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ON THE POSSIBILITY OF SELECTIVE BIOCHEMICAL REACTIONS INDUCED BY LASER RADIATION

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Summary

Two possible methods of selective excitation or breaking of macromolecular bonds in solution under ultrashort pulses of laser radiation are considered. The first method is based on the two-step excitation of a molecule under i.r. and u.v. radiation pulses, while the second one is on the direct action of i.r. radiation pulse upon the molecular vibration. Conditions for power and duration of pulses are found as well as those for removing a potential non-selective thermal effect. As an example, these influences upon various parts of a DNA molecule are considered.

Introduction

Beyond doubt, the use of laser radiation in biophysical and biochemical research (*e.g.* studies on the excited molecular electron state [1, 2]) holds much promise. But the possibility of selective action of optical radiation on biomolecules is less obvious. Methods of selective action of laser radiation on atoms and molecules in a gas phase have been found recently [3]. Experiments have been conducted for the scheme of selective breaking of molecular bonds under the action of i.r. and u.v. laser pulses [4], and on selective chemical reactions of particular molecules under visible laser light [5] and high power i.r. laser pulse radiation [6]. To what extent the methods of selective action on molecules in a gas phase thus far developed can be extended into molecules in a condensed state, specifically into complex molecules in a solution, is worthy of consideration. If there is a positive result one would hope for the possibility of laser stimulation of selective biochemical reactions.

In the general case of selective action upon molecules there are two conditions to be met: (1) the molecule chosen in a solution must have narrow lines of selective absorption; (2) the excitation selectivity must be preserved after laser radiation is absorbed during subsequent physical and chemical processes. It is rather difficult to meet these conditions in the case of complex molecules in solution at normal temperatures. First, the electron absorption spectra for complex molecules, lying usually in the u.v.

region, are almost the same for total classes of molecules. For example, all nucleic acids have a wide absorption band in the region of 260 nm owing to heterocyclic purine and pyrimidine bases, which is insensitive to other details of molecular structure [7, 8]. Therefore, the selectivity of molecular bond excitation can be achieved better in the i.r. region. However, in a condensed medium at normal temperatures the vibrational excitation should relax very quickly (in less than $T_1^{vib} = 10^{-11}$ s). It is difficult to rely on selective acceleration of biochemical reactions when the molecular vibration with its energy $\hbar\omega_{vib}$ is excited in a very short time $T_1^{vib} = 10^{-11}$ to 10^{-12} s; for the rest of the time the molecules are subjected to a thermal non-selective action with energy kT only just several times less than $\hbar\omega_{vib}$.

Nevertheless, there are at least two methods essentially possible for selective action of laser radiation on complex molecules complying with conditions (1) and (2) above. These methods have been successfully realized [3, 4, 6] with laser radiation acting on molecules in a gas phase (at 10 - 100 Torr pressures and pulse durations of 50 - 200 ns) and, as shown below, can be extended to condensed media with the laser pulse duration reduced 10^3 to 10^4 times, *i.e.* when passing to the picosecond range.

Method of two-step i.r.–u.v. excitation

Figure 1(a) illustrates the scheme of molecular selective two-step excitation based on the combination of selective excitation of molecular vibrational level under an ultrashort pulse (USP) of i.r. laser radiation and subsequent excitation of the electron unstable (or stable) state of vibration-excited molecules under the action of a USP of u.v. laser radiation. Under a USP of i.r. radiation the electron absorption band of a particular molecule, the vibrational band frequency of which coincides with that of the i.r. radiation, becomes shifted. The USP of the u.v. radiation causes the molecule to transfer to an excited electron state before the vibrational relaxation occurs. This method assures the vibration selectivity, solves the problem of rapid vibrational relaxation and, at last, enables the molecule chosen to attain an energy of several eV, which is sufficient for fast biochemical reactions.

Some conditions should be met to realize the method successfully. First, the vibrational excitation energy should be materially more than the thermal energy kT and the width of red wing $\hbar\Delta\omega_{wing}$ of the electron absorption band [4]:

$$\hbar\omega_{vib} \gg kT,\ \hbar\Delta\omega_{wing} \tag{1}$$

Secondly, a considerable proportion of the molecules should be subjected to vibrational excitation under the action of USP of i.r. radiation before the vibrational relaxation of excitation starts. From this we get the condition for the rate of stimulated transitions W_{IR} between molecular vibrational levels:

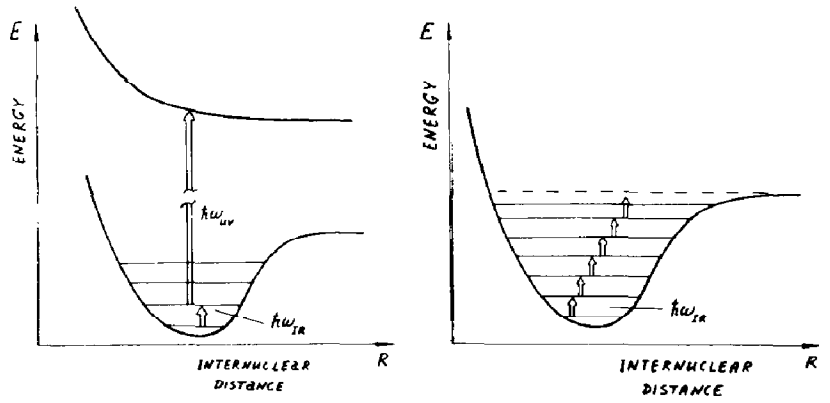

Fig. 1. Methods of selective action on a molecule by laser radiation: (a) two-step excitation under i.r. and u.v. radiation; (b) multiphoton excitation by a strong i.r. radiation.

$$W_{IR} = \sigma_{IR}\frac{P_{IR}}{\hbar\omega_{IR}} \gtrsim \frac{1}{T_1^{vib}} \tag{2}$$

where σ_{IR} is the cross-section of molecular absorption on vibrational transition, P_{IR} is the i.r. radiation power (W/cm^2). Thirdly, the electron excitation of a considerable part of vibration-excited molecules should come about before the vibrational relaxation as well. From this we get the condition for rate of stimulated transition W_{uv} under USP of u.v. radiation:

$$W_{uv} = \sigma_{uv}\frac{P_{uv}}{\hbar\omega_{uv}} \gtrsim \frac{1}{T_1^{vib}} \tag{3}$$

Finally, USP pulses of i.r. and u.v. radiation should be time-synchronized to the accuracy $\Delta\tau$ better than T_1^{vib}.

Let us consider the possibility of meeting conditions (1) - (3) taking as an example a specific thymine molecule which is a pyrimidine base of the DNA molecule. Figure 2 shows the u.v. absorption spectra of a thin thymine film at 300 K and 77 K [7] and i.r. absorption spectra at 300 K [9]. Condition (1) can be met when exciting i.r. bands in the frequency range 1500 - 1600 cm^{-1}, conditioned by covalent vibrations of the double bonds C=C, C=N and C=O in the ring. Figure 2(a) illustrates also the u.v. absorption shift expected when exciting the vibration with a frequency of 1600 cm^{-1} in a molecule at 300 K. For estimating the time of vibrational relaxation at 300 K it may be assumed that $T_1^{vib} = 10^{-11}$ s. (This value is, probably, typical of molecules in solutions [10].) Then the duration of USP of i.r. and u.v. radiation must be some times shorter than 10 ps. The cross-sections of the absorption transitions will be: for the i.r. band at 1600 cm^{-1} $\sigma_{IR} \simeq 10^{-17}$ to 10^{-18} cm^2; and for the u.v. band at 2600 Å $\sigma_{uv} \simeq 2.8 \times 10^{-17}$ cm^2. If this is the case it follows from relations (2) and (3) that the intensity of the pulses should comply with the following conditions: $P_{IR} \gtrsim 3(10^8 - 10^9)$ W/cm^2 and $P_{uv} \gtrsim 2.7 \times 10^9$ W/cm^2. With molecules cooled, say, to 77 K the steepness of the red wing of the u.v.

