

THE ROLE OF INTERFACES AND CHARGE OF A MOLECULE IN THE PROCESSES OF NONEQUILIBRIUM CRYSTALLIZATION OF PEPTIDE NANOBIMATERIALS

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Abstract: The influence of various factors on the processes of nonequilibrium crystallization of dipeptide (DPT) from its aqueous solutions has been studied. The nonequilibrium crystallization leads to the formation of spatially heterogeneous structures. Depending on the charge of the DPT molecule (neutral or anion), not only the morphology of the surface of the layers changes, but also its electronic structure. The important role is played by the properties of the substrate-layer interfaces, which are responsible for the formation of ferroelectrics on gold during the crystallization of the DPT anion. The structure of the solid layers is also significantly affected by charges on the substrate surface, which can partially or completely compensate for the charges of individual functional groups in the PT molecule. The observed effects of bipolar resistive switching in peptide layers and the formation of ferroelectrics can find practical application as a smart material of memristor organic electronics.

Keywords: CRYSTALLIZATION, PHASE TRANSFORMATIONS, SMART BIOMATERIALS, NANOPARTICLES, MEMRISTORS

1. Introduction

The use of biological objects and the principles of their functioning for the organization of matter at the molecular level - biomimetic - is an actual and intensively developing direction in nanotechnology. Discoveries in it gave impetus to the creation of new functional (smart) materials. The characteristic feature that actually makes them smart is their ability to respond to very slight changes in the surrounding environment. The uniqueness of these materials lies not only in the fast macroscopic changes occurring in their structure but also these transitions being reversible. It is assumed that such materials can replace metals as conductors of current, while retaining their inherent resistance and ductility. Also it was found that the hardness of peptide (PTs) nanospheres formed as a result of the self-organization of simple protected DPTs turned out to be higher than the hardness of steel. On the basis of PTs, a material with unique adhesion properties and providing super-strong adhesion was developed.

At present, in spite of the continuously increasing number of PTs studies, the regularities of their binding to solids and the formation of solid structures from them remain to be studied. In order to reliably control the formation of PTs based self-organized structures on solid surfaces, it is necessary to elucidate the roles of different factors in this process, such as: the molecule chemistry, its charge, the chemical nature of a substrate surface and the presence of various defects or foreign "structural" units on it.

Methods of colloid chemistry make it possible to create various ordered nanostructures on solid surfaces as a result of self-assembly and self-organization processes. At the same time, they are characterized by high efficiency, manufacturability and low costs. Unfortunately, the mechanisms for the formation of solid-phase nanostructures are still not disclosed. As a result, there is no possibility of complete control over the technological processes of their production (coatings, 3D printing, etc.).

There is a considerable interest in the processes of self-organization that occur during crystallization from a melt or solidification from a solution. First of all this is due to fundamental problems, in particular, to the problem of molecular design. However, the relationship between molecular structure and macroscopic supramolecular organization is very complex and poorly understood. Therefore, based solely on the structure of the PT molecule, it is very difficult to predict the final structure and properties of solid-phase coatings based on it in advance. So a high level of understanding of the underlying processes and mechanisms is required. All this requires more detailed studies of all stages of the processes of PTs crystallization and the influence of various factors on them.

Previously we investigated basically the synthesis of various bionanocomposites, including different PTs [1-3]. These studies have shown that PT composite materials and coatings with silver and gold nanoparticles (NPs) do have a variety of structural, electrical and tribological properties that make them promising for

large-scale practical applications in engineering. Also it was found that due to the specific zwitterionic structure of their molecule, it is possible to modify its chemical, electrical and structural properties simply by changing pH of solution. In this communication, we report the results of studying the influence of molecule charge and different interfaces on the structure and electrical properties of PTs materials to determine the structure-property relationship of the formed layers and the feasibility of practical application of DPT in engineering, for example, as a smart material of organic electronics.

2. Experimental section

In this report, a short DPT $\{HOOC-(CH_2)_2-CO-Glu-Lys-NH-(CH_2)_3\}_2$ was taken [3]. It belongs to the class of short PTs of neuroprotective effect and is a mimetic of nerve growth factor. Its structural formula is shown in Fig. 1.

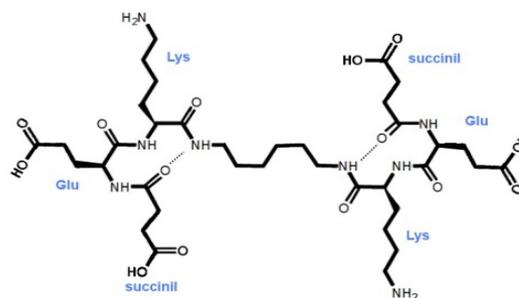


Fig. 1. Structure of dipeptide molecule.

