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# Natural vs synthetic auxin: Studies on the interactions between plant hormones and biological membrane lipids



Michał Flasiński\*, Katarzyna Hać-Wydro

Department of Environmental Chemistry, Faculty of Chemistry, Jagiellonian University, Gronostajowa 3, 30-387 Kraków, Poland

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## ABSTRACT

Analysis of the interactions between two representatives of plant hormones: synthetic (1-naphthaleneacetic acid, NAA) as well as natural (indole-3-acetic acid, IAA) and phospholipids occurring in biological membrane of both plant and animal cells was the subject of present studies. The aim of undertaken experiments was to elucidate the problem of direct influence of these plant growth regulators on phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) in monolayers at the air/water solution interface. The studied phospholipids differ not only as regards the structure of polar head-groups but also in the length of hydrophobic chains as well as their saturation degree. These differences result also in the main properties and functions of these phospholipids in biomembranes. The analysis of the results was based on the characteristics of the surface pressure ( $\pi$ ) – area (A) isotherms registered for monolayers spread on the subphase containing plant hormone and as a reference on the surface of pure water. Moreover, as a complementary technique, Brewster angle microscopy was applied for the direct visualization of the investigated surface films. The obtained results revealed that auxins effectively influence phospholipids monolayers, regardless of the lipid structure, at the concentration of  $10^{-4}$  M. It was found that for this concentration, the influence of auxins was visibly larger in the case of PCs as compared to PEs. On the other hand, in the case of auxins solution of  $\leq 10^{-5}$  M, the observed trend was opposite. Generally, our studies showed that the natural plant hormone (IAA) interacts with the investigated lipid monolayers stronger than its synthetic derivative (NAA). The reason of these differences connects with the steric properties of both auxins; namely, the naphthalene ring of NAA molecule occupies larger space than the indole system of IAA. Therefore molecules of the latter compound penetrate easier into the region of phospholipids' polar head-groups. Moreover, the NH group of the indole moiety is capable of hydrogen bond formation with the acceptor groups in the polar fragment of lipid molecules. We proved also that among the investigated phospholipids, the highest susceptibility toward auxin influence show these lipids, for which during compression, surface film increases the degree of condensation.

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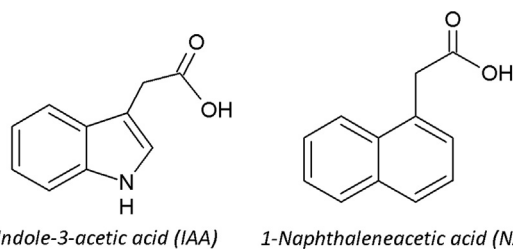
## 1. Introduction

It is widely accepted that plant hormones (auxins) are implicated in the majority of processes connecting with plant growth, proper development and reproduction. These compounds participate in the process of cell elongation, division, differentiation as well as fulfill a key role as mediators in responses to external environmental changes (Mooney, 2007). By regulating the concentration of phytohormones it is possible to control loss of leaves, formation and growth of roots, buds, flowers and fruits (Yoshida et al., 2012; Shimizu-Sato et al., 2009). Auxins are predominantly synthesized

in young leaves, having maximum capability of biosynthesis as well as in cotyledons, shoots and roots (Woodward and Bartel, 2005). Among plant hormones that attract special attention and are the subject of extensive studies, mostly as a result of their prevalence, special place belongs to indole-3-acetic acid (IAA) being the main natural auxin. It is also not without significance that IAA due to its involvement in the response to directional light was the first plant hormone discovered (Ljung, 2013). This omnipresent compound can be found in all members of plant kingdom as well as in bacteria, fungi, algae and even animals, where it gets with the diet rich in vegetables or can be synthesized from tryptophan (Furukawa et al., 2004; De Melo et al., 2004). Biosynthetic pathways of IAA in plants are still incompletely recognized, since both are responsible for these process genes, enzymes, precursors and intermediates are not fully elucidated. On the other hand, it is known that generally there are

\* Corresponding author. Fax: +48 12 634 05 15.

E-mail address: [flasinsk@chemia.uj.edu.pl](mailto:flasinsk@chemia.uj.edu.pl) (M. Flasiński).



**Scheme 1.** Chemical structures of the investigated auxins: IAA and NAA.

two possible routes of auxins biosynthesis: tryptophan-dependent and Trp-independent which based on the precursor of Trp (Woodward and Bartel, 2005; Korasick et al., 2013). Auxins which are synthesized largely in shoot apical region can be then transported downward to the base throughout the other plant cells, tissues and organs. In higher plants IAA can be stored in the form of conjugates ester-linked to sugars or amide-linked to amino acids and peptides (Hobbie, 1998). Auxins are essential for plants lifecycle but their overproduction or excessive exogenous administration may lead to negative effects including inhibition of root elongation, epinastic leaves and elongated hypocotyls (Mooney, 2007). Apart from IAA being the most frequently studied auxin, there are also other examples of compounds demonstrating similar properties and therefore classified as plant hormones. As far as the similarity in the chemical structure is concerned, one of the closest analogs of IAA is 1-naphthaleneacetic acid (NAA). This compound differ only as regards the system of aromatic rings present in the molecule (Scheme 1).

