PERSPECTIVE

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Prospects for the study of biological systems with high power sources of terahertz radiation

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Abstract

The emergence of intense sources of terahertz radiation based on lasers and electron accelerators has considerable potential for research on biological systems. This perspective gives a brief survey of theoretical work and the results of experiments on biological molecules and more complex biological systems. Evidence is accumulating that terahertz radiation influences biological systems and this needs to be clarified in order to establish safe levels of human exposure to this radiation. The use of strong sources of terahertz radiation may contribute to the resolution of controversies over the mechanism of biological organization. However the potential of these sources will only be realized if they are accompanied by the development of sophisticated pump–probe and multidimensional experimental techniques and by the study of biological systems in the controlled environments necessary for their maintenance and viability.

1. Introduction

There has been a considerable amount of experimental work on the study of biological systems using terahertz (THz) radiation [1–6]. However, until recently this has been limited to the use of low power laboratory sources with a typical output power of ∼100 μWatts [1–4, 6, 7]. The progress made in this field has been recently reviewed [1–4] and this indicates that significant advances will be made through the use of more powerful THz sources [5]. Such sources are now being developed based on the exploitation of high power lasers [5, 8] for the generation of THz and the demonstration that electron bunches passing through a magnetic field give rise to coherent emission when the bunch length is shorter than the wavelength of the emitted radiation [9, 10]. These developments are opening up new approaches to research with THz radiation and should lead to major advances in our understanding of biological systems and in particular may clarify theoretical ideas on the mechanism of biological organization. Although this field is now developing rapidly there are relatively few publications on the applications of intense sources of THz to biological systems [4 and references therein], [5, 11–17]. This short perspective will give only a brief overview of the field illustrated by a few recent examples of experimental results.

2. Theoretical background

There has been a considerable amount of theoretical work in this field over several decades and the subject is highly controversial. Much of the controversy concerns the hypothesis put forward by Frohlich [18–22] that long wavelength (THz) modes play an important role in biological self-organization and mediate the formation of a coherent state. The background to this controversy and references to previous work can be found in recent reviews [4, 23, 24]. Frohlich [19] argued that the self-organization of living systems is maintained by a flow of free energy through a coherent exited state maintained by metabolic processes. He predicted that under appropriate conditions biological systems can support coherent excitations in the range 10^9 to

10^{12} Hz. This hypothesis is very relevant to the question as to whether quantum mechanics plays a non-trivial role in living systems [25, 26] an idea that has been discussed by physicists for decades [19, 25–27] and largely dismissed by biologists because there is no conclusive experimental evidence. However this issue should be taken seriously given recent developments in the understanding of photosynthesis [28–31] and the demonstration that the human nose works by phonon assisted electron tunnelling [32, 33].

The suggestion that coherent THz modes are exploited in mechanisms of biological organization is appealing since living systems operate in thermal equilibrium but are maintained out of thermodynamic equilibrium by a flow of free energy released by chemical interactions. At room temperature kT is 6 THz so it is likely that rotational and vibrational modes excited in this frequency range will constrain chemical reactions and hence will have been selected by evolution to play a role in biological organization. A crucial issue is the lifetime of long wavelength modes of vibration in condensed matter and Adair has argued on theoretical grounds [27] that such modes would decay rapidly on picosecond timescales into the thermal bath of overlapping modes and hence not have time to play any significant role in biological organization. While there is some limited evidence for long-lived modes that mediate biological processes [34, 35] there is currently insufficient experimental evidence to support an idea with such far reaching implications as Frohlich's hypothesis that coherent vibrational states exist in biological systems. In a later section we suggest how this idea can be tested using the emerging strong sources of THz radiation.

3. Experimental capabilities

The experimental developments in this field were reviewed in an international symposium on the generation and application of THz radiation in Novosibirsk in July 2010 [36]. The key instrumental developments for the generation of high power THz radiation are the free electron laser (FEL) [5] and the use of accelerators to generate short bunches of electrons, as exemplified respectively by the THz FEL in Novosibirsk [8] and the energy recovery linear (ERL) accelerator at Jefferson Laboratory [10]. However while the development of intense sources is a necessary step in the investigation of the effects of THz radiation on biological systems it is not sufficient since it is also necessary to develop sophisticated experimental techniques and a suitable environment in which to maintain and irradiate biological material [4]. A beamline that delivers intense THz radiation into an environment suitable for experiments on biological material has recently been commissioned on the ALICE accelerator at Daresbury Laboratory (figure 1) [37]. ALICE is an energy recovery linear accelerator [38], based on superconducting technology, that was originally developed as the prototype for the proposal for a UK fourth generation light source, 4GLS [38–42] a project that has now been subsumed into the UK New Light Source (NLS) proposal [43]. ALICE is designed to have a bunch length of 0.6 ps, with the result that coherent emission is expected to be observed for radiation of frequencies up to ∼1 THz, and to yield a THz output with a high peak power, 70 kW, and a low average power of 24 mW. The accelerator is designed to operate at a 20 Hz repetition rate and deliver THz radiation in a macro pulse train of 8125 pulses each 0.6 ps wide taking 100 μm s. The accelerator has been commissioned and is currently working up to its design specification.

