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SYNCHROTRON RADIATION SOURCES FOR PHOTOBIOLOGY AND ULTRAVIOLET, VISIBLE AND INFRARED SPECTROSCOPY

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WHAT IS SYNCHROTRON RADIATION?:

Maxwell's equations show that an accelerating electrical charge emits electromagnetic radiation. The emission of radio waves by electrons oscillating within an antenna is a familiar example of this effect. Electrons moving through a vacuum can be accelerated radially by a magnetic field oriented perpendicularly to their direction of motion. The high energy electrons circulating within a synchrotron experience a centripetal accelleration each time they pass through a bending magnet. Electrons whose path is bent by the magnetic field emit photons along the direction tangential to their path. Thus at each bending magnet around a synchrotron ring, radiation is emitted in a fan-shaped distribution; the limits of the "fan" are the directions of travel of the electrons before entering and after leaving the bending magnet. The radiation covers a very broad spectral range as shown in Fig. 1; this is the most important feature of synchrotron radiation.

The shorter the wavelength, the greater the fraction of the radiation with linear polarization of the electric vector in the plane containing the curved orbit of the electrons, and the greater the extent to which the photons are confined in or close to this plane. For x-rays, synchrotron radiation is effectively plane polarized while for ultraviolet radiation, a significant fraction of the photons radiated above or below the orbital plane will be polarized with the electric vector perpendicular to the orbital plane.

The short wavelength limit of the synchrotron emission

spectrum is proportional to cube of the kinetic energy of the electrons and inversely proportional to the radius of curvature of their trajectory through the magnetic field. Thus high-energy photons are produced by high-energy electrons and intense magnetic fields. For a given wavelength, the photon flux is proportional to the number of electrons passing through the magnet per unit time. Thus, the number of ultraviolet, visible and infrared photons is strongly dependent on the number of electrons in the ring - i.e., the circulating current - and correspondingly independent of the energy of the electrons (provided their energy is greater than about 10^8 electron volts, which is the case for all modern synchrotron storage rings).

The "time structure" of synchrotron radiation is also important in uv, visible and infrared applications. Photons emitted when electrons pass through bending magnets carry with them energy which must be replaced if the electrons are going to travel around and around the storage ring for long times - typically several hours. The electrons are accelerated by passing through a hollow electrode to which a high voltage oscillating electric field is applied. The accelerating field must oscillate so that the electrons are attracted as they approach the electrode and repulsed as they leave. Typically an oscillating frequency in the range used for radio transmissions is used and the accelerating device is termed an "rf" cavity.

For the electrons to receive the correct "boost" each time they pass through the rf cavity and thus maintain a stable orbit, they must arrive at just the correct time relative to the sinusoidally changing rf field. Thus electrons travel around the ring in a number of "bunches"; the maximum number of possible bunches is given by pf/c where p is the distance the electrons travel in going all the way around the ring, f is the frequency of the rf field and c is the velocity of the electrons - which is only very, very slightly less than the velocity of light in vacuum. Light is thus generated in pulses. The duration of a pulse is the "length" of the bunch of electrons divided by c. The distribution of the electrons within the bunch and hence the temporal profile of the light pulse is approximately gaussian. For most storage rings, the full-width-at-half-maximum of the light pulse is one nanosecond or less.

The minimum time between flashes is achieved by populating all of the potentially stable bunches with electrons; the pulse-to-pulse separation is 1/f; values of 5 to 30 nanoseconds are typical. The maximum flash-to-flash separation is realized by loading all of the electrons into a single bunch resulting in a dark interval given by p/c. Typical values for this mode range from 10 nanoseconds to a few microseconds depending on the size of the synchrotron.

For some experiments it is desirable to have still longer interpulse periods. Besides using very fast shutters-such devices exist for use in the uv and visible but not for x-rays, it may be possible to perturb the path of the electrons so that light from only every n^{th} flash traverses the experimental optical system and reaches the sample (Blumberg, 1979). Since n could be adjusted experimentally, a broad range of interpulse periods could be achieved. This scheme has the advantage of working equally well for all wavelengths.

