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# THE EFFECT OF THE CHEMICAL STRUCTURE OF POLYENE MACROLIDES ON THE PERMEABILITY OF THE *CHLORELLA VULGARIS* PLASMA MEMBRANE



Investigations of the biochemical processes in living organisms require the use of various special inhibitors of chemical reactions. These inhibitors play a particularly important role, since they allow the observation of specific biochemical processes while inhibiting some reactions in the cycles of biochemical transformation. These substances are widespread in biochemical studies, e.g. chloramphenicol or cycloheximide used in selective inhibition of protein synthesis in the cells of microorganisms (Gale et al. 1972), m — chlorophenylhydrazone carbonyl cyanide (CCCP) or dinitrophenol as inhibitors of the phosphorylation processes in various microorganisms (Hall, Evaus 1972; Kun, Grisolia 1972), etc. In this group of active substances antibiotics are particularly important because of their very specific action in comparision with synthetic compounds, which are much less selective.

In the investigations of biochemical processes in living organisms an important role can be played by the substances which induce characteristic changes in the permeability of the plasma membrane. A number of such substances have been discovered and applied in biochemical investigations of bacteria and fungi for example, they include depsipeptides and antibiotics of the polyene macrolide group (Gale et al. 1972). So far, these substances have not been used in the biochemical investigations of algae.

This study was intended to determine the possibility of developing specific changes in the permeability of the plasma membrane of algae

caused by the action of substances from the polyene macrolide group. The investigations were conducted on a model organism of *Chlorella vulgaris*, which is a typical representative of eucariotic algae.

## CHARACTERISTICS OF THE BIOLOGICAL MODEL OF THE INVESTIGATIONS OF *CHLORELLA VULGARIS*

*Chlorella vulgaris* — the representative of eucariotic unicellular algae investigated in this study is a species classified as follows:

class — *Chlorophycea*
order — *Chlorococales*
family — *Oocystaceae*
subfamily — *Chlorelloideae* (Fott, Nowakowa 1969).

*Chlorella vulgaris* cells are spherical or ellipsoidal 2 to 10 μm in diameter. They are covered with a thin smooth cell wall without any roughness or relief. They contain a single chloroplast adhering to the plasma membrane. In young cells the chloroplast covers almost all the inside surface of the plasma membrane. The occurrence of a pyrenoid is not characteristic of this species. Young *Chlorella vulgaris* cells contain one nucleus, while older ones exhibit two or more nuclei (Retowsky 1965). The number of vacuoles depends not only on the age of a cell, but also on the culture conditions and can vary from one to several. *Chlorella vulgaris* reproduces through autospores forming inside the parent cell wall. The quantity of the autospores depends on the culture conditions and varies from 2 to 16 (Fott, Nowakowa 1969; Prokop Ričica 1968; Nečas 1970). During reproduction the parent cell wall is not subject to lysis, but breaks into several fragments (Fott, Nowakowa 1969).

The *Chlorella vulgaris* cell wall consists of α — cellulose and hemicellulose (which in turn is made of glucose, ramnose and xylose (Kreger 1962). The plasma membrane 15 nm thick adheres to the inside of the cell wall. Among others, sterols (ergosterol, ergostenol, chondrillasterol, and chondrillastenol) are present in the *Chlorella vulgaris* plasma membrane (Otsuka 1963; Tomita, Vomori, Minato 1969). Their presence determines the sensitivity of the microorganism to the action of polyene macrolides.

Chlorella vulgaris is an autotrophic organism. It grows well on poor inorganic substrates under conditions which favour photosynthesis (Döhler 1974; Nečas 1970). Furthermore, the organism is able to assimilate organic matter from the environment (Griffiths 1965; Modson, Thompson 1969; Tanner, Grunes, Kandler 1970; Tanner, Kandler 1967).

*Chlorella vulgaris* grows most intensively on an organic medium under conditions of weak light intensity (50 to 2000 Lx) (Zajic, Chin 1970).

*Chlorella vulgaris* is not sensitive to the variation of pH, light, and composition of medium and can easily adapt itself to new conditions (Steemann-Nielsen, Hansen, Jorgensen 1962; Zajic, Chin 1970).

