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Direct observation of UV-B radiation effect on antigen–antibody coupling using surface plasmon resonance

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Abstract

We studied the effect of UV irradiation on (non)specific bounding of bovine serum albumin (BSA) and mouse antiBSA immunoglobulin (IgG) using a surface plasmon resonance (SPR) apparatus Biosuplar-2 and special photodetectors. It was found that specific bounding of AG–AB complex is broken under UV irradiation. Contrary to this, UV irradiation of BSA layer before its contact with IgG did not demonstrate any peculiarities. On the basis of quantitative determination of kinetics of specific bounding and washing and precise measurement of photon flux, the product $\gamma\sigma$ of quantum yield γ and active centre cross-section σ was evaluated to be about 10^{-20} cm^2. As the dimension of amino acid residues tryptophan and tyrosine is about 1 nm, we have concluded that the quantum yield of reaction is very small (about 0.001). This indicates at presence of a barrier for direct photochemical reaction between the interacting substances.

Keywords: UV irradiation; Biomolecular interaction; Surface plasmon resonance sensor

1. Introduction

UV radiation is an important factor affecting living matter. Besides photosynthesis, it plays a great role in apoptosis processes, radiative damage of nucleic acids, gene mutation, etc. That is why changes in biologically active ultraviolet radiation reaching the Earth's surface are under permanent observation [1].

In recent years many publications have appeared in the biological literature dealing with the problem of the effect of UV-B radiation on photosynthesis in plants from the viewpoint of possibilities of transgenic engineering for creation of novel sorts of plants (see, e.g. [2–4]). There are also some monographs dealing with the effect of UV radiation on living cells [5], human health and environment [6,7].

UV irradiation was successfully used for production of proteins assemblies by coupling separate fragments [8]. UV radiation of wavelength 254 nm used in these experiments was not very intense (irradiance of 1–6 mW/cm^2), and the exposure time was several minutes. Another application of UV irradiation is cross-linking of a nylon membrane with nucleic acid probe [9,10]. Linkage proceeds predominantly through thymine residues that react with amine groups (present on the nylon membrane) when they are activated. Some of these bases therefore become covalently linked to the membrane surface and are unavailable for hybridization. Thus, UV linkage can destroy subsequent hybridization if the membrane is overexposed. The determination of the correct dose of UV radiation is the key problem to be solved for successful exposure of membrane. Typical doses are 1.2–1.6 kJ/m^2 for damp membranes and 0.12–0.16 kJ/m^2 for completely dry membranes [11].

Photoreactive cross-linking is widely used for identification of protein–protein interactions when investigating living tissues. This effect is based on activation of azido groups; it was studied using mass spectroscopy, chromatography or enzymatic assay [12]. The authors of that work used as a source of irradiation an 8 W quartz bulb placed at a distance of 15 cm from the samples studied.

However, all such researches were conducted by biological methods, without investigation of physical mechanisms of biomolecular interactions. Only in [8] a molecular model of interaction between two proteins was proposed which could be used for recognition of the molecular mechanism of UV-B radiation effect on inter-protein binding.

It should be noted that, though UV irradiation is widely used in biological experiments at molecular level, the results are usually obtained with indirect methods—mass

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spectroscopy, thin layer chromatography, etc. Besides, very often the illumination intensity is not determined accurately, and precise numerical data on the details of biological processes are not obtained.

Therefore, it seems of interest to realize a new experimental approach for direct observation of such processes at molecular level. This can be done with the devices operating on the basis of SPR. At present such devices are intensely applied for investigation of biomolecular interactions. Among them Biosuplar-2 seems to be best suited for this purpose. It has an open construction and enables one to measure concurrently the plasmon resonance angle at additional illumination [13,14].

2. Experimental

We used a complementary pair bovine serum albumin (BSA)–mouse anti-BSA IgG (both from Sigma) for investigation of the UV-B radiation effect on the specific binding processes in an antigen–antibody (AG–AB) molecular complex. An SPR device Biosuplar-2 (Micro-systems, Germany) served to detect the amount of adsorbed molecules, because increasing of plasmon resonance angle in the course of molecular binding is prorotional to the number of bound molecules per unit area (with coefficient of proportionality being 16.7′ per 1 ng/mm^2) [15]. The basic advantage of this device is a possibility to affect the molecular layers on the solution side, while registration of adsorption is made on the glass prism side. This allowed us to irradiate the AG–AB complex directly. The details of experiment are presented schematically in Fig. 1.