The THz radiation from ALICE is extracted from a mirror located in the accelerator which directs the radiation out through a diamond window and into the beamline (figure 1). The beamline directs the THz radiation into a diagnostics room and then up to the tissue culture facility which is on the next floor (figure 1). The tissue culture facility satisfies the requirements for work on cancerous tissue and is equipped with a CO_2 incubator and a microbiological safety cabinet. A switch mirror in the beamline makes it possible to direct the THz radiation through the base of either of these units for the direct irradiation of biological material in the conditions needed to maintain it in a healthy state.

4. Terahertz radiation and water

Realistic experiments on biological systems need to be performed in the liquid environments that are necessary for their maintenance and functional behaviour. However the hydrogen bonding network of water is a very efficient absorber of THz radiation: radiation at 1 THz is attenuated by a factor of $\sim 10^{18}$ by 1 mm of water [17]. Thus high peak powers are necessary in order for the THz radiation to penetrate liquid environments and reach biological systems. It is also important that the source have a low average power in order to minimize thermal effects which can have a major impact on biological systems. Federici [44] has reviewed the interaction of THz radiation with water and he and Wilmink and Grundt [4] provide formulas and parameters for calculating the temperature rise in biological systems due to THz exposure. These authors also summarize thermal effects in biological systems.

5. Brief survey of experimental results

While infrared spectra correspond to the motions of the nuclei in a molecule and provide characteristic signatures of particular molecular species that are not strongly influenced by the molecular environment, the longer wavelength vibrational modes in the THz range arise from the motions of the entire molecular structure and involve large masses and more shallow potentials and couple strongly to the molecular environment. This coupling to the environment means that it is unlikely that THz spectroscopy will develop into a comparable tool to infrared spectroscopy for molecular analysis except for very dilute and isolated systems such as found for molecules in space [45] or potentially in trace components found in exhaled human breath that might find applications in the diagnosis of disease. However the sensitivity of THz modes to the molecular environment is an important characteristic of this radiation which is finding increasing applications in the study of biological systems. Such modes have the potential to influence multiple levels of biological organization. Direct