In principle, *Chlorella vulgaris* is a fresh-water organism, but it is also met in brackish waters owing to its high saline tolerance. *Chlorella* organisms are often found in the coastal waters of the Gulf of Gdańsk, sometimes in quantity (Malewicz, Bojanowski, Popławski 1975). The special interest shown by both biologists and biochemists in this species results, among other things, from the part played by this algae in the primary production in natural water reservoirs and also from the possible use of *Chlorella* strains in the rapid production of high quality protein, which accounts for 50 to 60 per cent of the dry mass of the Chlorella cells (Volesky, Zajic, Knettig 1970).

## CHARACTERISTICS OF POLYENE MACROLIDES

Polyene macrolides are a numerous group of natural compounds with antibiotic properties produced by Actinomycetales.

Polyene macrolides belong to compounds with a complex chemical constitution, but all of them have the following common structural properties (Borowski et al. 1967; Borowski et al. 1971; Kinsky 1967; Lampen 1969; Lampen 1963; Otsuka 1963; Schaffner, Borowski 1961; Tanita, Vornori, Minato 1969).

i) They contain the polyene system of conjugated double bonds of four to seven, which form the chromophore. The chromophore structure forms a basis for the classification of these compounds. Thus polyene macrolides may be divided into tetraene, pentaene, hexaene and heptaene compounds, all depending on the quantity of the conjugated double bonds.

ii) They are macrocyclic lactones with 25 to 38 carbon atoms in the so-called macrolide ring. Two groups can be distinguished with reference to the time of the macrolide ring. The first includes the so-called „smal" polyenes with 25 to 27 carbon atoms in the ring, while the other contains the so-called „large" polyenes, the lactone ring of which is made up of 37 or 38 carbon atoms. The particle size of a certain antibiotic does not depend only on the size of the macrolide ring. Compounds of substantially different molecular weight, due to the concurrence of the substituents of various size are coupled with the macrolide ring within the same group.

Aside from the common structural properties mentioned above, the polyene macrolide particles also include a number of various functional groups connected with lactone, viz. isolated double bands or an additional diene system, methyl groups or longer carbohydrate chains, hydroxyl groups, ketone, epoxy, and carboxyl groups, and glycoside-bounded sugars which are derivatives of mannose, i.e. mycosamine, perosamine, ianose, and ramnose. In addition, these substances can also include an aldol-bounded aromatic ring viz. p-aminoacetophenon or its N-methyl derivative. Various functional groups of the above mentioned are present in different quantities, in particular polyne macrolides (Borowski et al. 1970; Borowski et al. 1971; Kinsky 1967; Lampen 1969).

Most diversified as to structure, is the heptaene group, from which two subgroups can be formed, viz. non-aromatic heptaenes and aromatic heptaenes (Borowski et al. 1971; Lampen 1969; Weissmann, Sessa 1967). The antibiotics of the first subgroup are characterized by the presence of one nitrogeneous moiety (this always being mycosamine) and do not contain aromatic fragments in their particles. The antibiotics of the other subgroup include two nitrogenous moiety one of them being the aldol —bounded p-aminoacetophenon or its N-methyl derivative, while the other is the aminosugar mycosamine or perosamine. Owing to the aromatic fragment and aminosugar present in the aromatic subgroup heptaene the group can be subject to further division (Schaffner, Borowski 1961).

Depending on substituents, polyene macrolides have different ionic character (Kinsky 1963). Therefore, the following polyenes can be singled out:

— neutral, without amino group and carboxyl groups,
— basic, with amino group, but without free carboxyl groups,
— amphoteric, with one carboxyl group and one or two amine groups in the particle.

Natural acidiferous polyene macrolides are not known as yet. The only known acidiferous semi-synthetic N-acyl derivatives of basic as well as amphoteric polyenes have been produced by introducing the radicals of mono- or bicarboxyl acids (Lechevalier et al. 1961; Ragni et. al. 1961; Schaffner, Borowski 1961).

Polyenes are not water-soluble, but form colloidal suspensions in water. Water-soluble are only salts of polyene derivatives (N-acyl, ester, and N-glycoside derivatives).

Polyene antibiotics act on Fungi, Protozoa, higher Algae, and animal cells, but do not affect bacteria and lower Algae.