SYNCHROTRON RADIATION AS A PROBE OF BIOLOGICAL STRUCTURE AND DYNAMICS:

Biological applications of synchrotron radiation involve ultraviolet, visible and infrared radiation as well as x-rays. This paper is mainly concerned with the former, but I will also mention the different ways in which x-rays from a synchrotron may be used to probe the structure and dynamics of biological materials. All of these applications are discussed in the volume edited by Castellani and Quercia (1979).

Biological applications of synchrotron radiation in the ultraviolet, visible and infrared (UVISIR) regions of the spectrum can be divided into two classes based on the use which is made of the time structure of the radiation. Experiments such as circular dichroism, magnetic circular dichroism, photoacoustic spectroscopy, and action spectroscopy make use of the broad spectrum and high intensity of synchrotron radiation. For these experiments, time structure is not important. In contrast, fluorescence lifetime and anisotropy experiments require a pulsing source. Of course, the broad spectrum and high intensity will also be valuable for the time-structure-dependent class of experiments.

Circular dichroism is one of the most important spectroscopic experiments which will benefit from the broad tunability of synchrotron radiation. Commercial dichromators with xenon arc sources operate marginally below 200 nm and not at all below 180 nm. CD experiments using hydrogen discharges have shown the spectral region below 200 nm to be extremely important in the study of proteins, nucleic acids, and sugars. The greater intensity available from synchrotron radiation will improve the precision and sensitivity of far ultraviolet CD experiments and probably permit kinetic measurements of changes of CD in this region. Synchrotron radiation may also be the best source for extending CD measurements into the infrared.

Circular dichroism is sensitive to the conformation of molecules. Thus the far ultraviolet CD spectrum of proteins

(165-240 nm) is sensitive to the secondary structure of peptide bonds (Johnson and Tinoco, 1972; Brahms and Brahms, 1980). In particular, the region from 165-200 is important for quantitating β sheet and reverse turns in proteins (Brahms and Brahms, 1980). In the case of nucleic acids, the strong band observed between 180 and 190 nm appears more sensitive to helical structure and base-base interactions than the more extensively studied 260 nm band (Sprecher et al., 1979). Far ultraviolet CD has also been important in studies of polysaccharides since all of the electronic absorption bands of these molecules lie below 200 nm. Johnson (1978) and Pysh (1976) have reviewed the early work on vacuum ultraviolet circular dichroism.

Magnetic circular dichroism (MCD) is sensitive to the configuration, i.e. the covalent and electronic structure, of a molecule; generally MCD, in contrast to CD, is insensitive to conformation. Far ultraviolet MCD will thus be useful in probing the higher excited states of biological molecules and assigning the origin of bands observed in absorption and CD. One important application will be in the nucleic acids where theoretical predictions have yet to be tested.

To date, CD experiments using synchrotron radiation have been performed at the Aladdin storage ring (Stoughton, WI, USA) by Patricia Snyder and her colleagues (Snyder and Rowe, 1980) and at the SURF II ring (Gaithersburg, MD, USA) by my group. The latter experiment will be moved to the National Synchrotron Light Source (Brookhaven, NY, USA) sometime in 1981. I expect that CD and MCD experiments will be built at several of the other synchrotron facilities.

Synchrotron radiation is a useful source for probing the biological effects of far ultraviolet radiation. In contrast to longer wavelength ultraviolet where the most important biological target is DNA, wavelengths below 185 nm are absorbed very near the surface of a cell. Experiments by Ito and his colleagues using radiation from the Tokyo synchrotron as well as conventional sources indicate that these short wavelengths can kill cells but do not induce mutations (Ito et al., 1980). Perhaps focused beams of synchrotron radiation can be used to probe surface structure. It will also be interesting to see if some organisms have evolved repair mechanisms for this type of damage.

Photoacoustic spectroscopy is another measurement which can be extended to shorter wavelengths by the use of synchrotron radiation. Dehydrated samples and materials adsorbed to surfaces can be analyzed.