In contrast to IAA, NAA is a synthetic compound, design to mimic properties of plant hormones; however there are subtle differences between activity of both compounds (Singh et al., 2009; Li et al., 2012; Yamamoto and Yamamoto, 1998). They may result predominantly from the differences in the steric properties of IAA and NAA, since the naphthalene moiety present in the latter compound occupies more space than the indole ring of IAA (Tan et al. 2007). Entering the plant cell, auxin molecules move across the plasma membrane by two possible mechanisms: the first one is passive (Gutknecht and Walter, 1980) (characteristic for more bulky NAA Delbarre et al. (1996)), involves the pH gradient across the lipid bilayer, whereas the second takes place through membrane-inserted protein carriers (Johri and Mitra, 2001). Regardless of the dominating way, cellular membrane comprises a specific barrier for unhampered flow of auxins' molecules. At the same time, according to some results existing in literature, phytohormones are able to interact with lipid components of biomembranes (Stillwell et al., 1989). There are relatively lots of studies reporting harmful effects caused by compounds belonging to the class of plant growth regulators (PGRs), including IAA and NAA (Celik and Tuluçe, 2007). One of the best confirmed example of the toxic effect displaying by auxins is the oxidative stress caused by these compounds which may lead to cell death. De Melo et al. show that 1 mM concentration of IAA examined in rat neutrophils and lymphocytes induce loss of membrane integrity, DNA fragmentation and other toxic effects leading to cell apoptosis (De Melo et al., 2004). The mechanism of such toxicity is connected with the production of reactive oxygen species (ROS) induced by IAA (Kawano, 2003). Similar mechanism was also observed in other studies performed in biological in vitro conditions (Celik et al., 2002; Folkes et al., 1999) and in model systems (liposomes) (Candeias et al., 1995; Jones and Paleg, 1984). Cytotoxicity of auxins was also tested in the context of their potential application in anticancer therapy (Greco et al., 2002; Morshed et al., 2005; Folkes and Wardman, 2003). There are also results of studies suggesting antifungal and antimicrobial activity of IAA,

what makes this compound good candidate for natural food preservative (Morshed et al., 2006). Despite the proven toxic effects of both IAA and NAA, as shown in numerous studies, there is lack of systematic knowledge regarding the direct impact of auxin on components found in plasma membranes of both plant and mammal cells. Admittedly, the influence of PGRs on lipid fractions extracted from cell cultures was analyzed in model systems, like liposomes (Laggner et al., 2003; Jones and Paleg, 1984) and Langmuir monolayers (Gzyl et al., 2004) but because of complexity of such multicomponent systems, it is difficult to draw coherent conclusions regarding specificity of the studied interactions. Such studies would be of great importance taking into account that auxins were confirmed to be active at the level of biological membranes. Interactions with lipid components of cellular membrane was also observed in the case of other substances having similar to plant hormones properties. For example, abscisic acid (ABA) was found to influence phospholipid organization in biomembranes which was closely correlated with the type of phospholipids' head-group and hydrocarbon chains (Stillwell et al., 1989).

The main purpose of the present work was to emphasize the differences in the interactions of natural (IAA) vs synthetic (NAA) phytohormones with phospholipids occurring naturally in plasma membranes of both plant and animal kingdom. In our studies we applied simple but efficient and broadly applied in such studies methodology based on the characteristic of phospholipids monolayers spread on subphase containing investigated bioactive substances (e.g. Flasiński et al., 2010; Travkova et al., 2013; Nunes et al., 2011). In this project we analyzed the influence of auxins' type and concentration on the characteristics of the investigated monolayers formed by molecules of phospholipids differing as regards the type of polar head-group as well as the saturation of hydrophobic acyl chains. Analysis of the experimental results was carried out on the basis of the obtained surface pressure ( $\pi$ ) – mean molecular area (A) isotherms. Additionally, the degree of monolayer condensation in the presence of different concentrations of auxins was quantified based on the compression modulus ( $C_s^{-1}$ ) vs the surface pressure dependency. As a complementary technique, Brewster angle microscopy (BAM) was applied in order to visualize the impact of auxins on the morphology of the investigated surface films.

## 2. Experimental

### 2.1. Materials

Phosphatidylcholines (PCs) applied in our studies: 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) as well as sphingomyelin: N-palmitoyl-D-erythro-sphingosylphosphorylcholine (C16-SM) as well as phosphatidylethanolamines (PEs): 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (POPE), 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (SOPE) and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were synthetic products of the highest purity available ( $\geq 99\%$ ) in leading lipid manufacturer: Avanti Polar Lipids, Inc. Both auxins, i.e. indole-3-acetic acid, abbr. IAA (98%) and 1-naphthaleneacetic acid, abbr. NAA ( $\geq 99\%$ ) were synthetic compounds purchased from Sigma-Aldrich. In routine experiments 100–200  $\mu\text{L}$  of 0.25 mg/mL lipid solution in chloroform/methanol 9/1 (v/v) mixture was deposited onto water subphase with the Hamilton micro-syringe, precise to 1.0  $\mu\text{L}$ . The monolayers were left after spreading for 10 min before the compression was initialized with the barrier speed of 20  $\text{cm}^2/\text{min}$ . Chloroform of spectroscopic purity (99.9% stabilized by ethanol) as well as methanol (99%) were provided by Sigma-Aldrich. Auxins were dissolved in ultrapure water of the resistivity  $\geq 18.2 \text{ M}\Omega \text{ cm}^{-1}$  obtained from a Milli-Q system.

### 2.2. Methods

The experiments were performed with the NIMA (UK) Langmuir trough (total area = 300  $\text{cm}^2$ ) placed on an anti-vibration table. The surface pressure was measured using Wilhelmy plate made of filter paper (ashless Whatman Chr1) connected to an electrobalance. The constant temperature during experiments (20  $^\circ\text{C}$ ) was controlled thermostatically with the circulating water system. All the

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