As a result of the investigations of the action of polyene macrolides carried out to date, the consequences of this action have been explored

better than the molecular processes of the phenomena involved. It is well known that these antibiotics bond with the sterol compounds of the plasma membrane of the cell sensitive to their influence, thus bringing about the destruction of the cell, which ceases to play the part of a selective osmotic barrier both for the metabolites inside the cells and for substrate substances. The damaged cell is unable to continue its proper vegetative functions and is thus fated inevitably to die. Among polyene macrolides, N-succinylperimycin acts in a specific manner on Saccharomycetes cells; under certain conditions this action can be reversed. Damage to the plasma membrane of a Saccharomycetes cell due to this antibiotic is limited to initation of the leakage of potassium ions only, from the cell. Under optimum conditions the damage can be repaired by the cell (Borowski, Cybulska 1967).

The extens of damage to the membrane which is measured by the size of the particles which manage to pass through the permeable membrane by free diffusion, depends on certain elements of the chemical constitution of a polyene (the effect of the size of the macrolide ring of which has been determined more definitely), its concentration, and duration of action, and also on the temperature and pH of the environment (Andreoli, Monahan 1968; Kinsky 1967; Kinsky 1967; Lampen 1969; Lampen, Arnow 1963; Sessa, Weissmann 1968).

The knowledge of the mechanism of the action of polyene macrolides gained prior this study was obtained from studies on model organisms, such as Saccharomycetes, Candida, Neurospora, Trychomonas, Leishmania, Mycoplasma, and animal cells (mostly erythrocytes). The models include representatives of all types of organisms sensitive to the action of polyene macrolides with the exception of algae, which were not studied systematically hitherto.

The investigation of the effect of polyene macrolides on eucariotic algae would not only add to the existing knowledge, but would also provide further information about the phenomena taking place during the action of these antibiotics, especially in view of their individual biological character.

## THE COURSE OF THE INVESTIGATIONS AND DISCUSSION OF THE RESULTS

A *Chlorella wulgaris* Beijerinck (A 88a) strain obtained from the cultures of the Czech Academy of Science was used in the investigations.

The strain was maintained on agar media with inorganic bases at room temperature and in weak light (1.000 Lx). A population of *Chlorella*

*vulgaris* from autotrophic or photoheterotrophic cultures was used in the tests.

The *Chlorella vulgaris* suspension used in the experiments contained about 80 per cent of cells 3—7 μm, in size; 1 mg of *Chlorella vulgaris* dry mass contained abount $7.10^6$ cells.

All the tests were conducted under the conditions of optimum growth. The optimum growth parameters (light, pH, temperature) were chosen on the basis of bibliographical data and checked experimentally, investigating among other things, the photosynthesis rate as an indicator of cell growth (Strickland, Parson 1965). It was found that the optimum temperature of heterotrophic growth on a basis containing bactopeptone and glucose was 30°C.

The pH index close to the neutral value applied in the tests was within the optimum range for *Chlorella vulgaris.*

The so-called "doubling time" of the *Chlorella vulgaris* cells incubated under the test conditions was worked out experimentally by the method of microscope-aided counting of the number of cells. It was found to be about 8 hours.

To establish the conditions of some tests it was essential to determine the mean concentration of potassium ions in cells. This concentration was determined by the flame photometry method and found to equal about 40 mM.

Upon observing the photosynthesis level as an indicator of the growth of cells it was found that the concentrations of all the chemical reagents used in the tests do not either inhibit or stimulate the growth of the *Chlorella vulgaris* cells.

The chosen polyene macrolides and their derivatives represent all the most important structural groups and the groups of physico-chemical properties. The differences are due to the following factors:

— the size of the macrolide ring,
— the size of the whole molecule (as characterised by molecular weight),
— the degree of unsaturation in the chromophore,
— the structure of nitrogenous moiety,
— ionic character,
— dispersion in water medium.

The chosen substances are presented in Table 1 together with the characteristics of their chemical and physicochemical properties. These substances were subject to biochemical tests to determine the sensitivity of the *Chlorella vulgaris* cells to the action of polyene antibiotics and also to establish the character of changes in the permeability of the plasma membrane. The sensitivity of *Chlorella vulgaris* to the action of polyene antibiotics was determined by finding the lowest concentration

Table 1

Polyene macrolides and their derivatives chosen for the tests, representative for the structural groups and the groups of physico-chemical properties *