When studying the processes of crystallization of DPT layers, the methods of probe microscopy and spectroscopy (AFM, STM, STS), IR and Raman spectroscopy, XPS, interference microscopy and dynamic light scattering were used. The experiments were carried out at pH values 4.2 (neutral molecule) (DPT-0) and 7.6 (anion) (DPT-1) by adding K_2CO_3 solution. In each experiment 10 ml of DPT aqueous solution at different pH was deposited at room temperature on the surface of polycrystalline gold films, as well as on the cleaned glass and freshly prepared mica and HOPG (ZYH with misorientation angles of $3.5^\circ-5^\circ$) surfaces. The deposited DPT layers were dried in air at room temperature. Two methods of deposition were used: drop casting and spin coating at 2000–3000 rpm, resulting in thin and ultrathin layers, respectively. The maximum height of the formed thin layer at the boundary of the drop was $\leq 0.5 \mu m$.

3. Results and discussion

The evaporating drop of a colloidal solution deposited on a solid surface is a complex strictly non equilibrium system in which the degree of supersaturation continuously changes, local gradients of

concentrations and temperatures appear, and processes of mass transfer of the solute to the drop boundary proceed. The final structure of the composite layer, which can not be predicted in advance, will be determined by the action of a variety of forces, particle sizes and concentration of reagents. Therefore, the structures of thin layers formed in the center and on the rim of the drop can differ significantly. The limited volume of the article does not allow us to present and consider all changes in the structure and morphology of the formed solid layers that are observed moving the probe from the center of the drop to its periphery. Thus, when moving in the radial direction from the center to the edge of the drop, along with the change in structure, the thickness of the layer also increases and reaches its maximum at drop boundary where the rim is formed. Only the most characteristic structures in our opinion are listed here.

At the initial stages of crystallization, the self-assembly of DPT-0 NPs begins on the gold surface (Fig.2a). Their average size is ca. 50 nm and the aspect ratio (height/diameter) is 1/10. They are assembled into separate units, the forms of which are very diverse.

The layers of DPT-1 on the surface of gold also consist of separate flat NPs, but in this case they form chains with a width of 400-600 nm, oriented in a certain direction (Fig. 2b). The directed aggregation of DPT-1 NPs into anisotropic chain-like structures proceeds probably according to the mechanism of bridge formation between adjacent NPs via non-covalent interparticle interactions.

In the of DPT-1 layers on graphite, a growth of monolayers is observed. Their height is ≤ 0.9 nm (Fig.2c). This is very close to the calculated value for an anion of 0.7 nm [4].

Fig. 2d presents 3-d image of ultrathin DPT-1 layer on mica. In this case the individual mesocrystals are growing. Their height is less than 80 nm, and the angles formed by the crystal faces are 60° and 120° . These structural features indicate on one side a higher degree of perfection of such mesocrystals and on the other side, that the growth front is formed by certain crystal facets. In addition to mesocrystals, individual filaments are also can be seen. The height of these filaments does not exceed 4 nm.

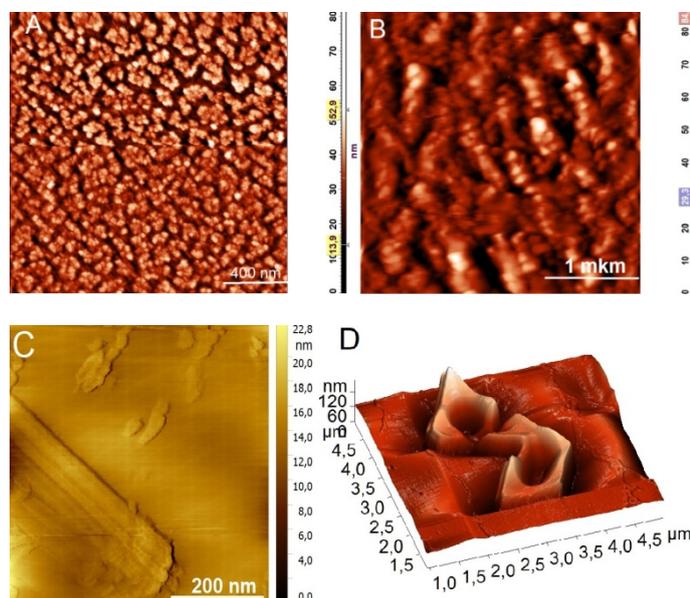


Fig.2. Examples of self-assembly of DPT molecules in ultrathin layers, DPT-0 (a), DPT-1 (a,b,c) - on gold (a,b), graphite(c) and mica (d)