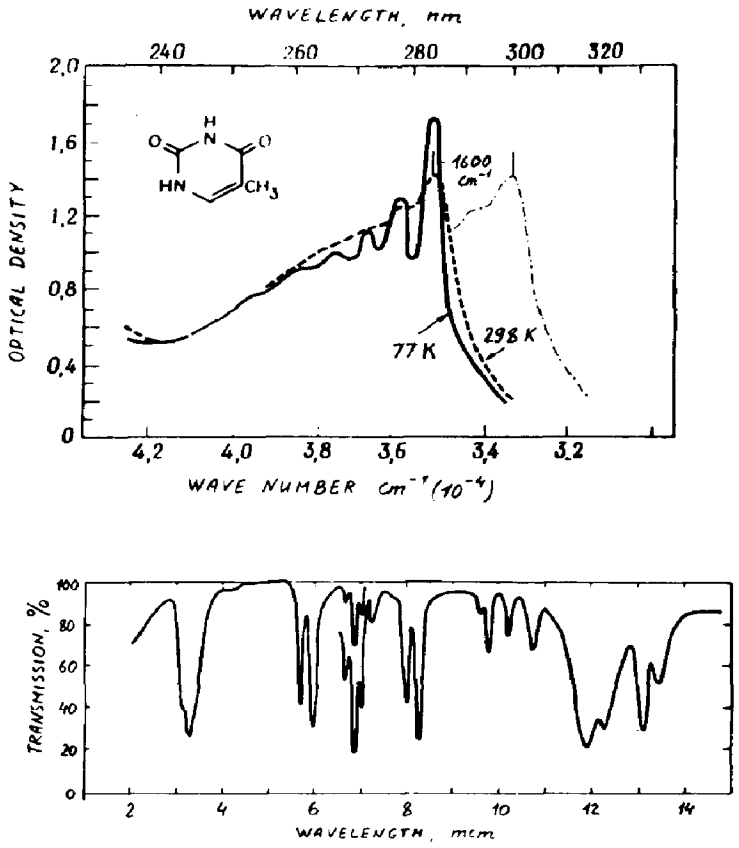

Fig. 2. U.v. and i.r. spectra of a thin film of a DNA base, thymine (after Smith and Hanawalt [7] and Blout *et al.* [9]).

band increases, which makes it easier to meet condition (1); the most important point is that the vibrational relaxation rate declines, which allows the pulse duration to increase and the power to reduce in proportion.

Method of excitation by a strong i.r. field

Figure 1(b) gives a simplified scheme for selective excitation of molecular vibrational levels under the action of USP of i.r. resonance radiation. Under a strong i.r. pulse a resonance multiquantum excitation of high vibrational levels and a molecular bond break may occur. Such a possibility was experimentally discovered recently in studies of molecular collisionlesss dissociation in a gas under a strong field of resonance i.r. radiation [6, 11]. To accomplish the process of multiquantum excitation the rate of induced single-quantum transitions between vibrational levels W_{IR} must comply at least with the condition:

$$W_{IR} = \sigma_{IR} \frac{P_{IR}}{\hbar\omega_{IR}} \geqslant \frac{1}{T_2^{vib}} \qquad (4)$$

where T_2^{vib} is the time of transversal relaxation for vibrational levels, *i.e.* the dephasing time of molecular vibrations induced by the external strong i.r. field. Usually the dephasing time T_2^{vib} is far shorter than the relaxation time of vibrational energy, T_1^{vib}. For example, according to the measurements made by Kaiser *et al.* [10, 12] for symmetric vibrations of the covalent bond of CH_3 in a molecule of CH_3Cl_3 at 300 K (ν = 2900 cm^{-1}), T_2^{vib} = 5 ps, T_1^{vib} = 20 ps. Because of this condition (4) is more rigid than (2) and necessitates a higher intensity and a shorter duration of i.r. pulse. For the molecular bond break in a solution under the action of i.r. pulse to be efficient its duration should be no more than a few ps. Its power should be within the range 10^9 - 10^{10} W/cm^2, *i.e.* over the same range as the breaking of molecular bonds in a gas medium demands [6, 11].

Non-selective thermal effect

The power values of USP of i.r. and u.v. radiation essential for stimulating biochemical reactions by the methods considered lie far below the threshold power which results in damage and strong heating of the condensed medium ($\sim 10^{12}$ W/cm^2). Yet, in any biochemical reaction, especially those *in vivo*, one should avoid even a slight (several degrees) heating of the medium under radiation. This calls for a number of additional experimental conditions to be met.

First, the concentration of molecules in a solvent must be rather low so that the energy converted to heat during the inevitable relaxation of excited molecules is sufficiently small. In order that the medium heating does not exceed ΔT the molar concentration of excited molecules M should comply with the condition:

$$M \leqslant \frac{\rho c}{e_T N_0} \Delta T \tag{5}$$

where e_T is the energy of each molecule under excitation converted to heat, c and ρ are the specific heat and the solvent weight respectively, N_0 is the concentration of solvent molecules. Since the energy required to excite a molecule or to break its bonds, ranges from fractions of an eV to several eV and the portion of the energy converted to heat must be, perhaps, of the same order, we may take $e_T \simeq 1$ eV as a rough estimate. Then for aqueous solutions we have $M \lesssim 0.78 \times 10^{-3} \cdot \Delta T$ instead of that in condition (5).