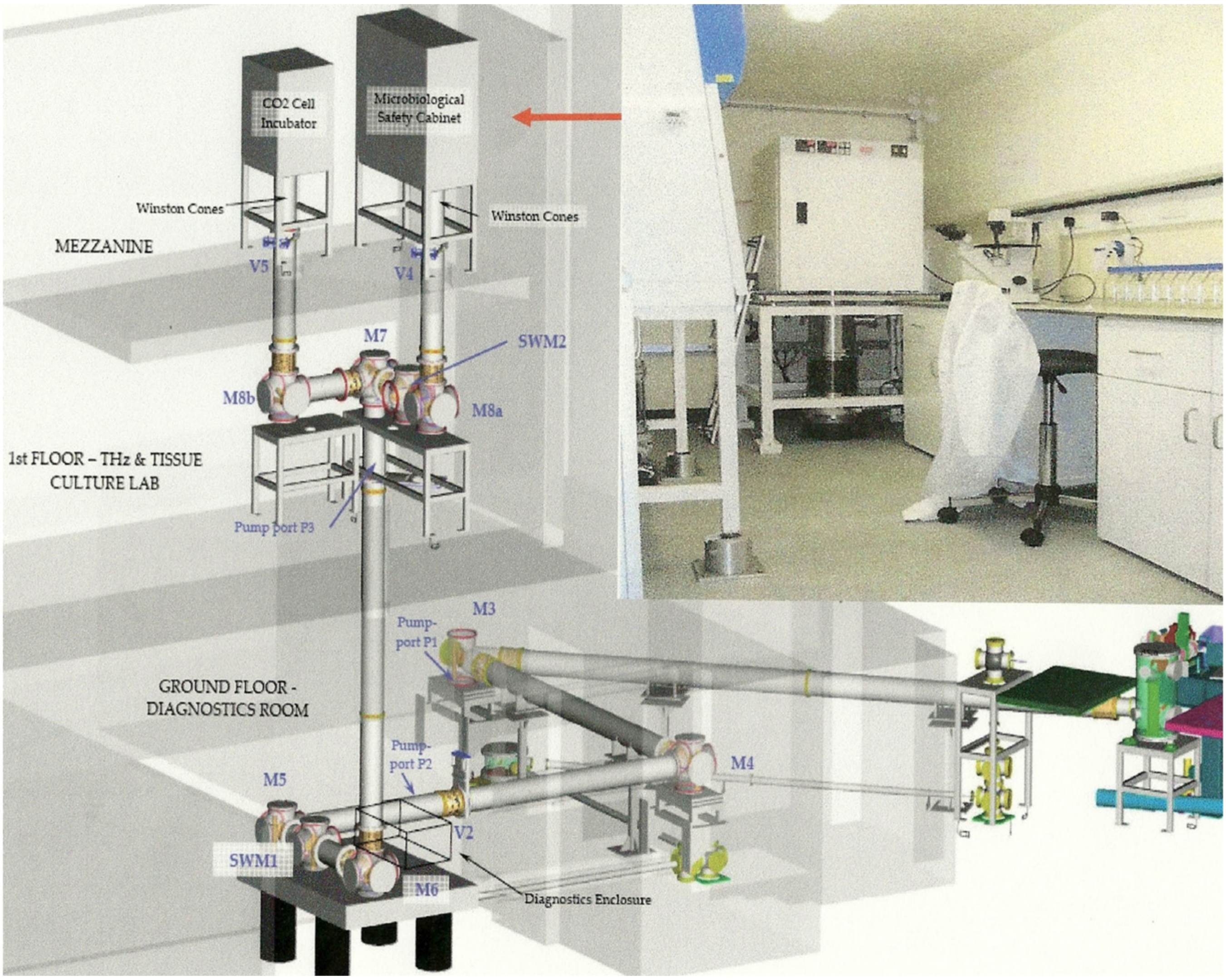

Figure 1. The beamline on the ALICE accelerator enters from the bottom right and directs THz radiation to the tissue culture facility. Once in the tissue culture facility the THz beamline splits into two with one component entering through the base of a CO_2 incubator and the second through the bottom of a microbiological safety cabinet on. The THz output from the beamline can be switched between these lines for the irradiation of live human tissue. A photograph of the tissue culture facility is superimposed at the top right of the figure.

interactions at the molecular level may affect cell behaviour, which in turn will influence cell–cell interactions thereby affecting the development and homeostasis at the level of a whole organism.

6. Biological molecules

6.1. Proteins

Biological molecules carry out their functions in an environment that is warm, crowded and wet. They are expected to have a large number of normal modes of vibration in the THz range and these modes will be broadened by interaction between molecules and with the hydrogen bonding network of water. At room temperature one expects that vibrational modes with energies up to ∼6 THz will be excited thermally and this expectation is borne out in research with low power sources which reveal broad featureless THz spectra formed from overlapping modes, as shown for the proteins hen egg white lysozyme and horse heart myoglobin [46]. The THz absorption spectrum of solvated bovine serum albumin (BSA) shows a similar dense overlapping spectrum of vibrational modes that increases monotonically with increasing frequency and shows no evidence of distinct spectral features [47]. However the authors showed that departures from the spectrum predicted by simple models of disordered inorganic solids indicate that, as expected, the THz spectrum is influenced by the structure of the protein [47]. Proteins carry out their functions by subtle changes in conformation which are expected and found to involve vibrational and rotational frequencies in the THz range and to be mediated by water [48–51]. Thus THz modes of vibration are implicated in the primary event of vision [52], the acceptance of oxygen by haemoglobin [53] and many other important biochemical processes as discussed by Laman *et al* [50]. The functional importance of the interaction between biological molecules and water is a major impediment to the use of THz radiation to probe some important biological processes such as the interactions between components of the extra-cellular matrix where it is necessary to study interactions between very low concentrations of molecules of the order of micrograms per millilitre. The enormous absorption in this spectral range by water makes such studies almost impossible with low power sources. More subtly the use of high power THz sources to overcome the absorption of water runs the risk of raising the temperature of the system and thus drastically changing the interactions that are being monitored. Furthermore the hydrogen bonding network that gives rise to the strong THz absorption of liquid water is a very nonlinear function of the concentration of water molecules with the implication that in the crowded environment of the cell the interactions between proteins and the various kinds of 'bound water' may be very different to those monitored for proteins in solution. The work of Prohofsky [54] and of Heugen *et al* [48] gives insight into this problem and gives references to determinations of the water solvation shell around proteins.

6.2. DNA

Theoretical work [55, 56] predicts a large number of intrinsic resonances in the THz range in DNA that arise from excitations of the sugar-phosphate backbone of the molecules in the range roughly between 0.3 to 3.0 THz. THz experiments with low power sources have been used to detect single and double stranded DNA sequences [57, 58] and a label free method of detecting DNA hybridization has been developed based on coating resonators embedded in thin film microstrip waveguides with micrograms of DNA in solution [59–61]. The technique depends on detecting the change in the frequency dependence of the dielectric constant of the solution resulting from the hybridization of DNA strands. The sensitivity of the technique illustrates how, as found for proteins, THz modes of DNA couple strongly to the hydrogen bonding network of water.

