THE PLASMAMEMBRANE SENSITIVITY OF PLANT CELLS TO UV-B IRRADIATION

Ismat S. Ahmadov, Aygun N. Nasibova, Rovshan R.Khalilov

Khazar University, Baku State University, Azerbaijan

Чувствительность плазматических мембран клеток растений на УФ-Б облучений. *В данной статье исследованы влияние УФ света на электрические параметры мембран клеток водных растений.Получено что при облучений листьев растений сильно изменяется МП клеток. Наблюдается два типа деполяризации МП, сильная и кратковременная а затем медленная деполяризяция.Выяснено, что первая фаза деполяризации МП результат ингибирование редокс активности мембран, а вторая фаза подавление функции H^+-ATPasa.*

Ключевые слова*: мембранный потенциал, редос система, УФ свет, растение*

Intrudaction

From a biological viewpoint, UV-B radiation is by far the most significant part of the terrestrial ultraviolet spectrum and the levels of radiation in this waveband reaching the surface of the Earth are largely controlled by ozone, a gas which comprises approximately one molecule out of every two million in the atmosphere. Global UV radiation is dependent on a number of factors, which include solar angle, time of day, season, *geographical latitude*, cloud cover, and the stratospheric concentration of ozone. It was realized in the early 1970's that man-mad chemicals (chlorofluorocarbons, airplane and rocket exhaust products, fertilizers, etc.) released into the atmosphere can deplete stratospheric ozone, with a concomitant increase in the amount of solar ultraviolet radiation reaching the earth's surface. During the last three decades, much attention has been paid to the reduction in the concentration of ozone. Decreases in the straospheric concentration of ozone cause increases in the ultraviolet radiation, particulary in the UV-B portion of the spectrum, from 290 to 320 nm. It is UV-B radiation whose fluence will be most greatly increased by ozone depletion, and it is also UV-B radiation which is absorbed with high probability by organic macromolecules such as DNA, causing damage which interferes with the natural function of the molecule (Nachtwey and Rundel, 1982).

Summery of the effects of solar ultraviolet radiation on plants (Teramura, 1983)

Plant characteristics	Enhanced UV radiation
Photosynthesis	Decreases in many plants
Leaf conductance	No effects in many plants
Water use efficiency	Decreases in most plants
Dry matter production and yield	Decreases in many plants
	Decreases in many plants
Leaf area	Decreases in many plants
Specific leaf weight	No effects
Crop maturity	May inhibit or stimulate flowering in some plants
Flowering	Species may vary in degree of response
Interspecific differences	Response varies among cultivars
Intraspecific differences	Plants become less sensitive to UV but not tolerant to drought
Drought stress	

The responses of plants to UV irradiation include physiological, biochemical, morphological and anatomical changes (table). In general UVR deleteriously affects plant

growth, reducing leaf size and limiting the area available for energy capture. These findings have been achieved mainly through studies in greenhouses and exposure to artificial sources of ultraviolet radiation. Extrapolation to changes on crop yield as a result of increases in terrestrial solar UVR is difficult, and in those few field trials conducted outdoors the results were variable (Tevini and Teramura 1989).

Furthermore, the effects of natural UVR on plants will be influenced by other stresses such as water shortage, mineral deficiency and increased concentrations of carbon dioxide. It is of note that increased ambient levels of CO2 (the greenhouse effect) have a beneficial effect on plants (Lemon 1983) but this may not necessarily compensate for the anticipated deleterious effects of increased ambient UVB as a consequence of ozone depletion.

Plant cells have many different UV-sensitive components, among which the membrane system is very important from the viewpoint of the biological significance of UV action. The mechanism governing the effect of UV radiation on plant cell membrane systems is therefore a problem that is being actively studied. Certain regularities applying to changes in the structure and function of plant cell membrane systems during exposure to UV have been clarified at the present time (Doughty and Hope, 1976; Caldwell, 1984; Imbrie and Murphy, 1984-1992; Novacky and Ullrich-Eberius, 1982) The most general manifestation of injury to the membrane is breakdown of its barrier and transport functions. In addition to this, there are data indicating direct influence of UV radiation on the enzyme complexes of membrane systems. However, it is still difficult to ascertain the sequence of events in the event of cell injury via aa membrane mechanism. Determination of the sequenses of these events largely depends on clarifying initial responses of membrane system to UV radiation.