Value (5) does not allow for a possible heating of the solvent by i.r. and u.v. radiation owing to its own absorption. For example, the aqueous solvent has a strong absorption in the region of OH vibrations ($\sim 3\,\mu$m) which makes it difficult to act upon other molecular vibrations in this frequency range. To remove the heating medium those i.r. and u.v. frequencies should be chosen for selective action for which the absorption of solvent per unit length will comply with the conditions:

$$\varkappa_{IR} \lesssim \frac{c\rho}{\epsilon_{IR}} \Delta T \quad \text{and} \quad \varkappa_{uv} \lesssim \frac{c\rho}{\epsilon_{uv}} \Delta T \tag{6}$$

where ϵ_{IR} and ϵ_{uv} are the energy densities of USP of i.r. and u.v. radiation (J/cm^2) respectively. Since $\epsilon_{IR} \simeq \tau_{IR} P_{IR}$ and $\epsilon_{uv} \simeq \tau_{uv} P_{uv}$, where the powers required are given by relations (2) and (3), and the pulse durations $\tau_{IR}, \tau_{uv} \lesssim T_1^{vib}$, then in the most rigid case $\tau_{IR} = \tau_{uv} = T_1^{vib}$ and conditions (6) reduce to:

$$\mathcal{H}_{IR} \lesssim \frac{c\rho\sigma_{IR}}{\hbar\omega_{IR}} \Delta T \quad \text{and} \quad \mathcal{H}_{uv} \lesssim \frac{c\rho\sigma_{uv}}{\hbar\omega_{uv}} \Delta T \tag{7}$$

For the above stated case of the two-step action of a thymine molecule, estimates (5) take the form: $\mathcal{H}_{IR} \leq 1.3\ (10^2 - 10^3) \cdot \Delta T$ (cm^{-1}) and $\mathcal{H}_{uv} \leq 1.5 \times 10^2 \cdot \Delta T$ (cm^{-1}). These conditions can be complied with when the solvent and wavelengths used are properly selected.

On selective action upon DNA molecule

In order to gain some insight into potentialities and restrictions of the methods described let us consider their application in the most interesting and difficult case, *i.e.* in a selective action upon a DNA molecule. The DNA molecule is a double helix formed by hydrogen bonds between two identical polynucleotide chains. Each chain consists of only four successive mononucleotides or bases (thymine, adenine, guanine and cytosine) connected by phosphate bridges. This offers some possible types of radiation action upon a DNA molecule.

When the hydrogen bonds NH . . O and NH . . . H between bases of two chains (thymine and adenine, cytosine and guanine) are broken we can stimulate untwisting of the double DNA helix. Vibrations at 1720 cm^{-1} correspond to the bond between cytosine and guanine in a native DNA, while vibrations at 1700 cm^{-1} correspond to that between thymine and adenine [13]. The breaking of hydrogen bonds when denaturing DNA results in disappearance of these vibrations. To accomplish a selective breaking of hydrogen bonds in DNA one can try to act with a strong i.r. radiation at wavelengths of these bonds (5.814 μm and 5.888 μm) with pulse duration of about 10^{-12} s and power of $10^9 - 10^{10}$ W/cm^2. Such pulses can be obtained when modes of a high pressure CO laser are mode-locked.

The breaking of a polynucleotide chain can be stimulated by acting on the phosphate group which connects mononucleotides. The PO_2 group has an antisymmetric covalent vibration about 1230 cm^{-1}; when it is acted upon the length of the PO bond varies [13]. To conduct a direct breaking of the phosphate bridge one can try to act on DNA by radiation at 8.13 μm with pulse duration in the picosecond range and power of $10^9 - 10^{10}$ W/cm^2 again. The radiation of a parametric tunable picosecond-pulse oscillator is best suited to this purpose.

It is possible to act on a particular base of DNA. To gain the selectivity of action it is good practice to apply the method of two-step i.r.–u.v. excitation since u.v. spectra of all purine and pyrimidine bases are similar.

For this purpose, vibration bands specific to each base should be selected. The analysis of vibrational spectra has shown that this is possible because the molecular structures of the bases differ markedly, even though they have many common features.

To get mutations in a certain position in a DNA helix it is necessary to perform the spatial localization of action. Theoretically the laser radiation makes possible the localization of excitation with an accuracy of its wavelength only (in the order of μm portions for u.v. action and some μm for i.r. action). The methods of selective action above considered do not provide the spatial localization inside the DNA molecule, since the latter, being coiled, occupies a space usually less than 10^{-6} m^2. The length of the uncoiled chain of DNA molecule may be as long as 1 mm in specific cases, and then some spatial selectivity can be achieved.

The example considered illustrates a potential limitation of selectivity when biological molecules, consisting of reiterative identical elements, are acted upon by laser radiation. But the problem of selective action on individual mononucleotides in DNA is too complicated for the rather simple methods discussed here. Nevertheless, these methods are quite appropriate to many other simpler problems of biochemistry, *e.g.* for the bulk formation of special radicals *in vivo*. Of course, one should try to apply these methods in the cases when ordinary electron absorption spectra do not exhibit the wanted photochemical selectivity.

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