6.3. Membranes

The lipid membranes that surround cells play a crucial role in cell signalling and in mediating the interaction between the cell and its environment. Membranes are complex structures that incorporate a wide variety of proteins, some of which are organized in 'membrane rafts' [62, 63] and some of which control channels through the membrane by which molecules and ions enter and leave cells. There has been a significant amount of theoretical and experimental [62–66] work on the dynamic properties of membranes and they are expected to interact strongly with the dipole moments of water. There is also evidence that interactions between proteins in membranes are mediated by phonons in the THz range [67]. Research in this field has reached a high level of sophistication with the development of a variety of fluorescence markers and pump–probe and sum-frequency optical techniques that have made it possible to recognize a number of distinct membrane potentials that are implicated in cell signalling and cell interactions mediated by interactions with water [62–66]. The addition to the existing panoply of techniques of precise time structured sources of high peak THz power capably of penetrating water without incurring large thermal effects have the potential to make a major contribution to this field by scanning the THz spectrum of water and monitoring the response of membrane fluorescence markers.

A recent study of liposomes, which are artificial phospholipid bilayers and good mimics of the basic structure of cells, showed that irradiation, in solution, with 0.13 THz radiation from a microtron driven FEL facilitated a chemical reaction between molecules isolated inside the liposomes and molecules in the solution [68]. This demonstrates that membrane permeability or damage is influenced by THz modes and is consistent with Frohlich's prediction that the natural frequencies of membranes will be of the order of 10^{11} Hz. This experiment made it possible to investigate the effects of high peak power while maintaining a low average power and avoiding heating effects.

The coupling of the THz modes of proteins, DNA and membranes to excitations of water and to each other is also expected to include sugars, the other major biomolecular species, and in these conditions it is difficult to extract useful information on biomolecular interactions in all but the simplest systems using THz spectroscopy alone. Laman *et al* [50] have developed a method of overcoming the environmental broadening and overlapping of features that are observed in conventional Fourier transform infrared spectroscopy and THz time domain spectroscopy of biological systems by studying thin molecular films using high resolution waveguide THz spectroscopy. Kim *et al* [49] have probed dynamic interactions in the THz range technique using the technique of kinetic THz absorption (KITA) which monitors the changing THz electric field pulse shape on a picosecond time scale as a chemical reaction proceeds on a longer timescale. By comparing circular dichroism (CD) results, which are sensitive to secondary structure, with results from KIKA, which are sensitive to the protein-hydration-water interactions, they showed how closely protein dynamics and solvent dynamics interact during protein folding. These conclusions are supported by the work of Knab *et al* [51].

It is clear that the study of biological molecules with THz radiation would benefit considerably from the development of more powerful sources of THz radiation capable of overcoming the strong absorption of THz by water. Provided such sources combine high peak power with low average power it should be possible to study the important interactions between such molecules and their liquid environment without changing these interactions through the induction of thermal effects. In the longer term this field will benefit from the development of techniques that combine THz sources with other probes of molecular vibration in pump–probe or multidimensional studies. This will require the design of accelerator based facilities using short electron bunches to drive a range of light sources providing well characterized short pulses of light on fast timescales, as proposed in recent years for new UK light sources, 4GLS [69] and NLS [70], the FLARE project in The Netherlands [71], the Biglight facility [72] in the USA and as is being realized at the Jefferson Laboratory [73]. The illumination of specimens with time structured and synchronized sources of radiation in different frequency ranges opens up a number of sophisticated techniques in which THz radiation can be used to initiate reactions or pump specific vibrational and rotational modes and the corresponding conformational changes can be monitored by techniques such as infrared, ultraviolet and CD spectroscopies and extended x-ray absorption fine structure (EXAFS).

One approach is to combine THz radiation to induce conformational changes with reflection anisotropy spectroscopy (RAS) a technique that has been shown to monitor real time conformational changes in flavoproteins absorbed at a metal liquid interface driven by electron transfer reactions in an electrochemical cell [74]. RAS can also be used monitor the hybridization of single stranded DNA, tethered to a diamond surface, with the complementary strand in solution [75]. The combination of intense THz sources with RAS offers a method of investigating whether an intense burst of THz radiation can drive conformational change in proteins and investigating further the known tendency of THz radiation to interfere with the protein recognition process [76] and induce conformational change in proteins [77]. These techniques could also be used to investigate whether intense THz radiation can cause dynamic separation of double stranded DNA as suggested by recent theoretical work [78]. A scheme has been put forward for performing RAS measurements on a nanosecond timescale using light from a bending magnet on the ERL that was the basis of the 4GLS design [40].