An additional UV irradiation was made with a quartz tube PRK-4 (electrical power of 220 W). A quartz lens

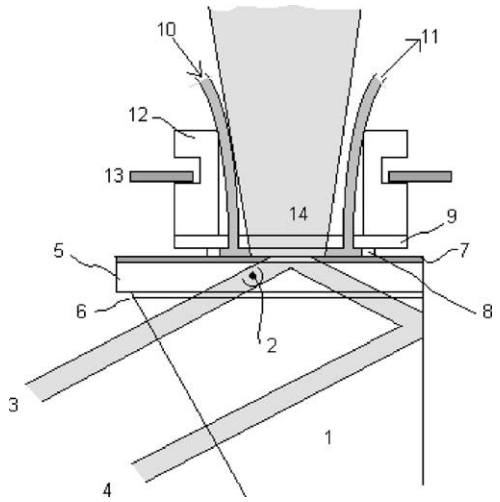

Fig. 1. Schematic of the experimental setup: (1) glass prism ($n = 1.61$); (2) axis of rotation; (3) incident laser beam; (4) outgoing laser beam; (5) glass plate ($n = 1.61$); (6) immersion oil; (7) thin gold film; (8) silicon rubber o-ring; (9) polished quartz plate; (10, 11) solution input and output; (12) cell body; (13) steel spring; (14) area of UV illumination.

($f = 10$ cm, $d = 8$ cm) and optical filter system were used to decrease the intensity and separate the required wavelength range. The distance between the tube and sample was 15 cm. The illuminance of the sample surface was measured by special calibrated photodiodes developed at ISP NASU [16]. A measuring cell has a thin (1 mm) quartz window with two small holes for solution injection and removal (Fig. 1). Solutions were replaced manually with a syringe. The kinetics of molecular adsorption on the gold surface was measured in absence of liquid flow.

In our experiments we used doubly distilled water; soybean trypsin inhibitor (STI) (“REANAL”, Hungary); bovine serum albumin, polyclonal mouse antibodies against BSA and glutaraldehyde (“Sigma”, USA). The solutions—phosphate buffer (50 mM phosphate buffer, pH 7.2) (PBS) and glycine buffer (0.1 M, pH 2.2), inorganic salts, acids and alkalis used by us were no worse than of analytical grade of purity.

The BSA agents were immobilized at the surface of a gold film using STI [17]. This technique has been developed by us to simplify the procedure of gold surface modification traditionally used by the leading manufacturer of the affinity-based biosensor technology—Biacore AB, Sweden. Our technique is quicker and does not require application of nonaqueous solutions.

The STI layer on the gold chip surface was formed by immersion in the STI solution (0.5 mg/ml in 0.1 M sodium-acetate buffer, pH 5.5) for 60 min. Then the chip was incubated during 30 min in 5% solution of glutaric aldehyde and washed in 0.01 M sodium-acetate buffer. Then the chip was put into the device (Biosuplar-2) and pressed onto the cell.

3. Experimental results and discussion

The typical result of the experiment is shown in Fig. 2A. As soon as the base line has been established in buffer solution, the BSA solution (120 μg/ml) in PBS was injected into the cell (time point 0). Due to molecular binding between BSA and treated gold surface, we see a smooth signal increase (while kinetics was monotone), and the signal reaches a stationary value (approximately of 10–15″, or 0.8–1.2 ng/cm^2). At the point 1 pure PBS was put into the cell. One can see that the response level practically does not change. This fact indicates at strong fixation of BSA molecules on the treated gold surface. The difference between the response values at the points 0 and 1 corresponds to maximum surface filling by BSA molecules. At the point 2 the surface with adsorbed BSA molecules came into contact with the antibody molecules. Obviously there is intense binding between antibodies and immobilized antigens that reaches filling limit at the point 3 where buffer solution has been injected again. The maximum response of 3.2–3.3 ng/mm^2 evidences that the mass of bound antibodies became about 2.5 times over that of antigens.