Makrolidy polienowe i ich pochodne wytypowane do przeprowadzenia badań, reprezentowane dla grup strukturalnych i grup własności fizykochemicznych

| | Polyene Polien | Molecular weight Ciężar cząsteczkowy | Chromophore Chromofor | The size of the lactone ring Wielkość pierścienia makrolidowego | Molecular formula Wzór cząsteczkowy | Ionic character Charakter jonowy | Nitrogenous moiety Fragmenty azotowe | Water solubility Desperacja w roztworze wodnym |
|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| 1 | nystatin $A_1$ | 925 | tetraene | 37 C | $C_{47}H_{75}O_{17}N$ | amphoteric | mycosamine | non soluble |
| 2 | nystatin $A_3$ | 1095 | tetraene | 37 C | $C_{53}H_{85}O_{22}N$ | amphoteric | mycosamine | non soluble |
| 3 | pimaricin | 661 | tetraene | 25 C | $C_{33}H_{47}O_{13}N$ | amphoteric | mycosamine | non soluble |
| 4 | rimocidin | 727 | tetraene | 27 C | $C_{38}H_{63}O_{13}N$ | amphoteric | mycosamine | non soluble |
| 5 | filipin III | 654 | pentaene | 27 C | $C_{35}H_{58}O_{11}$ | neutral | no | non soluble |
| 6 | lienomycin | 1200 | pentaene | — | | amphoteric | amin group | non soluble |
| 7 | hexamycin | unknown | hexaene | unknow | — | unknown | unknown | non soluble |
| 8 | amphotericin B | 930 | heptaene | 37 C | $C_{47}H_{75}O_{17}N$ | amphoteric | mycosamine | non soluble |
| 9 | mixomycin | 1100 | heptaene | — | $C_{58}H_{88}O_{18}N_2$ | amphoteric | mycosamine p-N-methylaminophenyl | non soluble |
| 10 | candicidin D | 1200 | heptaene | 41 C | $C_{62}H_{96}O_{22}N_2$ | amphoteric | mycosamine p-aminophenyl | non soluble |
| 11 | perimicin | 826 | heptaene | 39 C | $C_{46}H_{78}O_{14}N_2$ | basic | perosamine p-aminophenyl | non soluble |
| 12 | N-acetylamphotericin B | 972 | heptaene | 37 C | $C_{49}H_{77}O_{18}N$ | acidic | mycosamine | non soluble |

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|
| 13 | N-acetyl-candicidin D | 1250 | heptaene | 41 C | $C_{64}H_{98}O_{23}N_2$ | acidic | mycosamine p-aminophenyl | non soluble |
| 14 | Na-succinyl-perimicin | 948 | heptaene | 39 C | $C_{50}H_{82}O_{17}N_2$ | acidic | perosamine p-N-methylaminophenyl | soluble |
| 15 | N-glucosyl-nystatin $A_1$ | 1087 | tetraene | 37 C | $C_{50}H_{85}O_{23}N$ | acidic | mycosamine | soluble |
| 16 | N-glucosyl-nystatin $A_3$ | 1257 | tetraene | 37 C | $C_{59}H_{95}O_{27}N$ | acidic | mycosamine | soluble |
| 17 | N-glucosyl-amphotericin B | 1092 | heptaene | 37 C | $C_{53}H_{85}O_{23}N$ | acidic | mycosamine | soluble |
| 18 | N-glucosyl-candicidin | 1350 | heptaene | | $C_{68}H_{106}O_{27}N_2$ | acidic | mycosamine p-aminophenyl | soluble |
| 19 | N-glucosyl-linomycin | 1350 | pentaene | unknow | unknown | acidic | amin group | soluble |
| 20 | N-glucosyl-pimaricin | 813 | tetraene | 25 C | $C_{39}H_{57}O_{18}N$ | acidic | mycosamine | soluble |

* Chemical data of the compounds given herein have been taken from the relerant literature on unpublished information from the Department of Drugs Technology and Biochemistry of the Gdańsk Technical University.

of a given antibiotic which inhibits the growth of cells (MIC). The MIC value was tested for its dependence on the composition of the medium, an important factor being the dependency of these values on the presence of sodium and potassium ions. The ratio of the minimum concentrâtion of the antibiotic inhibiting the growth of cells on the potassium base to the sodium MIC was assumed as a tentative criterion to estimate the damage to the *Chlorella vulgaris* plasma membrane (Table 2). A more accurate criterion was the investigation of the repair capacity of cells. On the basis of the criteria used to estimate the characteristic effects, two groups were separated from among the polyene macrolides tested viz:

— specifically acting polyenes,

— non-specifically acting polyenes.

