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3D-AFM-NANO-STRUCTURAL ANALYSIS AND SURFACE FEATURES OF INTERFACIAL LAYERS OF SOLID-PHASE GRAMICIDIN-A ANTIBIOTIC

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ABSTRACT

Although gramicidin family are a polypeptide with alternating L- and D-amino acids. The branching of polypeptide linkage needs special stereo –orientation to be applied as therapeutic antibacterial cream or ointment .The present 3D-AFM-investigations introduce important conclusive remarks enhance scientific community to understand why gramicidin family as antibiotic cream or ointment are structurally suitable with special surface topography enhance it to be applied as cream or ointment.

Key Words:- Gramicidin-A, Nano-Structural Features, 3D-AFM, Cream, Grains Size, Surface.

INTRODUCTION

It is well known that Gramicidin is a polypeptide with alternating L- and D-amino acids, sharing the general formula: formyl-L-**X**-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-**Y**-D-Leu-L-Trp-D-Leu-L-Trp-ethanolamine X and Y depend upon the gramicidin molecule. There exist valine and isoleucine variants of all three gramicidin species, and 'X' can be either. Y determines which is which; as Y gramicidin A contains tryptophan, B phenylalanine, and C tyrosine. Also note the alternating stereochemical configurations (in the form of D and L) of the amino acids; this is vital to the formation of the β-helix. The chain assembles inside of the hydrophobic interior of the cellular lipid bilayer to form a β-helix. The helix itself is not long enough to span the membrane but it dimerizes to form the elongated channel needed to span the whole membrane. (Ketchem RR *et al.*, 1997; Izumiya N *et al.*, 1979; He K *et al.*, 1994; Koeppe RE and Andersen OS, 1996; Oiki S *et al.*, 1994; Oiki S *et al.*, 1995; Mattice GL *et al.*, 1995; Smith R *et al.*, 1989; Nicholson LK and Cross TA, 1989; Levitt DG *et al.*, 1978; Andersen OS *et al.*, 2007; Myers VB and Haydon DA, 1972; Russell EWB *et al.*, 1986).

The structure of the gramicidin head-to-head dimer in micelles and lipid bilayers was determined by solution and solid-state NMR. In organic solvents and crystals this peptide forms different types of non-native double helices.

Over the years, many different compounds that target specific bacteria have been developed, both from natural sources and through synthetic efforts. These compounds can be categorized in different ways. Some compounds lead to bacterial cell death and are called bactericidals, whereas others merely arrest bacterial cell division and are called bacteriostatics (Andersen OS *et al.*, 1998; Cotten M *et al.*, 1999; Jude AR *et al.*, 1999).

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Obviously different compounds classes can be distinguished based on the origin of the bacteria they target. Often antibiotics are subdivided into those that act against Gram-positive (Weiss LB and Koeppe RE, 1985; Mouritsen OG and Bloom M, 1984; Killian JA *et al*., 1996; Andersen OS and Koeppe RE, 2007; Jordan JB *et al*., 2006; Durkin JT *et al*., 1990) bacteria exclusively, those that target only Gram-negative bacteria Durkin JT *et al*., 1993; Sun H, 2003. Fonseca V*et al*., 1992; Lundbæk JA and Andersen OS, 1999; Miloshevsky G and Jordan P, 2006) and those that act against both. Perhaps the most comprehensive subdivision is the one that takes into account the molecular mechanism that is at the basis of the antibacterial action of antibiotics.

The major goal of the present manuscript is introducing focused informative conclusions on the structural surface features fitting of gramicidin family and how much it is fitted to their applications which normally applied as antibiotic ointment/cream.

EXPERIMENTAL

Sample Source

A commercial structurally well confirmed sample of highly pure solid-phase gramicidin A (a hydrophobic linear polypeptide) in solid phase was supplied from EDWIC company of pharmaceutics (EGYPT) and applied as model for testing micro-structural features and surface topology of gramicidin family.

Nano-/Micro-Structural Investigations

Scanning electron microscopy (SEM): measurements were carried out along ab-plane using a small amount of sample powder by using a computerized SEM camera with elemental analyzer unit Shimadzu (Japan).Atomic force microscopy (AFM): High-resolution Atomic Force microscopy (AFM) is used for testing morphological features and topological map (Veeco-di Innova Model-2009-AFM-USA).The applied mode was tapping non-contacting mode. For accurate mapping of the surface topology AFM-raw data were forwarded to the Origin-Lab version 6-USA program to visualize more accurate three dimension surface of the sample under investigation. This process is new trend to get high resolution 3D-mapped surface for very small area ~ 0.1x0.1 μm^2.

