acac)_2 + L^2 + Tyr\}$ on surfaces of modified silicon (figs. 7(a, b). Spontaneous organization process, i.e., self-organization, of researched triple complexes on surfaces of modified silicon are driven by the balance between intermolecular, and molecule-surface



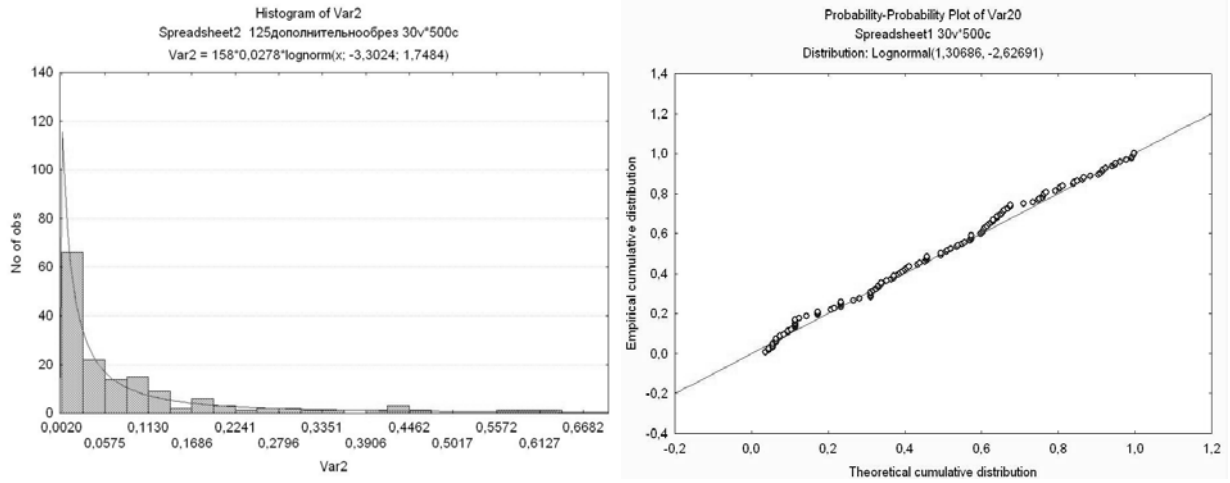
(a) (b)

Fig. 6 a—the AFM three-dimensional image (5.0 × 5.0(μm)) of the structures (h~80-100 nm) formed on a surface of modified silicone based on triple complexes $Ni(acac)_2{\cdot}NMP{\cdot}PhOH$, b—AFM three-dimensional image (3.5 × 3.5(μm)) of the structures (h~12 nm) formed on a surface of modified silicone based on binary complexes $\{Ni(acac)_2{\cdot}NMP\}$.



(a) (b)

Fig. 7 The AFM three-dimensional image (2.0 × 2.0 (μm)) of the structures (h~25 nm) (a) and three-dimensional image (0.3 × 0.6 (μm)) of the structures (h~50 nm) (b), based on triple systems {Ni(acac)$_2$+MP+Tyr}, formed on a surface of modified silicone.



(a) (b)

Fig. 8 *a*—histogram of volumes of the particles based on systems {Ni^{II}(acac)$_2$+MP+Tyr}, *b*—the empirical and theoretical cumulative Log-normal distribution of volumes of the particles based on systems {Ni^{II}(acac)$_2$ + MP + Tyr}, formed on a surface of modified silicone.

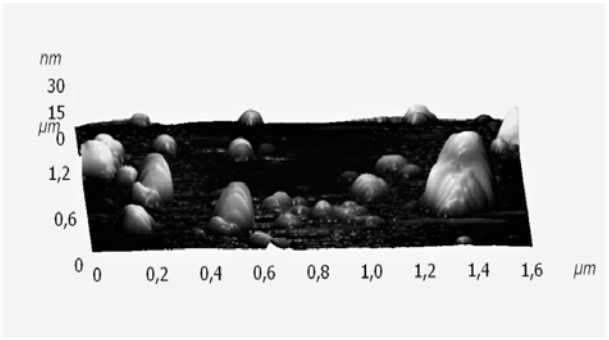
interactions, which may be the consequence of hydrogen bonds and the other non-covalent interactions [22].

Histogram of volumes of the particles based on systems {Ni(acac)$_2$ + NMP + Tyr}, and also the empirical and theoretical cumulative Log-normal distribution of volumes of the particles based on systems {Ni(acac)$_2$ + NMP + Tyr}, formed on the surfaces of modified silicon, are presented on figs. 8a and 8b. As can be seen, distribution of volumes of the particles in this case is well described by a Log-normal law.

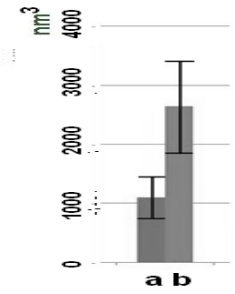
In the case of binary systems {Ni(acac)$_2$ + Tyr} we also observed formation of nanostructures due to H-bonds. But these nanoparticles as well as particle based on Ni(acac)$_2$·NMP complexes (Fig. 6b) differ on form and high (h~10-12 nm) from the nanostructures based on triple systems {Ni(acac)$_2$ + NMP + Tyr} (h~25-50 nm) (fig. 7).