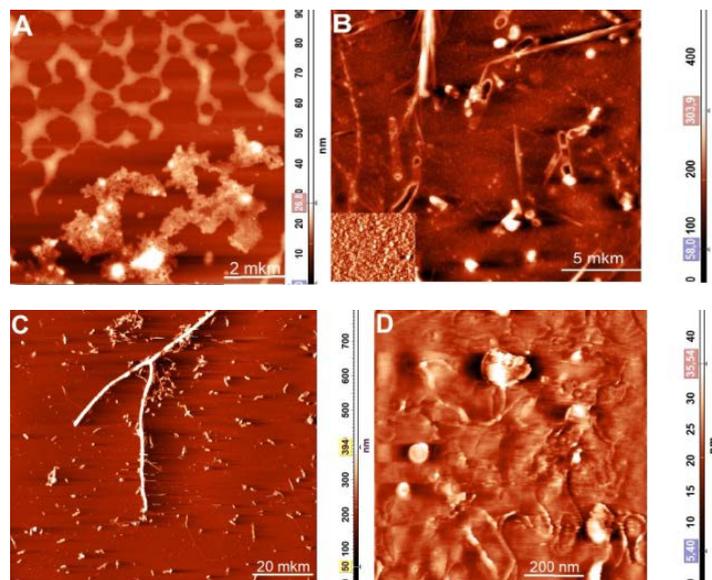


Fig.3. AFM images of thin layers of DPT-0 on the surfaces of mica (a), gold (b), glass (c) and graphite (d).

In thin layers of DPT-0 on the surface of mica the coalescence of islands are observed. They have equal thickness about $\approx 2-3$ nm (Fig. 3a). Between the individual islands bridges are formed. In this case, structures similar to honeycombs appear. Near the rim there is a coalescence of individual particles of micron size with the formation of a continuous layer with a height up to 35 nm. It should be noted that the growth of such aggregates is observed only at the edges of the drop.

On the surface of gold, the formation of various linear 3-d chains or short rods begins atop of a layer consisting of tightly packed "pancakes" which can be seen at higher resolutions (left corner of Fig. 3b). There is a certain similarity between the DPT-0 structures on mica and gold. Indeed, at the center of the drop on mica, we also see layers consisting of tightly packed NPs, similar to Fig.3b, and only upon moving toward the drop boundary, the growth of individual 3-d aggregates begins. Their length can reach ≥ 10 microns. The morphology of thin layers of DPT-0 on the glass surface can be characterized as having elongated short chains formed by individual flat particles as main structural elements of self-assembling (Fig. 3c). These building blocks forms 100-mkm-long chains composed of individual flat particles with an average length of ca. 800 nm, a width of 1.2 mkm and a height of 100-150 nm. Similar to the gold surface the chains are also built atop of the layer consisting of tightly packed NPs. Thin layers of DPT-0 on graphite completely cover its surface, forming a continuous layer, which has no pronounced relief (Fig. 3d).

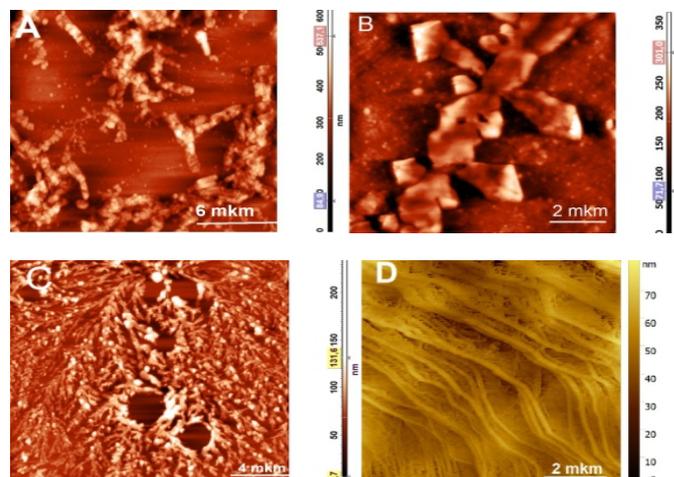


Fig.4. AFM-STM images of thin layers of DPT-1 on the surfaces of mica (a), gold (b), glass at pH 10 (c) and graphite (d).