RESULTS & DISCUSSIONS

Micro-Structural Measurements

Fig.2 displays scanning electron micrograph captured for gramicidin sample as powder with two different sectors .As it clear in Fig.2 no in homogeneties were observed on the surface's layers or in between grains boundaries .The black arrows refer to different pore sizes which is experimental conditions dependent . The average grain size was estimated and found to be ranged in between 0.65-3.7 μm which reflect complexity of poly peptide linkages present in gramicidin A antibiotic as model of gramicidin family.

The density of pores per micrometer square is dependent on the stereo-chemical configurations of poly amino-acids that linked together with peptide linkage whether it L- or D- .Furthermore existence of cyclic hetero-molecules moieties within different amino-acids could also affect in the pores densities throughout the surface topography.

Nano-Structural Investigations

The nano-structural properties of gramicidin sample were tested by using atomic force microscope applying non-contacting tappling mode imaging. As it clear in Fig.3a-c which displays three different imaging with different parameters and resolution to clarify internal nano-features of investigated gramicidin A sample. Fig.3a displays 2D-AFM-tapping non-contacting mode of gramicidin-A for scanned area 1 μm^2 .In this range of imaging gramicidin-A sample shows regular compacted arrays due to their specialty in stereo-configuration of poly-peptide chains which must oriented in specific positions to eliminate steric effect of cyclic hetero-cyclic moiety present in constituent poly amino acids [16,17,18 and 19] .The arrays is repeated after ~ 0.05 μm as clear in Fig.3_b but the heights is shifted to lower depth ~0.1μm as shown in the magnification tool bar in Fig.3b . The imaging in two dimensions enhanced more and more via 3D-imaging which could be possible with AFM-microscopy. Fig.3_c shows 3D- AFM-tapping mode imaging for Gramicidin A (scanned area 0.01 μm^2) .It was observed that there are no violation in the surface heights gradient due to scanned area is very narrow to display any differences on the surface topology. The average of grains numbers and its size was calculated using AFM-analyzer and found to be 80 μm which is slightly smaller than detected by SE-microscopy. The smallest grains sizes found in AFM-microscopy could be attributable to that atomic force microscopy can scanned very tiny area ~0.01 μm^2 which is too difficult to be measured by SE-microscopy.

For accurate mapping of the surface topology AFM-raw data were forwarded to the Origin-Lab version 6-USA program to visualize more accurate two and three dimension surface of the sample under investigation.Fig.4 shows the possibility of mapping the whole scanned area with very precise and accurate results .As it clear in Fig.4 the horizontal and vertical profile of the surface are

correspondence to the two perpendicular (Cartesian axes) yellow perpendicular axes .Applying this trend of investigation one can scan and visualize real contour image in 2D and 3D as shown in Fig.$5_{a,b}$ with very high resolution and accuracy in calculating surface's parameters.

Fig.5a,b can introduce accurate analysis of the surface's topography such that color gradient is heights dependent and from the key of the mapping figure one can calculate maximum heights and minimum one by just looking to the key of the figure .

Fig 1. Amino acids sequence of Gramicidin- A antibiotic

formyl-L-Val[1]-Gly[2]-L-Ala[3]-D-Leu[4]-L-Ala[5]-
-D-Val[6]-L-Val[7]-D-Val[8]-L-Trp[9]-D-Leu[10]-L-Trp[11]-
-D-Leu[12]-L-Trp[13]-D-Leu[14]-L-Trp[15]-$NHCH_2CH_2OH$.