Considerable advances have been made recently with two dimensional infrared and photon echo techniques [28–31, 79, 80] using laboratory sources. These techniques are able to monitor coupling of vibrations of very different frequencies no matter how far apart and can be used for the finger printing of proteins and for the determination of the dynamics of catalytic sites. However current laboratory instruments cannot produce sufficient powers to study collective motions such as α helical stretches, β sheet movements and more global and longer range structural motions. Accelerator based sources of THz and infrared radiation used in combination have the potential to revolutionize this field and provide considerable insight into the relationships between the structure, dynamics and functions of proteins.

These approaches based on the high intensities available with accelerator based light sources will be particularly important in research on the extra cellular matrix [81]. This consists of a large number of sugars and proteins in dilute amounts that interact and facilitate physical, chemical and organizational processes that are important in a very wide range of diseases. Progress in this field is severely limited by the low power levels available with a wide range of laboratory light sources due to the need to study very low concentrations, $\sim\mu$g ml^{-1}, similar to that which occurs in natural systems and which is inaccessible with conventional laboratory equipment. The high power levels available with the next generation of THz sources will make it possible to study the basic interactions between components of the extra cellular matrix at the dilute concentrations necessary in order to elucidate the function of this important biological system.

Recent research with the THz FEL at the Budker Institute has indicated that high intensity THz radiation has the potential to make a major contribution to proteomics the aim of which is to identify, characterize and isolate the complete proteomes of living things. This subject is limited by the difficulty of separating proteins from cells and membranes. Considerable progress is being made with the matrix-assisted laser desorption/ionization technique (MALDI) in which lasers are used to ablate proteins into gas phase and study the components with mass spectroscopy. This process leads to the fragmentation of proteins the structure of which is then deduced from the mass spectroscopy analysis of a number of fragments. The Budker group have shown (figure 2) that irradiation with high power THz radiation is able to desorb large molecules into gas phase without fragmentation as demonstrated by the fact that following subsequent deposition the enzyme horseradish peroxidase retained its functional activity [82]. This approach may prove to be a powerful addition to the MALDI technique. The soft non-destructive ablation of molecules with intense THz radiation has also been used to transfer target DNA sequences to aerosol phase and has the potential to reduce noise out of genetic analysis using of DNA microarrays [83] by standardizing the production of biochips [15].

7. Complex biological systems

It is unlikely that the processes that are postulated to occur in biological systems by the Frohlich hypothesis will be revealed by experiments at the molecular level. The hypothesis is essentially concerned with the mechanism of biological organization and its influence will be felt in more macroscopic systems such as cells, bacteria, plants and animals. Living systems maintain an organized active state that operates out of thermodynamic equilibrium. If the Frohlich hypothesis is correct and long wavelength vibrational modes play a key role in maintaining this organized state then irradiation with THz modes would be expected to interfere with these processes with unpredictable but potentially serious consequences.

7.1. Cells

Cells carryout many functions in living things and among the more crucial ones are the control of the population of proteins by organizing the expression of genes coded in DNA and the duplication of DNA for cell division. The possibility that THz radiation can influence either of these processes must be taken seriously given that life in earth has evolved in an environment of exceedingly low exposure to THz radiation and that the exposure of humans to higher power levels of radiation in this frequency range is likely to increase significantly in the future.

Berns *et al* [14] studied the effect on the ability of mammalian tissue culture cells at different stages of the cell cycle to incorporate radioactively labelled tritiated thymidine after exposure to 100 pulses of 200 μm THz radiation from a FEL delivering pulsed radiation with a power level of 1.3 kW per pulse. Since the transport of the cells from the incubation facility to the FEL required a 3 h journey this research put a significant premium on the development of robust protocols for maintaining the viability of the cells. Experiments were