On mica the self-assembly of DPT-1 particles takes place with the formation of aggregates of various shapes (Fig. 4a). The growth of various crystals oriented in two perpendicular directions occurs on the surface of gold atop a layer of densely packed particles (Fig. 4b). At pH 10 on glass substrate, DPT-1 chains build dendrite structures (Fig. 4c). The angle between the branches of dendrites is 30-40°. The formation of such structures was never observed upon DPT-0 crystallization.

In thin layers of DPT-1 on the graphite surface the growth of extended fibrous structures of different widths is observed (Fig. 4d). At a higher resolution, it can be seen that these strands have different widths. The ultra thin DPT-1 layers crystallize on the graphite surface in accordance with the mechanism of 2-d (layer-by-layer) growth of monolayers, so the topography of the clean surface of graphite is copied.

The local tunneling current-voltage characteristics (LTCVC) DPT-0 and DPT-1 layers on gold and graphite are very different (Fig.5).

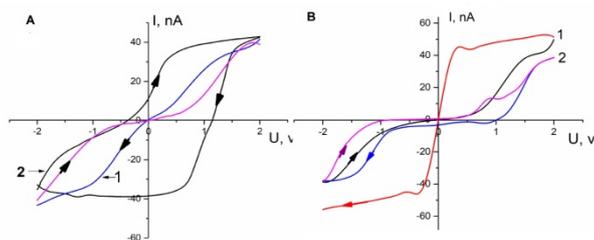


Fig.5. LTCVC of DPT-0 (1) and DPT-1 (2) on the surface of gold (A) and graphite (B)

The greatest differences are observed on a gold substrate. In this case, the width of the energy band (E_g) at points on the surface, located at a distance of less than 200 nm, can vary from 0 to 3 eV, the change in the form of LTCVC on different samples is caused by a change in the local work function or the local density of electronic states (LDOS) of the substrate-DPT-tip system. For all LTCVC, the nonlinear form is characteristic and is conformed the model of currents, limited by the space charge.

Regardless of the nature of the substrate and molecule charge, the effect of bipolar resistive switching is observed at all LTVACs, i.e. presence of areas of high (OFF) and low (SET) resistance, depending on the forward or reverse direction of the current. When a molecule becomes an anion, the order of their succession also changes. Thus, the switching effect depends mainly on the charge of the DPT molecule, not on the substrate. The most interesting result is the presence of hysteresis of LTCVC in the DPT-1 layers on the gold surface (Fig.5a). This indicates the existence of residual polarization and the formation of a ferroelectric. An increase in the crystallization temperature of DPT to 90° C on the gold surface does not change the shape of the CVC of the DPT-1 layers and, consequently, its electronic structure. In the case of DPT-0 layers, they become dielectrics with $E_g \geq 3$ eV. However, in this case it does not become a ferroelectric.

The significant local nonuniformity of the electronic structure of DPT layers was found. It is primarily associated with the chosen method of material deposition. Indeed, the evaporation of water from the surface of the drop begins at its periphery, and ends at its center. Therefore, during the evaporation process local DPT concentrations in the drop volume continuously change. In addition, with a decrease in the drop area, the rate of evaporation of water sharply increases; the crystallization conditions change and the formed layer is disordered. So the thickness of the DPT layer and morphology of its surface at the center and at the rim are significantly different. When measuring LTCVCs, a change in the thickness of the layers will also lead to a change in the local strength of the electric field. Thus, the formed layers would have a local lateral disorder in both the interface region and the volume of the layer. In this case the charge injection process also becomes laterally heterogeneous and acquires a filamentary character.

To summarize the experimental results including spectral data, firstly it is necessary to consider the conformation of the neutral (DPT-0) and ionized (DPT-1) molecule and their interactions with gold, graphite, mica and glass surfaces. The total charge of DPT molecule depends on pH, because it contains amino acids with ionic side groups. Molecule is neutral when pH reaches the value of 3-4 and is characterized by four stabilizing intramolecular hydrogen bonds. Then with increasing pH, DPT becomes negatively charged due to the acid dissociation of two terminal carboxylic groups at first, and at pH > 10, the negative charge reaches the value - 4 due to the acid dissociation of two carboxylic groups at the side chains of the glutamic acid residues. The pH range of 7-8 corresponds to DPT molecule having one or two ionized terminal carboxyl groups; and two uncharged amino groups of lysine residues. They can participate in coordination with the surfaces of different substrates. Conformation of an isolated anion in a strongly basic environment is stabilized by eight intramolecular hydrogen bonds. Because of the repulsion of like-charged groups, the DPT chain stretches and its radius of gyration increases. Therefore, the height of the anion decreases in comparison with the height of the neutral molecule (1.2-1,5nm). This is what we observe in the ultrathin layer on the graphite surface.