The polyenes which act specifically on the *Chlorella vulgaris* plasma membrane cause only such insignificant damage which can be fully repaired by a cell under optimum conditions.

The repair capacity of cells in this case is irrespective of the concen-

Table 2

The influence $K^+$ and $Na^+$ ions on the sensitivity of *Chlorella vulgaris* cells to polyene macrolides

Wrażliwość komórek *Chlorella vulgaris* na działanie makrolidów polienowych w obecności jonów $K^+$ i $Na^+$

| Antibiotic | Minimal inhibitory concentration of polyene tested in growth medium containing: | | |
|---|---|---|---|
| | 25 mM $K^+$ | 0,1 M $Na^+$ | A/B |
| | A mcg/ml | B mcg/ml | |
| Filipin III | 10 | 10 | 1 |
| Pimaricin | 4 | 4 | 1 |
| N-glucosyl-pimaricin | 100 | 100 | 1 |
| Rimocidin | 10 | 10 | 1 |
| Nystatin $A_1$ | 4 | 2 | 2 |
| Nystatin $A_3$ | 8 | 4 | 2 |
| Lienomycin | 10 | 5 | 2 |
| N-glucosyl-lienomycin | 20 | 10 | 2 |
| Hexamycin | 10 | 5 | 2 |
| N-glucosyl nystatin $A_1$ | 10 | 3 | 3 |
| N-glucosyl-nystatin $A_3$ | 10 | 3 | 3 |
| Mixomycin | 0,4 | 0,1 | 4 |
| N-succynylperimicyn | 1 | 0,2 | 5 |
| N-acetyl candycidin D | 12 | 2 | 6 |
| Candicidin | 0,4 | 0,05 | 8 |
| N-glucosyl-amphotericin B | 15 | 2 | 8 |
| Amphotericin B | 2 | 0,2 | 10 |
| N-acetyl amphotericin B | 10 | 1 | 10 |
| N-glucosyl-candicidin D | 3 | 0,3 | 10 |
| Perimicin | 0,5 | 0,02 | 25 |

tration of a given antibiotic or the duration of the contact between the antibiotic and the cell.

Among the antibiotics of non-specific action, two subgroups can be singled out: polyenes of immediate action and progressive-action polyenes. The first subgroup includes those antibiotics which bring about irreversible damage to the *Chlorella vulgaris* plasma membrane which cannot be eliminated by the cell and thus results in its death. The second subgroup consists of those antibiotics which, while used in small concentrations and for short periods of time, destroy a certain part of cells in a reversible manner and thus enable regeneration. By increasing the anti-

Table 3

Classification of the polyene macrolide tested according to extent of damage to the *Chlorella* plasma membrane

Podział badanych makrolidów polienowych według typu działania na komórki *Chlorella vulgaris*

| Specificaly acting antibiotics Antybiotyki o działaniu specyficznym | Non-specificaly antibiotics Antybiotyki o niespecyficznym działaniu | |
|---|---|---|
| | immediate natychmiastowym | progressive prosperującym |
| Amphotericin B | Filipin III | Nystatin $A_1$ |
| N-acetyl amphotericin B | Pimaricin | N-glucosyl-nystatin |
| N-glucosyl-amphotericin B | N-glucosyl-pimaricin | Nystatin $A_3$ |
| Mixomycin | Rimocidin | N-glucosyl-nystatin |
| Candycidin D | | Heksamycin |
| N-acetyl candycidin D | | Lienomycin |
| N-glucosyl-candycidin D | | N-glucosyl-lienomycin |
| Perimycin | | |
| N-succinylperimicin | | |

biotic concentration or extending the contact period we obtain all *Chlorella vulgaris* cells which are unable to regenerate, i.e. the final effect is non-specific damage. Table 3 shows the division of the tested polyene antibiotics into subgroups characterized by the effect on *Chlorella vulgaris* cells.