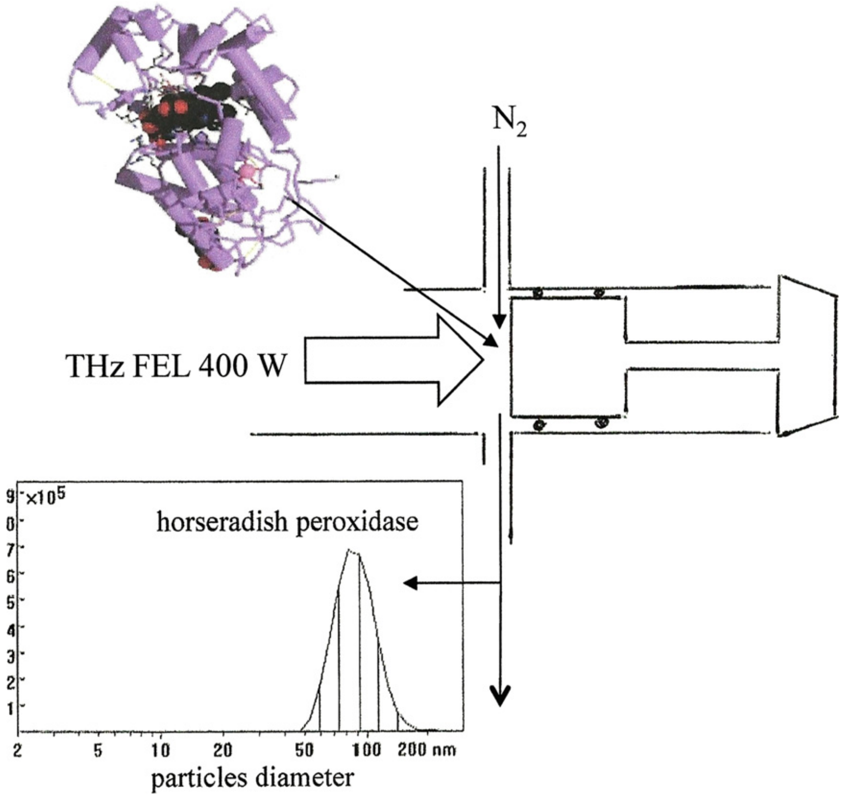

Figure 2. The scheme adopted on the THz FEL at the Budker Institute for the damage free ablation of large molecules into a gas flow. It has been demonstrated that after 'landing' the enzyme horseradish peroxidase retains its functional activity. Figure reproduced with permission from a lecture by Dr Sergey Peltek (Russian Federation, Novosibirsk, Institute of Cytology and Genetics).

performed on 40 cell cultures, twenty of which involved cells carefully synchronized to be exposed at a key stage in the cell cycle. It is clear that exposure to THz radiation significantly inhibited the incorporation of thymidine and that the extent of this inhibition depended on the stage of the cell cycle at which the cells were irradiated. This is consistent with the idea that exposure to 200 μm radiation affects DNA directly and that the likely absorption site is the DNA molecule. They conclude that due to the low fluences and the thermal diffusion time between pulses it unlikely that the effect is thermally mediated. The difficulty of these experiments and the conclusive result draws attention to the need to control the environment of the cells before, during and after the exposure and to design the experiment to yield a statistically significant result.

The evidence for genotoxic effects arising from irradiation with THz radiation is mixed and has recently been analysed by Alexandrov *et al* [78]. They quote the findings of an international study, the 'THz-Bridge' project [84] which concluded that 'under some specific conditions of exposure, change in permeability of membrane of liposomes was detected and an indication of genotxoity was observed to occur in lymphocytes'. Taking into account the conclusions of the THz-bridge project and more recent results [76, 85, 86] they conclude that the available experimental data strongly suggest that THz radiation can affect biological function only under specific conditions such as high power and/or extended exposure and/or specific frequency. They go on to consider the influence of the THz field on the breathing dynamics of double stranded DNA and, based on a model of the spontaneous formation of spatially localized openings of a damped and driven DNA chain, find that linear instabilities lead to dynamic dimerization while true strand separations require a threshold amplitude mechanism. They conclude that a specific THz radiation exposure may significantly affect the natural dynamics of DNA and thereby influence intricate molecular processes involved in gene expression and DNA replication. They find that the main affect of THz radiation is to resonantly influence the dynamic stability of double stranded DNA. While an analysis of this approach by Swanson [87] concluded that it is not relevant to physically realizable situations its conclusions are supported by the work of Bock *et al* [16] who found evidence that extended exposure to broad band THz radiation centred on ~10 THz results in specific changes in the function of cellular DNA with the activity of some genes being enhanced and others suppressed.

7.2. Nerve cells

It is known that exposure to quite low power levels of 60 GHz radiation can influence the activity of neurons [88] and clearly for safety considerations alone such effects need to be investigated with the high power sources now being developed in the THz region. The long wavelengths of THz radiation means that the spatial resolution obtained with conventional microscopy techniques will be severely limited by diffraction. However the diffraction limit can be overcome using near field techniques provided the source is sufficiently intense. Van der Valk and Planken obtained a resolution of $\lambda/200$ at

~0.15 THz using a near field technique [89]. This approach enabled Masson *et al* [90] to exploit the difference in THz absorption of Na and K chloride solutions to provide the contrast needed to observed physiological relevant changes in the intra and extra cellular concentration of K and Na chloride in and around living neurons. The development of more intense THz sources may make it possible to extend such experiments to yield images at a wider range of THz frequencies and to observe dynamic effects.