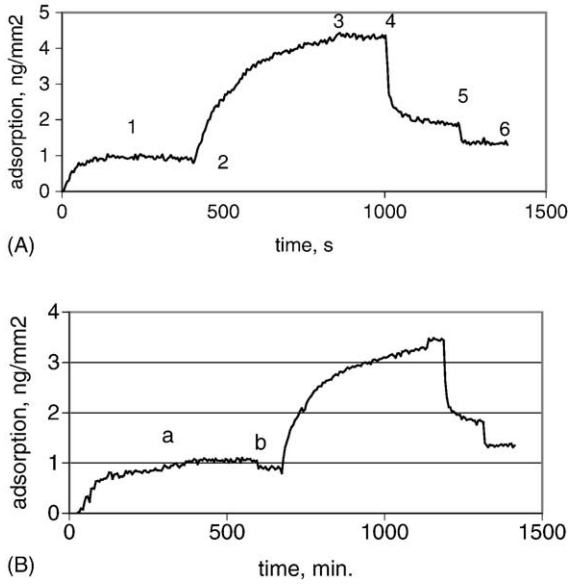

Fig. 2. (A) SPR sensogram of specific BSA–antiBSA interaction without UV irradiation (details in the text); (B) the same sensogram after UV irradiation between the points *a* and *b*.

At the point 3 buffer solution was added again, and no changes in the response were observed. This demonstrates formation of the AG–AB complexes at the gold surface. Another evidence of such complexes appearance is decrease of adsorbed mass after injection of glycine buffer (pH 2.2) into the cell (at the point 4) that breaks the specific AG–AB bonds. After injection PBS solution, the signal returned almost to the previous level (see the point 6). The difference between the SPR responses at the points 4 and 6 corresponds to the portion of specifically bound biomolecules.

It is known that response of SPR sensor to adsorption of biomolecules is proportional to their molecular mass and surface concentration. The masses of BSA and IgG molecules are known (about 67 000 and 160 000, respectively). Therefore, the ratio between the numbers of AG and AB molecules is $T = (1.2\,\text{ng}/67\,000)/(3\,\text{ng}/160\,000) = 1$. When IgG solution is put under repeated exposure, the sensors response has nearly the same value as in the first time.

Here, we have two stable states of the molecular system: antigen (BSA) layer in contact with PBS (the sensogram portion between the points 1 and 2) and specifically bounded complex AG–AB (the sensogram portion between the points 3 and 4). Hence we have two ways for testing the effect of UV radiation on such system: to irradiate molecular layer between the points (i) 1 and 2 or (ii) 3 and 4.

First we tried to irradiate the BSA molecular layer with focused light of maximal energy during 5 min (from the point *a* till the point *b*, Fig. 2B). One can see that the result is negative—the experimental curve is almost the same as in Fig. 2A, except a small signal variation during illumination (between 300 and 600 s). The experiment was repeated at least three times with the same result.

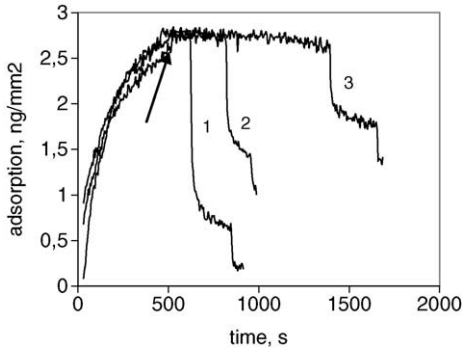

Fig. 3. Sensogram portions corresponding to specific binding (rising part) and washing (falling part) for different UV irradiation time: 1 min (1); 3 min (2); 7 min (3).