Upon analysing the dependence of the extent of damage to the *Chlorella vulgaris* plasma membrane on the chemical constitution and physico-chemical properties of polyene macrolides it was found that the kind of the changes induced in the membrane depend closely on the size of the macrolide and the degree of chromophore saturation. All the polienes with small rings investigated displayed a non-specific character and belonged to the subgroup of immediate action. The other subgroup of non-specific action antibiotics consists of substances with large rings including a chromophore with 4 to 6 conjugated bonds. Characteristic of the polyenes which cause specific, fully reversible damage to *Chlorella vulgaris* plasma membrane are a large lactone ring and the highest level of chromophore unsaturation. Moreover, it can also be stated that the specific action of the substances on *Chlorella vulgaris* cells does not depend on the ionic charakter of polyene, its molecular weight, the structure of the nitrogenous moiety or dispersion in water. Table 4 presents the correlation between the chemical constitutional characteristics and the physico-chemical properties of polyene macrolides on the one hand and the type of changes in *Chlorella vulgaris* plasme membrane on the other hand.

Table 4

Chemical characteristics of specific and non-specific polyene macrolides
Chemiczna charakterystyka makrolidów polienowych o różnej specyficzności działania

| Characteristic polyene | Type of action Rodzaj działania | | |
|---|---|---|---|
| | specific | non-specific niespecyficzne | |
| | | immediate natychmiastowe | progressive postępujące |
| The size of the lactone ring | "large" | "small" | "large" |
| Molecular weight | 826—1350 | 651—813 | 925—1257 |
| Chromophore | heptaenes | tetraenes pentaenes | tetraenes pentaenes hexaenes |
| Nitrogenous moiety | mycosamine perosamine p-aminophenyl p-N-methyl-aminophenyl | mycosamine none | mycosamine aminogroup |
| Ionic character | acidic amphoteric neutral | acidic amphoteric neutral | acidic amphoteric |
| Water solubility | soluble nonsoluble | soluble nonsoluble | soluble nonsoluble |

Irrespective of their effect on the damage to the plasma membrane, the polyene macrolides tested show a different activity with respect to *Chlorella vulgaris* cells. The antibiotics with seven conjugated double bonds in the macrolide ring, so-called heptaenes, are most active. They inhibit the growth of *Chlorella vulgaris* cells while in concentrations of 0.02 to 0.2 mcg/ml. The remaining antibiotics acted only in higher concentrations (Table 2).

All the polyene derivatives tested, both glicoside and acyl, are less active than the original substance by a factor of two to forty. The only one of the antibiotics tested which has very little affect on *Chlorella vulgaris* cells (with MIC of 100 mcg/ml) is the glucose derivative of pimaricine (Table 2).

On investigating the repair of *Chlorella vulgaris* cells after damage it was found that the process requires that certain strictly defined envi-

ronmental parameters be maintained. A prerequisite of this process is the presence of potassium ions in the medium in concentrations close to isotonic, together with the availability of air. Complete repair under these conditions takes place during 24 hours incubation at a temperature of 30°C. Light is not necessary for the repair process. *Chlorella vulgaris* cells repaired the specific damage with the same speed under heterotrophic growth conditions (and also photoheterotrophic — mixotrophic and autotrophic ones). Of the conditions given for the repair process, only the necessity for the presence of potassium ions in the isotonic concentration for Saccharomycetes cells (Borowski, Cybulska 1967) has been found to date.

In order to obtain detailed information on the differences in the action of polyene macrolides on *Chlorella vulgaris* cells the Author observes the migration of potassium ions through the plasma membrane of an organism damaged by antibiotics with different specific effects.

The radioactive isotope $^{42}K^{+}$ made it possible to observe both the effect of potassium ions on *Chlorella vulgaris* cells and their transport at various temperatures, with different concentrations of potassium salts (in the hypotonic and hypertonic medium), and under conditions in which the generation of the metabolic energy needed to cover the energy demand of the active transport processes was inhibited. The absolute contents of potassium ions in the cells were measured together with the parameter of the ionic exchange between the cells and the medium. The process of the generation of metabolic energy was inhibited by creating anaerobic conditions without light or by using the CCCP-inhibitor of the oxydative and photosynthetic phosphorylation in *Chlorella* cells.

It was determined that the primary symptom of the effect of polyene on *Chlorella vulgaris* was the leakage of potassium ions from the cell to the hypotonic solution. The rate of this leakage depended upon the kind and concentration of substance and environmental conditions.