Materials and Methods

The study was conducted on photosynthesizing cells of leaves of the higher water plants – Canadian waterweed (*Elodea canadensis* Rich.) and spiral wild celery (*Vallisneria spiralis* L.) Before the experiment, the leaves were kept in a flow chamber in artificial pond water (APW), composition of which included 1.0 mM NaCl, 0.1 mM KCl, and 0.1 mM $CaCl_2$. A continuous flow of APW made it possible to preserve the normal physiological state of leaves. The membrane potential and membrane impedance were studied using intracellular microelectrode technology, as described in detail by Aliev et al. (1984). The microelectrodes constitute a special glass capillaries filled with 3M KCl. The main source of UV radiation was a DRT-230 high-pressure mercury lamp, which had a linear emission spectrum. The distance from the lamps to the exposed object was 0.25 m. Radiation intensity was 32 Wm^{-2} . Glass filters (UFS and BS) were used in studying the action spectrum of membrane potential depolarization.

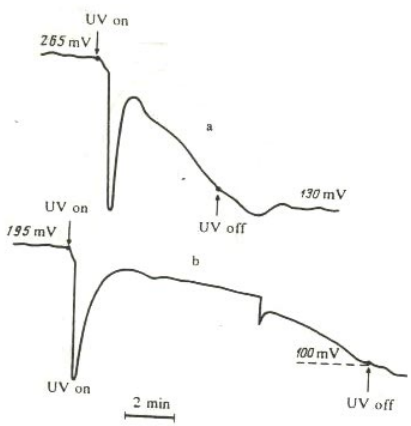


Fig.1 Changes in MP of waterweed leaf cells (a) and wild celery leaf cells (b) during UV exposure

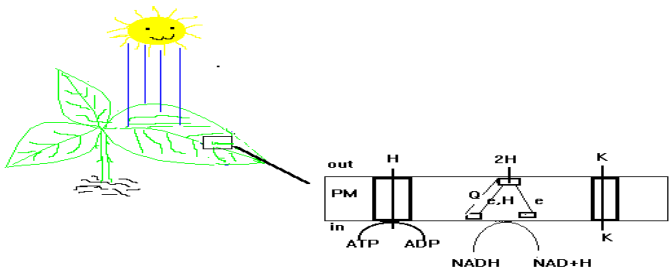
Experimental results and discussion

A complex change of membrane potential was detected when leaves of the water plants were exposed to UV. Fast and strong depolarization of the membrane potential occurred during the first minutes of exposure. Regardless of continuing exposure, the membrane potential returned to the starting level, after which a slow phase of depolarization set in (Fig.1). Thus, UV can evoke two types of depolarization, rapid and reversible depolarization at first, and then slow depolarization. It was interesting to study the action spectrum of these two types of depolarization.

To this end, we used different sources of UV and UFS and BS glass filters to investigate the kinetics of membrane potential changes during exposure to UV. Detailed study of this fast phase of membrane potential changes indicates that a fast and reversible depolarization occurred in cells during brief (15 - to 25 sec) exposures to UV with 290 nm wavelength. Depending on the starting membrane potential level and the time of exposure the depth of depolarization here attained more than half the value of the membrane potential. It is interesting to note that input impedance of the membrane and intercellular electrical couplings (conductance of plasmodesmata) did not change during depolarization development or after repolarization processes. We identified that the action spectrum of fast depolarization of membrane potential at the level of 300-330 nm means that, in this case, UV affects the component of a redox chain of the plasma membrane directly. The action spectrum of the slow phase of depolarization at the level 280-300 nm means that it affects a protein natural component of plasma membrane, which is H^+ -ATPase complex.