7.3. Bacteria

An obvious area in which the communication mechanisms envisaged by the Frohlich hypothesis might be found is in the complex behaviour of bacteria which communicate with each other and their environment in quorum sensing and biofilm formation. It is well known that much of the signalling is mediated chemically using pheromones [91, 92]. However there is evidence that bacteria also communicate using electromagnetic signals over distances substantially larger than cellular dimensions. This subject has been reviewed by Trushin [93] who provides extensive references to the results of this research, the majority of which does not use THz radiation directly. The experiments are difficult because often the effects are small and the natural variability of biological systems makes it difficult to obtain statistically significant results. However it has been shown that bacteria are sensitive to sound and also emit sound [94] and, in an extension of the Frohlich hypothesis, Norris and Hyland [95] have shown that these results can be explained in terms of intercellular integration mediated by THz modes. They suggest that enzymes can emit radiation in this range and that the chromosomes that receive these waves alter gene activity. They identify a particular subunit of RNA polymerase as a candidate for the emitter of electromagnetic waves in the sender bacterial population. Trushin [93] also gives references to papers reporting cell density effects arising from direct irradiation with microwaves and THz radiation. All these studies will benefit from experiments with high intensity THz radiation provided temperature effects and environmental conditions can be controlled.

7.4. Yeast

One of the earliest tests of the Frohlich hypothesis were carried out by exposing yeast to THz radiation and monitoring the subsequent growth of the yeast colony [96]. Hadjiloucas *et al* [24] review previous work and report the results of the exposure of yeast colonies to radiation in the 0.20 to 0.35 THz range using radiation from a backward wave oscillator. They observed a statistically significant enhanced growth rate after exposure to 0.341 THz at a power level of ~60 μW mm^{-2} over 2.5 h. Thermal effects were limited to a temperature rise of ~2 °C. This research would benefit considerable from the development of more intense sources since, as expected, the THz intensity was significantly attenuated by the medium in which the yeast was grown.

7.5. Plants

The process of photosynthesis in which photons are used to split water, release O_2 and create hydrocarbons from CO_2 is the basis of most of life on earth. The process is remarkably efficient and it has recently been demonstrated that in some systems at least [28–31] this efficiency arises from quantum mechanical coherence in the basic chemical and vibrational processes involved. The application of a two dimensional photon echo technique [31] using laboratory sources has demonstrated the presence of quantum mechanical coherence at ambient temperature in the coupling between the motions of a large number of the molecular antenna and reaction centres involved in the photon capture and exciton transport processes in *Rhodopseudomonas acidphila,* a eukaryotic light harvesting algae that functions at low light levels. The energy density of sunlight striking the earth is such that if artificial photosynthetic systems could be developed they could make a major contribution to the generation of CO_2 free energy and for this reason alone it is important to understand natural processes in as much detail as possible. However laboratory sources are weak and have a limited frequency range and the extension of 2D techniques to longer wavelengths using intense and tunable THz sources would make a major contribution to assessing the role of longer range couplings and coherence in photosynthesis.

7.6. Animals

There are very few published studies of the effects of the exposure of animals to intense THz radiation. An experiment was performed on male mice using radiation from the THz FEL at the Budker Institute [86]. It was found that the mice perceived the presence of THz radiation after a 15 min exposure. A 30 min exposure induce significant anxiety in the animals that persisted into the following day. The physiological explanation for these effects is unclear and the subject is clearly at very early stage. There is also a study of the effects of irradiation with 81.5 μm radiation from a laser on the generation of mutations in fruit flies [97]. It was found that exposure during the larval stage reduced the number of mutations relative to controls.

8. Testing the Frohlich hypothesis: the potential of intense sources of THz radiation

We currently have no satisfactory detailed explanation of how living things maintain themselves in an active ordered state in a world governed by the second law of thermodynamics. Frohlich's ideas offer an explanation in terms of the creation of coherent excited states maintained by metabolic processes and involving long range THz modes of vibration and it is reasonable to suppose that such a mechanism would be favoured by Darwinian evolution. Such a far reaching idea has been subjected to considerable, and somewhat inconclusive, theoretical analysis [16, 18–23, 25, 27, 87, 98–101] particularly with respect to the mechanisms employed by DNA in performing its biological functions [16, 78, 87, 98, 99]. However while theory is unlikely to provide a definitive assessment of the hypothesis it can play an important role in suggesting the parameters required for experimental tests. One obvious target for such experiments is DNA since this is the information carrying molecule in living things. Another is the ribosome which is the factory for producing proteins though less is currently known about the dynamic activity and vibrational characteristics of ribosomes.