Another effect was observed when UV illumination was switched between the points 3 and 4 (Fig. 2A). The result is presented in Fig. 3. Here on a bigger scale are presented only those parts of sensograms which are related to IgG binding and washing. UV-B irradiation started at the position of pointer and was switched off after 1, 3, and 7 min (curves 1–3, respectively). One can see that UV radiation itself does not cause any changes of the sensogram, i.e. the complex AG–AB does not decay under the action of UV radiation in the phosphate buffer ambience (pH 7.7). Contrary to this, the result of glycine buffer (pH 2.2) injection (to break specific binding) depends on the duration of UV irradiation: increase of the time of irradiation reduces the number of AB molecules that are released due to break of the specific bonds by the glycine buffer. Thus, the portion of nonspecifically bound IgG molecules increased.

Fig. 4 represents the dependence of the amount of specifically (non-covalently) bound IgG molecules on the time of UV irradiation. It is obvious that the amount of such molecules is decreasing, and this response depends almost exponentially on the time of irradiation. The solid line corresponds to the function $N = 2.5(\text{ng}/\text{mm}^2)\exp(t/8(\text{min}))$ which shows the best fit to the experimental data.

The main result of experiments is discovery of transformation of weak specific intermolecular binding in the AG–AB complex into more strong binding that cannot be broken by increased pH or ionic force of the buffer solution. The

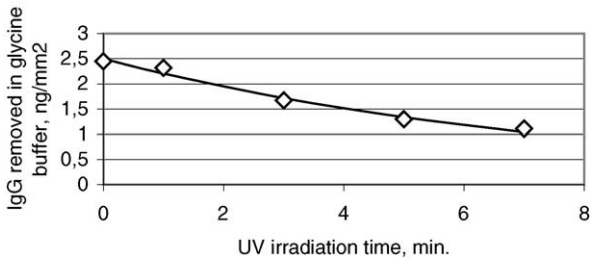

Fig. 4. Dependence of the amount of specifically bound IgG molecules on UV irradiation time (diamonds) and fitted exponential curve $N = 2.5(\text{ng}/\text{mm}^2)\exp(t/8(\text{min}))$ (solid line).

Table 1
Spectral dependence of irradiance and energy dose (exposure of 3 min)

Spectral range	Wavelength (nm)	Irradiance (W/m^2)	Energy dose (kJ)
UV-C	200–280	11.88	2.14
UV-B	280–315	75.31	13.55
UV-A	315–380	104.56	18.82
Visible	380–780	150.7	27.12
Total		342.5	61.65

number of transformed specific bonds is found to be related to the dose of UV irradiation. At the exposure of 7 min, almost half the AG–AB complexes lose the ability to form specific bonds. It seems to be of importance to determine the range of light spectrum responsible for this transformation of weak specific bonds into more strong bonds.

We made an analysis of the effect of UV radiation from different spectrum ranges on the above effect. First of all, we considered the spectrum of the light source used—a PRK-4 lamp with linear characteristics. Light spectrum includes visible light, as well as A-, B- and C-ranges of UV light. The spectrum of the PRK-4 lamp was measured with calibrated FRD-5 photodetectors (that are sensitive to UV rather than visible light) and FD photodetectors produced by Chernovtsy Plant "KVANT". To separate different spectral ranges, we used optical filters with narrow radiation transmission bands that are able to extract the necessary ranges of UV-A, UV-B and UV-C. The measurement data of the irradiance and dose for the exposure of 3 min are presented in Table 1. Obviously irradiations with the lamp in UV-A and UV-B ranges are comparable, but that in visible range is much stronger.

Using interference filters with sharp short wavelength boundary in the region of 280–380 nm, we investigated how the discovered effect depended on UV irradiation from different spectral regions. Fig. 5 presents the amount of specifically bound AB molecules (which are removed after the treatment in glycine buffer (pH 2.2) solution) as a function of the spectral range of irradiation. It is obvious that visible light ($\lambda > 380$ nm) and UV-A radiation ($315 < \lambda < 380$ nm) do not affect the nature of chemical binding in the AG–AB complexes. The number of released AB molecules does not depend on irradiation wavelength. At the same time we can see a dramatic decrease of the amount of specifically bound AB molecules after exposure to UV-B radiation ($280 < \lambda < 315$ nm). This fact indicates at the dominant role of this UV radiation from this spectral region.