The results of our experiments with UV radiation showed that the initial chromophore for UV light absorption is molecular of quinones. There are many compounds in the plasma membrane of plant cells, which can absorb the light with λ = 300 - 330 nm some albumens, flavonoids, lipids ets. However, the most intensive absorption takes place for quinones (plastoquinone, Q_A, Q_B). They are the intrincsinc components of membrane electron transfer chains. The molar absorption coefficient for quinones in this region is ε = $2x10^5$ dm^3 .mol^{-1}.cm^{-1}, which is 100-1000 times higher than the absorption coefficients of all other ETC components. The electron transfer chain (as a redox-system) plays a significant role in the formation of membrane potential. So, the observed changes in membrane potential can be attributed with the blocking of the ETC work (as it was stated in number of additional experiments on the measurements of interecell-resistance, short light impulses do not cause disturbance of membrane integrity).

Thus it is not impossible that quinones absorbing the light can bring about some



changes in membrane structure or contents and then the changes of the membrane potential reflects the disturbances. To check this hypothesis the following experiments were done.

What is more, the results of experiments with UV light allowed us to explain some aspects of functions of proton pumps of plasmamembrane of plant cells. It can now be

concidered proved that the main role in generation of the plant cell membrane potential is played by electrogenic proton pumps, whose contribution makes up more than half of the MP of plant cells. There are two conceptions as to the nature of electrogenic H^+-pumps. Numerous experimental data indicate the existence of H^+-ATPase enzyme complex on the plasmalemma of plant cells. Together with this, the idea of a redox-active nature of the H^+-pump has been actively developed. The existence of redox system on plant cell plasma membranes is likewise substantiated by experimental data in the studies of a number of authors (Rubinstein and Stern, 1986; Ivankina and Novack,1988; Trockner and Marre, 1988; Marre and Moroni, 1989; Dahse and Botger, 1992)

In works devoted to this question, it is postulated that both these types of H^+-pump can fuction on the plasma membrane of plants. Moreover, there are data indicating that they are interrelated, although final proofs of this relationship are so far lacking.

Dahse and his colleagues observed the rapid and reversible depolarization of MP on cells of Elodea by action diuron (DCMU) and ferricyanide (FeCN) (I.Dahse and et.al, 1987; M.Bernstein, I.Dahse et.al.,1989). The results of these investigations show that during action of DSMU and FeCN on the cell of Elodea a rapid and reversible depolarization at first, then slow depolarization take place. Similar results we obtained on cells of Elodea during UV irradiation (Khalilov, Akhmedov, 1992).

Adhering to the idea of parallel existence of the H^+-ATPase and redox-active types of H^+-pump on the plant cell plasmalemma, we suggest the following explanation for the effect (UV can evoke two types of depolarization of MP, rapid and reversible depolarization at first, then slow depolarization) we obtained during UV exposure. The action spectrum of the fast UV responce in the region of 290-330 nm means that UV in this case directly affects the nonprotein component of the plasmalemma. This may be a component of a redox-active complex. Evidently, UV with wavelength of 290-330 nm alters the function or structure of a component of the redox system. This component probably is molecular quinone. By exiciting and altering the form of quinone, UV brings about inactivation of redox system. Inactivation of the redox system in turn leads to MP depolarization and acidification of the cytoplasm, which stimulates a pH_i -dependent H^+-pump of the H^+-ATPase type. The initial strong MP depolarization during development of the fast UV response therefore undergoes repolarization and returns to the starting level irrespective of stoppage or continuation of exposure. This can be regarded as an argument for interrelated functioning of the redox system and the H^+-ATPase type of H^+-pump . As for the slow depolarization phase in the wavelength region of 260-300 nm, it coincides with the absorption spectrum of protein molecules. It may therefore be assumed that the H^+-pump of the H^+-ATPase type is inactivated at the same time as the redox system with continuation of UV exposure. Imbrie and Murphy also cite data indicating inactivation of ATPase.

Thus, the results of our experiments are in agreement with the idea that the H^+-extruding complex of the plant cell plasma membrane consists of two types of interrelated electrogenic H^+-pumps: an H^+-pump of redox-active nature and that of the H^+-ATPase enzyme complex. The approach we suggest makes it possible to exert selective influence on these two mechanisms of the H^+-extruding complex by employing UV with different wavelengths.

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Для контакта:
Д-р Исмет Ахмедов, Кафедра Биологических науки, Университет Хазар, тел: (99412) 421 69 79, e-mail: ismet522002@yahoo.com , iahmadov@khazar.org