It is not possible with THz techniques to target specific components of complex biological systems. However if vibrational modes in this frequency range are the basis of biological organization then disrupting the intrinsic THz spectrum of a biological system by irradiating with an external THz source should disrupt biological order, where ever it is located, and have far reaching consequences for its subsequent behaviour and viability. Many experiments have been undertaken with this objective but so far, in instances where effects due to THz irradiation have been observed, it has not been possible to be completely sure that the results have not been influenced by mundane mechanisms such as transient local heating, thermal shock due to experiments being conducted at room temperature in non-physiological environments or the absorption of THz energy by biological molecules including their interactions with the hydrogen bonding network of water in its various biological forms.

Since the Frohlich hypothesis concerns the mechanisms by which living systems function it is desirable to carryout experiments in the physiological conditions necessary for their maintenance and growth, typically supported in a growth medium in a CO_2 incubator maintained at 37 °C. Ideally the source of THz radiation should be directed into the incubator as in the design on the ALICE accelerator at Daresbury (figure 1). Stem cells are ideal subjects for such experiments since they are particularly sensitive to changes in their environment and there are established techniques for monitoring changes in their behaviour. The aim should be to arrive at experimental conditions, probably by trail and error, in which irradiation with THz leads to significant changes in the future development of cells [14, 16] rather than their death since the former result is more indicative of the subtle changes expected in the mechanisms of biological organization.

The peak power of the THz source is an important consideration for a number of reasons. Firstly 10% to 30% of the THz radiation incident on a biological system is reflected and as discussed above the attenuation by the water based growth medium will be very high [4, 44]. Since the attenuation of THz radiation by living tissue is similar to that of water [4] the peak power of the incident radiation needs to be high in order for the radiation to reach the loci of biological activity. Theoretical considerations also suggest that the peak power of the source is a key parameter in the design of experiments. It is clear that vibrational modes are the key processes by which DNA is able to carryout its functions and it has been suggested that nonlinear processes are important in creating localized regions of 'unzipped' DNA that are precursors to DNA duplication and the transfer of information for the expression of genes [78, 98–101]. If this is correct than the effectiveness of an external source in coupling to such modes and driving them into 'unnatural activity' will increase with the peak power of the source. However it is difficult to deduce the peak power required in such experiments since this depends on the assumptions made and the model adopted in analysing the vibrational behaviour of the molecule. Adair for example concludes that biological noise generates internal fields of $\sim$0.1 V m^{-1} [27, 87] while Sataric and Zdravkovic [99] estimate endogenous fields to lie in the range 10^3 V m^{-1} to 10^5 V m^{-1}. The legal limit for human exposure to THz radiation is a power density of 5 – 10 mW cms^{-2} [102] which Swanson [87] deduces to correspond to $\sim$30 V m^{-1}. In the circumstances and, given the losses due to reflection and the attenuation of THz by both the growth medium and tissue, the peak power of the source should be as high as possible. In the event that THz exposure is found to kill stem cells then the strategy should be to reduce the peak power and the exposure to the point where the cells survive and then search for long term changes in their subsequent development.

The average power of the THz source used in experiments to test the Frohlich hypothesis is also an important consideration. This should be as low as possible to avoid thermal effects. Thermal effects will be linearly dependent on exposure time so it is important to keep this short by employing a pulsed source with short pulse lengths and low repetition rates so as to allow thermal effects to dissipate between pulses. Continuous wave sources run the risk of establishing a thermal equilibrium at a raised temperature and it should be noted than small rises in temperature stimulate cell growth and metabolism [4], observations that should not be confused with the direct effects of exposure to THz radiation. The energy balance in an experiment should be assessed carefully and any temperature rise measured and calculated [4].

The intense THz sources that are now being developed should make it possible to design definitive tests of the Frohlich hypothesis. A positive outcome of any experimental test of the hypothesis would have a major impact on the theoretical basis of the biological and medical sciences. If the hypothesis is falsified then this will draw attention to the fact that we have no detailed theoretical understand of the mechanism of biological organization.

9. Conclusions

Clearly the development of intense sources of THz radiation has considerable potential to facilitate advances in the study of biological systems and may eventually resolve controversies over the mechanism of biological organization [103]. However the full potential of these sources will only be realized if they are accompanied by the development of sophisticated pump—probe and multidimensional experimental techniques and, most importantly, by the study of biological systems in the controlled environments necessary for their maintenance and viability.

Evidence is accumulating that THz radiation does influence biological systems and it is important to clarify these effects in order to establish safe levels of human exposure to this radiation. However the difficulties associated with maintaining specimens in appropriate environments during THz irradiation, the need to correct for thermal effects and the natural variability of biological systems means that there is no clear view yet of the nature and importance of the influence of intense THz radiation on biological systems. The development of strong sources of THz radiation, with high peak powers and low average powers, when combined with facilities to study biological systems in physiological environments has the potential to foster considerable progress in this field.

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