Fig 2. SE-micrographs captured for Gramicidin A with magnification bar 5 µm

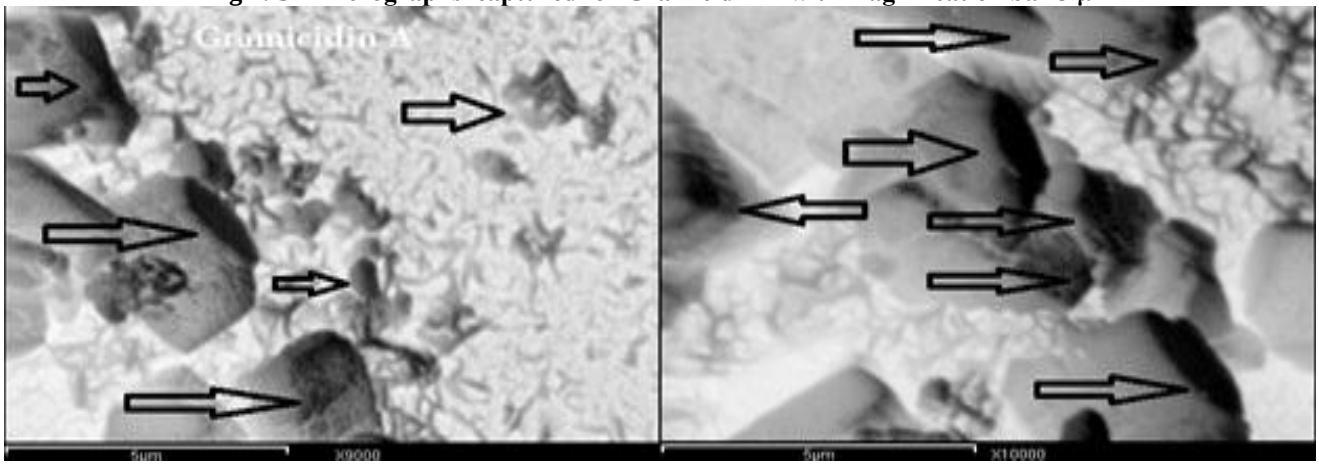


Fig 3a. 2D-AFM-tapping non-contacting mode of Gramicidin A for scanned area 1 µm^2,
3b. High Resolution 2D-AFM-tapping image of Gramicidin A for scanned area 0.01 µm^2
3c. 3D- AFM-tapping mode imaging for Gramicidin A (scanned area 0.01 µm^2)