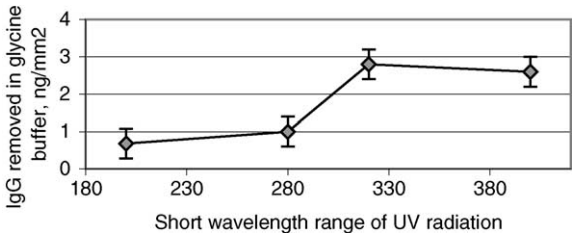

Fig. 5. Action spectrum of the effect of UV breaking of specifically bound AG–AB complexes.

From our experimental data we can find the number of protein molecules bound to the surface with specific bonds and the number of molecules which changed the binding character under the action of UV light. In fact, it is known [15] that SPR response to binding or release of molecules at gold surface is determined by the mass of the adsorbed molecular layer M_a (ng/cm^2), and there is a linear correlation between the molecular mass M and angular shift of SPR minimum. Thus it is easy to calculate the surface concentration of macromolecules $N_a = M_a A/M$, where $A = 6.02 \times 10^{23}$ is the Avogadro's number. The calculated numbers of BSA and anti-BSA molecules are presented in Table 2. One can see that the numbers of BSA and anti-BSA molecules (without UV irradiation) are almost the same. This allows one to conclude that under natural conditions about 100% AG–AB complexes have specific binding.

Decrease of the number of specifically bound IgG molecules N (cm^{-2}) at the first time point ($t = 0$) may be expressed with the reaction $dN/dt = \gamma \Phi N \sigma$, where γ is the quantum yield, Φ is the photon flux ($cm^{-2}\,s^{-2}$) and σ is the capture cross-section (cm^2). Assuming the energy of UV-B photon ($\lambda = 280$ nm) to be about 6.6×10^{-19} J and taking into account the data from Table 1, we may estimate the flux of UV-B photons as $2.65 \times 10^{16}\,cm^{-2}\,s^{-1}$. The dN/dt and N values may be obtained using Fig. 4 and Table 2. Our calculation gives for $\gamma\sigma$ about $10^{-20}\,cm^2$.

Decrease of the number of specific bonds can be related to interaction of high-energy UV photons with some fragments of amino acid components that results in formation of covalent binding of amino acid residues in the interaction area. The most probable absorbers of UV energy may be tryptophan and tyrosine (both are present in BSA and anti-BSA macromolecules). Thus we can expect a remarkable transformation of a portion of molecules in the immediate vicinity of those fragments. If we suppose that quantum yield

Table 2
The numbers of BSA and IgG molecules at the surface (without UV irradiation)

Surface modification	Protein	Molecular mass	Surface mass of immobilized protein (ng/mm^2)	Surface concentration of the immobilized protein molecules ($\times 10^{10}\,mm^{-2}$)
STI, GA	BSA	67000	1.1 ± 0.1	1.2 ± 0.1
STI, GA	IgG	160000	3.2 ± 0.3	1.05 ± 0.1

for this reaction is about 1, then the capture cross-section of active centre is about 10^{-20} cm^2, i.e. its size should be about 10^{-10} cm (0.001 nm). This is an unreal value because the size of ammonium acid residue of tryptophan and tyrosine is about 1 nm. If so, the quantum yield should be about 0.001. At present one cannot prove or refute any of these two possibilities, and further experiments are required to reveal the chemical mechanism of transformation of weak AG–AB specific binding into strong coupling.

4. Conclusions

Using the surface plasmon resonance technique, we studied experimentally the direct effect of UV irradiation on specific binding in the model complex BSA–anti-BSA. We found the effect of breaking of specific bonds under the action of UV-B radiation. It was found that that this effect depends on the exposure and wavelengths. It has no threshold and is caused exclusively by UV-B irradiation. The exponential dependence of the number of damaged AG–AB complexes on the UV dose has been shown. Assuming the first order kinetics, the product of the quantum yield and active centre cross-section was calculated to be 10^{-20} cm^2. We suppose that similar approach may be applied for investigation of DNA damage under UV-B irradiation.

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