Owing to amphiphilic character of molecule the presence of water-soluble functional groups of PT molecule (antennas) and hydrophobic hydrocarbon chain, DPT chains self-organize differently on the substrates of various polarities.

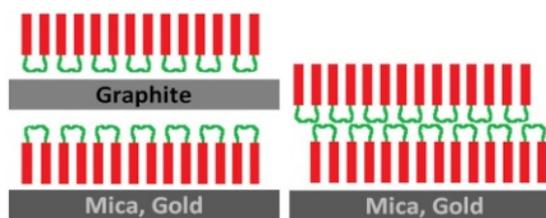


Fig.6. The scheme of DPT monolayers orientation on graphite, gold and mica surfaces, PTs antennas - red, hydrophobic linkers -green [4]

The materials we studied differ not only in their structure and chemical composition, but also in the surface charge. Indeed, the surfaces of graphite and glass are electrically neutral, and on the surface of gold a positive charge arises only when DPT-1 is deposited (image charge). The surface of mica, which is a classical dielectric, contains an equal number of positive and negative charges. If two terminal carboxylic groups are negatively charged two antennas of DPT are anchored on mica surface through hydrogen bonding and electrostatically. Remaining part of the molecule including its central hydrophobic unit stays non-adsorbed due to the high surface charge of muscovite (Fig.6).

Therefore, when DPT-0 and DPT-1 are deposited on the surface of gold and mica, the nature of their interactions will be significantly different. Initially neutral surface of gold during adsorption of DPT-0 remains neutral and DPT chain adsorbs entirely, and upon adsorption of anion DPT-1 is charged positively. All this contributes to the localization of the DPT-1 molecule on the surface of mica and gold.

The silanol SiOH groups on the silica surface can participate in hydrogen bonding with PT chains. The interaction between molecules is weak hydrogen bonding between amino- and carboxylic groups and silanol groups of the silica. The surface of graphite is the most inert and hydrophobic. After depositing the PT, it becomes hydrophilic, in contrast to, for example, mica and gold where initially hydrophilic surfaces become hydrophobic (Fig.6). The influence of the substrate is manifested in the way in which the growth mechanism of DPT layers changes from a layer-by-layer on graphite to a growth of individual 3-d aggregates with their subsequent coalescence on the surfaces of mica and gold. On the non-charged surface of graphite DPT chain adsorbs entirely [3]. Here COOH and NH₂ groups build intramolecular hydrogen

bonds. Because of the van der Waals character of the interaction between the chain and the surface, the strength of the adsorption is moderate.

We have previously found out that self-organization in PT layers [1-2] is observed in case of weak particle-substrate interactions at poor wetting conditions and at low rate of solvent evaporation. Apparently, the stronger interactions between negatively charged carboxyl groups of DPT and positively charged mica and gold surfaces due to a large number of charges prevent the formation of extended ordered structures.

The crystallization processes are largely dependent not only on the nature of the substrate, but also on the surface roughness and the method of deposition used. As a consequence, at different areas of the same substrate, the various structures can be observed.

4. Conclusion

Our experimental results demonstrate that the structure and morphology of the thin layers of the DPT composites depend significantly on the properties of the substrate-layer interface. Otherwise, on different substrates, we would observe the same morphology of relatively thick layers. As a result, a diverse set of crystal morphologies is observed including disc-like particles, dendrites, chains and mesocrystals at all stages of DPT crystallization. Depending on the charge of the DPT molecule (neutral or anion), not only the morphology of the surface of the layers changes, but also its electronic structure. This is due to the fact that the crystallization of DPT-0 leads to the formation of an organic crystal with covalent and hydrogen bonds, and DPT-1 to the formation of a DPT-1 salt with an ionic bond type. The important role is played by the properties of the substrate-layer interfaces, which are responsible for the formation of ferroelectrics on gold during the crystallization of the DPT anion. The structure of the solid layers is also significantly affected by charges on the substrate surface, which can partially or completely compensate for the charges of individual functional groups in the PT molecule. It is known, that polymorphism plays an important role in the manufacture of drugs and products of fine organic chemistry. Since DPT we studied is also a drug, its various polymorphs can exhibit different bioavailability, stability, melting point, solubility. The effect of bipolar electrical switching allows usage these structures as elements of two-terminal resistive nonvolatile memory. Regardless of the specific mechanism for the appearance of CVC hysteresis DPT -1 can find practical application in memristor organic electronics.

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