The antibiotics of the non-specific action group bring about the more rapid leakage of potassium ions as compared with the "specific" polyenes. For example, after being exposed to the action of N-succynylperimycin (in a concentration of 25 mcg/ml) for 60 minutes the cells still contained about 30 per cent of the original potassium contents, while the cells damaged by filipin III lost all potasium ions under the same conditions. Higher concentrations of the antibiotic speed up the leakage of potassium ions from the cells.

The action of polyene macrolide on *Chlorella vulgaris* cells defined by the incipient time of potassium outflow requires that energy be supplied. Metabolic energy is required to bond the specific antibiotic, N-succynylperimycin. Under anaerobic conditions and withouth light or in

the presense of a phosphorylation inhibitor *Chlorella vulgaris* cells are not sensitive to the action of N-succynylperimycin. However, it is enough to restore the ability of the cells to synthesis ATP, e.g. by changing from anaerobic conditions into aerobic conditions, for this substance to act on the cells. The process with "non-specific" polyenes requires only heat, i.e. incubation must be at a temperature of over 0°C.

The leakage of potassium ions from the *Chlorella vulgaris* cells damaged by polyene macrolides is not an enzymic process, irrespective of the extent of the damage, but follows the patterns of free diffusion through the membrance pits formed by the antibiotic. The temperature coefficient of this process ($Q_{10}$) found in this paper was close to one.

The leakage of potassium ions by free diffusion from the cells damaged by a polyene of the "specifically acting" subgroup was accompanied by an active transport of these ions, which compensated their loss to a greater or lesser degree. The compensation depended on the damage to the membrane, since this factor controls the rate of diffusion.

Of the antibiotics tested only N-succynylperimycin destroys the *Chlorella vulgaris* cells to such a minor extent that they maintain their capacity to keep the physiological ion concentration at a certain level in plasma while on a hypotonic medium (40 times smaller than isotonic) in spite of the continuous leakage of the ions to the medium. The *Chlorella* cells damaged by other "specific acting" polyenes are capable of compensating the loss of potassium ions only partially by intensive active transport from the environment. Cells damaged by N-succynylperimycin maintain their ability to control the plasma concentration of potassium ions also in the hypertonic solution of these ions, which would indicate their active elimination from the cells under these conditions.

The conclusions presented as to the character of the effect of polyene macrolides on *Chlorella vulgaris* cells can be summarized by pointing out that the antibiotics develop pits in the plasma membrane of the cells. The pits permit uncontrolled diffusion of metabolites, the rate of this diffusion depending upon the structure of the antibiotics. The results obtained from the investigation of the dependence of the cell damage repair ability on the antibiotic constitution indicate that some difference must exist in the size of the pits, irrespective of ther quantity. Non-selective polyenes develop the greatest pores, while the selective polyenes form the smallest ones. The lack of repair of the *Chlorella vulgaris* cells damaged by a non-selective polyene (with the concentration of potassium ions close to isotonic) is due to the leakage of metabolites larger than potassium ions. This was confirmed in the tests on the migration of particles larger than potassium ions carried out with

selected polyene macrolides. The particles chosen were those of unmetabolized sugar (L-sorbose) and calcium ions. It was shown that both L-sorbose and calcium ions diffuse freely through the *Chlorella* plasma membrane damaged by a non-selective antibiotic, while in the cells damaged by a specifically acting polyene the particles migrate through the plasma membrane only by means of enzymatic systems.

From the conclusions presented so far, it can be seen that the active transport of potassium ions in cells damaged by selective polyenes is not disturbed. In order to investigate more fully the effect of polyene macrolides on the membrane transport of *Chlorella vulgaris* the antibiotics were tested, based on some other metabolites, as to their facilitated diffusion (acetate and carbonate) and the active transport of organic metabolites (sugars and aminoacids) to damaged but living cells. The results obtained permit the Author to conclude that polyene macrolides do not affect the uptake of metabolites by facilitated diffusion. Both acetate and carbonate are taken by damaged *Chlorella* cells at the same rate as from control cells.

It was, however, found that the polyene-damaged cells inhibit the accumulation of glucose and aminoacids. This fact that organic metabolites, are not actively accumulated by damaged *Chlorella vulgaris* cells can be explained in three ways. Firstly, this can be the result of insufficient chemical energy needed to cover the energy demand for the active transport of the metabolites. This insufficiency can be caused by the participation of the cell in the intensive active transport of potassium ions aimed at maintaining $K^+$ homeostasis. There is a systematic loss of potassium ions in *Chlorella vulgaris* cells damaged by N-succynylperimycin due to their leakage to the medium and therefore the stabilization of the concentration of these ions on the physiological level requires more intensive active transport of potassium, which in turn absorbs all the energy synthesised in metabolic processes.