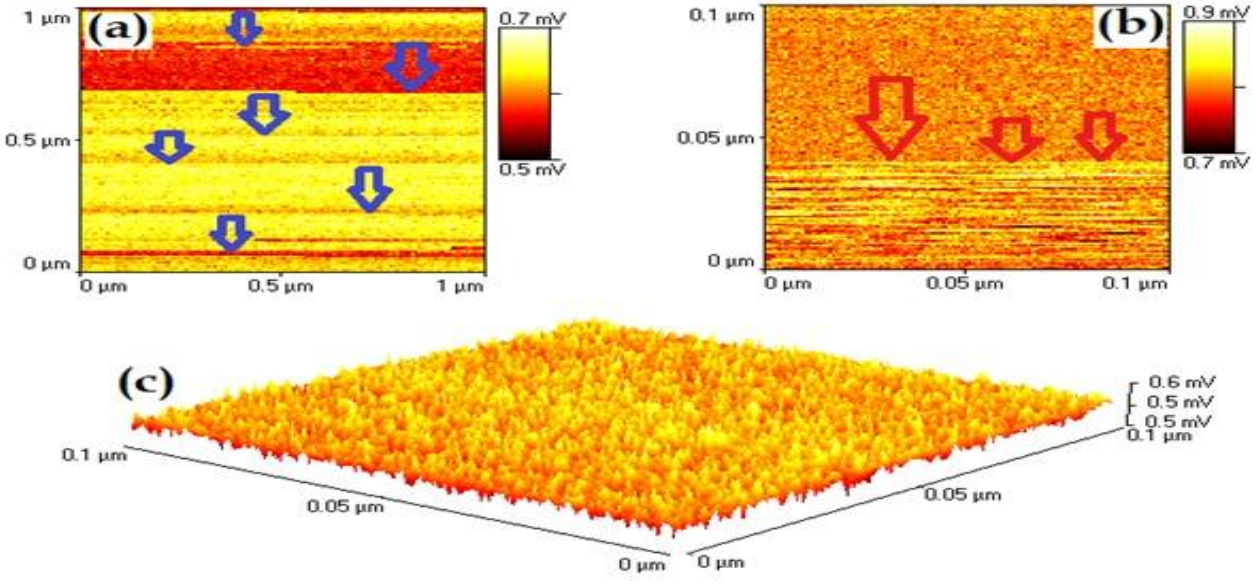


Fig 4. Mapping of the gramicidin-A surfaces through AFM-raw-data

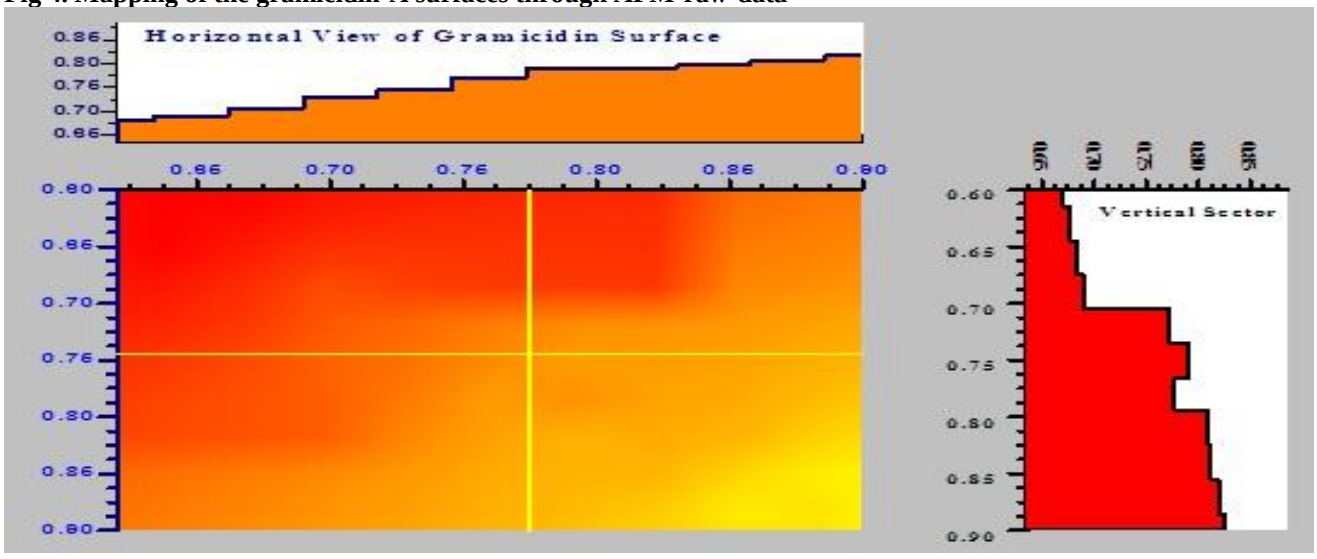


Fig 5a. 2D-contour diagram for Gramicidin–A surface's antibiotic

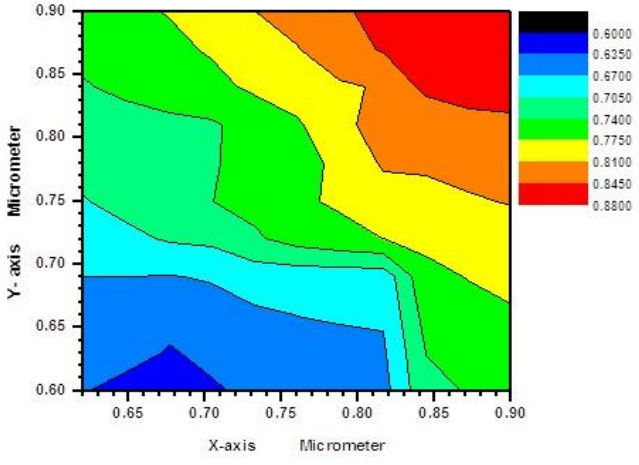
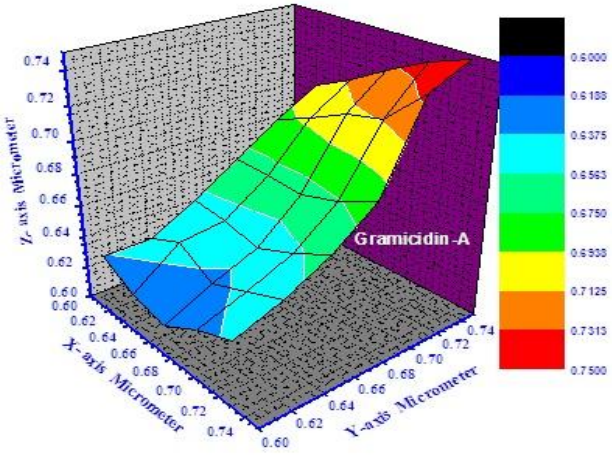


Fig 5b. 3D-contour diagram for Gramicidin–A antibiotic



CONCLUSION

The conclusions inside present article can be summarized in the following points;

- Gramicidin-A has special microstructure with very huge surface area.
- Gramicidin-A as member of gramicidin family has specific oriented configuration internal microstructure of poly-peptide chain to be fitted and suitable to be applied as interfacial cream/ointment antibiotic.
- Atomic Force Microscopy is efficient and accurate tools to predict by surface topology and nano-structural features of complicated solid surfaces (poly-peptide compounds).

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