We recently received confirmation of the formation of triple complexes {Ni(acac)$_2$·NMP·Tyr} by UV spectroscopy (in press).

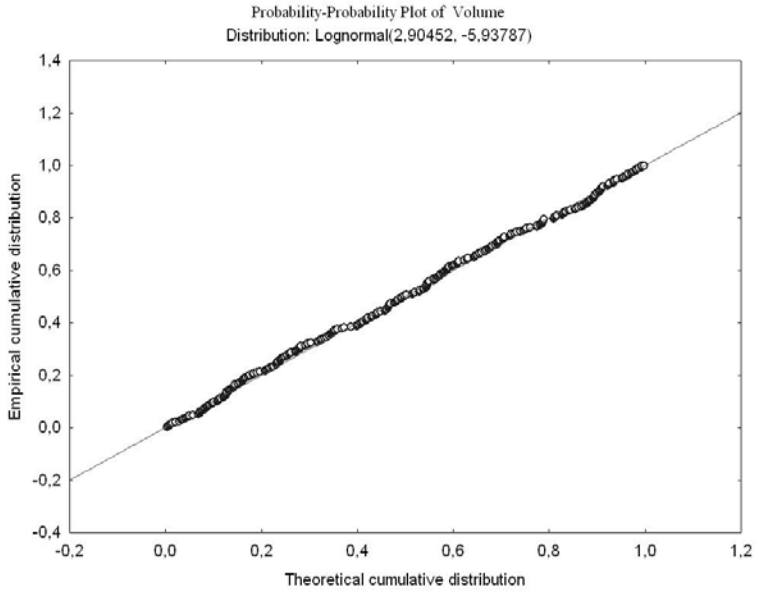
So the formation triples complexes {Ni(acac)$_2$·NMP·Tyr} (Tyr = L-Tyrosine) and stable macrostructures based on Ni(acac)$_2$·NMP·Tyr, we established with AFM, testified in favor of the role Tyr-fragment as a regulatory factor in the Ni(Fe)ARD action.



(a)



(b)



(c)

Fig. 9 (a) The AFM three-dimensional image (1.2 × 1.6 (μm)) of the nanostructures structures (h~30 nm) based on triple systems {Ni(acac)$_2$ + His + Tyr} formed on a surface of modified silicone. (b) Diagram of the mean values of volumes of the particles based on binary—{Ni(acac) $_2$+ His}(a) and triple systems {Ni(acac)$_2$ + His + Tyr} (b) (the diagram marked 95% confidence interval). (c) The empirical and theoretical cumulative Log-normal distribution of volumes of the particles based on system {Ni(acac)$_2$ + His + Tyr}.

Active site of Ni-ARD, as a member of cupins, includes histidine ligands. The formation of stable supramolecular structures due to intermolecular H-bonds based on triple systems {Ni(acac)$_2$ + His + Tyr}, (His = L-Histidine) that we found recently by AFM (Fig. 9), is another fact in favor of our proposed model of Ni-ARD action.

In the case of triple systems {Ni(acac)$_2$ + His + Tyr} we also observed growth of self-assembly of supramolecular macrostructures due to intermolecular (phenol–carboxylate) H-bonds and, possible, the other non-covalent interactions [18-22] (fig. 9(a)). The volumes of particles are based on binary and triple systems (with His ligand) differ significantly (fig. 9 (b)). As can be seen, also in this case distribution of volumes of the particles based on triple system {Ni(acac)$_2$ + His + Tyr} is well described by a Log-normal law (fig. 9(c)).

At the same time, it is necessary to mean that important function of Ni-ARD in cells is established now. Namely, carbon monoxide, CO, is formed because of action of nickel-containing Dioxygenase Ni-ARD. It was established, that CO is a representative of the new class of neural messengers, and seems to be a signal transducer like nitrogen oxide, NO [1, 11, 12].

4. Conclusions

The formation of supramolecular nanostructures due to intermolecular H-bonds based on catalytic active iron $Fe^{III}_x(acac)_y18C6_m(H_2O)_n$, and nickel complexes $Ni_2(AcO)_3(acac)\cdot NMP\cdot 2H_2O$, $\{Ni(acac)_2\cdot L^2\cdot PhOH\}$ (L^2 = NMP) can be observed earlier with AFM method. This indicates high probability of supramolecular structures formation due to H-bonds in the real systems, namely, in the processes of alkylarens oxidation [4, 5, 21].

Since the investigated complexes are structural and functional models of Ni(Fe)ARD Dioxygenases, the data could be useful in the interpretation of the action of these enzymes.

We assumed the participation of Tyr-fragment, which is in the second coordination sphere of Ni-ARD, as one of possible mechanisms of reduce in enzymes activity in Ni(Fe)-ARD enzymes operation, and we received experimental facts in favor this assumption. So with AFM we first observed the formation of supramolecular macrostructures based on model triple complexes $Ni(acac)_2\cdot NMP\cdot PhOH$ and triple complexes $\{Ni(acac)_2\cdot NMP\cdot Tyr\}$ that included L-Tyrosine as extra ligand, on a surface of modified silicone due to intermolecular (phenol–carboxylate) H-bonds and, possible, the other non-covalent interactions. The formation of stable supramolecular structures due to intermolecular H-bonds based on triple systems $\{Ni(acac)_2$ + His + Tyr$\}$, (His = L-Histidine), is another fact in favor of our proposed model of Ni-ARD action.

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