A second reason may by a lack in the demand for both types of metabolites resulting from the inhibition of polysaccharide and protein biosynthesis in *Chlorella vulgaris* cells due to the lack of the necessary energy.

Thirdly, an antibiotic can also act directly on the enzymatic transport system of the substances, but this seems to be the least possible reason.

The investigations of the effect of polyene macrolides on photosynthesis have shown that these antibiotics do not act directly on the enzymatic system of *Chlorella vulgaris* photosynthesis. Photosynthesis in the cells damaged by N-succynylperimycin in a potassium-containing environment, continues with the same intensity as in the con-

trol cells. The inhibition of photosynthesis in *Chlorella vulgaris* in the presence of polyene macrolides which belong to the non-specifically acting group (Table 3) appears to be a secondary effect.

The results obtained in this study permit the Author to conclude that the action of some polyene macrolides causes specific changes in the permeability of the *Chlorella vulgaris* plasma membrane, which are limited only to the free diffusion of potassium ions. Systematic studies of the dependence of the *Chlorella vulgaris* cell damage on the chemical structure and physico-chemical properties of a given antibiotic made it possible to determine the active polyene macrolides and the conditions in which they are active. Being defined as specifically acting antibiotics, they can be used as specific reagents in the investigations of the role of potassium in the biochemical processes depending on the presence of this ion in *Chlorella vulgaris* cells.

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# ZALEŻNOŚĆ MIĘDZY BUDOWĄ CHEMICZNĄ MAKROLIDÓW POLIENOWYCH A ICH WPŁYWEM NA ZMIANY PRZEPUSZCZALNOŚCI BŁONY PLAZMATYCZNEJ *CHLORELLA VULGARIS*

Streszczenie

Opracowano nowy model dla prac nad mechanizmem działania makrolidów polienowych — *Chlorella vulgaris*. Przeprowadzono na tym modelu systematyczne studia nad zależnością pomiędzy budową chemiczną oraz własnościami fizykochemicznymi tych substancji a charakterem zmian wywołanych w błonie plazmatycznej tego organizmu.

Stwierdzono, że stopień uszkodzenia błony plazmatycznej *Chlorella vulgaris* zależy od budowy chemicznej antybiotyku, a w szczególności od wielkości pierścienia makrolidowego oraz od stopnia nienasycenia chromoforu. Ciężar cząsteczkowy antybiotyku, jego charakter jonowy, budowa fragmentu zawierającego azot, a także stopień dyspersji w wodzie nie mają wpływu na stopień uszkodzenia błony plazmatycznej *Chlorella vulgaris*.

Stwierdzono, że działanie wszystkich makrolidów polienowych na komórki *Chlorella vulgaris* rozpoczyna się od specyficznych zmian struktury błony plazmatycznej tego organizmu umożliwiających wolną dyfuzję jonów potasu. Część antybiotyków ogranicza swoje działanie do tego etapu i antybiotyki te nazwano „specyficznie działającymi". Pozostałe antybiotyki powodują głębsze zmiany błony plazmatycznej *Chlorella vulgaris*, umożliwiając wolną dyfuzję większej ilości metabolitów. Stwierdzono, że specyficzne uszkodzenie błony plazmatycznej jest przez komórki *Chlorella vulgaris* reperowane i że warunkiem tego procesu jest obecność potasu w stężeniu izotonicznym oraz obecność tlenu.

Badano proces migracji w uszkodzonych komórkach jonów $K^+$, $Ca^{++}$, sorbozy, glukozy, aminokwasów, węglanu i octanu. Stwierdzono, że ucieczka jonów $K^+$ w wyniku działania polienu nie jest procesem enzymatycznym, lecz przebiega na drodze wolnej dyfuzji. Antybiotyki polienowe nie działają bezpośrednio na enzymatyczny układ aktywnego transportu jonów $K^+$. Makrolidy polienowe nie mają wpływu na pobieranie przez komórki *Chlorella vulgaris* metabolitów na drodze ułatwionej dyfuzji, hamują natomiast aktywne pobieranie metabolitów organicznych.

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