Fluorescence spectroscopy uses to advantage both the broad

spectrum and the pulsing nature of synchrotron radiation. Excitation spectra can be extended to at least 185 nm (where water starts to absorb). At about 220 nm, for example, phenylalanine absorbs as strongly as tryptophan and tyrosine, in contrast to longer wavelengths where absorption by phenylalanine is usually unimportant in photochemical and photophysical processes if tryptophan or tyrosine is present. The ability to extend excitation spectra to shorter wavelengths will also be valuable for measurements of phosphorescence and delayed fluorescence.

The time structure inherent in synchrotron radiation can be used to measure fluorescent lifetimes (Alpert and Lopez-Delgado, 1976), time resolved polarization anisotropies (Munro et al., 1979) and time resolved emission spectra. These measurements reveal molecular dynamics with sub-nanosecond resolution. The advantages of using synchrotron radiation compared to flash discharge or pulsed laser radiation are the ease with which any excitation wavelength can be chosen, the excellent temporal stability of the pulse and the high repetition rate.

The most dramatic impact to date of synchrotron radiation on structural biology has involved Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy. This technique permits determination of the radial distances from a unique atom to its neighbors - e.g., the distances from the iron in an iron-sulfur protein to the sulfur ions surrounding it. Samples can be either dehydrated or in solution; crystals are not required. Since interatomic distances can be determined with subangstrom precision, EXAFS spectroscopy is an important complement to diffraction methods. For a review see Shulman et al. (1978).

Synchrotron radiation may become an important tool in x-ray crystallographic studies of biological structure by virtue of the changes in a diffraction pattern which occur for x-ray wavelengths above and below the absorption edge of a unique component - again consider the iron ion in an iron-sulfur protein. Changing the wavelength of the x-rays may provide the same information which presently must be obtained from tedious isomorphic substitutions. As for small angle x-ray scattering, the higher fluxes of collimated radiation obtainable with synchrotron radiation have made possible the measurement of changes in scattering associated with time dependent events - e.g., the contraction of a muscle - with a resolution of milliseconds.

X-ray microscopy may become a practical reality as a consequence of the degree of collimation inherent in synchrotron radiation. For biological materials the principal advantage of x-ray microscopy is the relative opacity of organic materials and

transparency of water between the carbon absorption edge (4.36 nm) and the oxygen absorption edge (2.33 nm). Thus biological materials could be studied with higher resolution than is achievable with visible radiation, but without the dehydration and staining required by electron microscopy. The theoretical resolution of an x-ray microscope is below that achievable with an electron microscope by about an order of magnitude. The experimental challenge facing the designers of these instruments is finding some means of focusing soft x-rays; at present the use of Fresnel zone-plates seems to be the best approach. For recent reviews, see the volume edited by Wright (1980).

SYNCHROTRON LIGHT SOURCES

The generation of electron synchrotron storage rings which presently are being used as light sources was originally built to perform experiments in nuclear and particle physics. Several of the older, lower energy storage rings were converted to "dedicated" radiation sources when they became obsolete for their original mission. These facilities are useful sources of short wavelength ultraviolet and soft x-rays. Higher energy rings, the ones at Hamburg and Stanford for example, are still used for elementary particle research. Simultaneously, they serve as sources of synchrotron radiation in what is called a "symbiotic" mode of operation. The higher energy of the electrons in these rings means that they are good sources of hard x-rays as well as longer wavelength radiation.

A new generation of storage rings designed specifically to serve as sources of synchrotron radiation is nearing completion in several European countries, Japan and the United States. As an example of the facilities which will be available at these new "dedicated" synchrotron radiation centers, I will describe the National Synchrotron Light Source (NSLS) which is being built at Brookhaven National Laboratory in the United States; it is located on Long Island about 100 km east of New York City. The NSLS will have a total of 44 ports. Of these, 28 will receive radiation from a high energy storage ring, the electrons in which will have an energy of up to 2.5×10^9 electron volts. These ports will be used primarily for experiments which require hard x-rays. A smaller, lower energy storage ring (the electrons will have energy of 7×10^8 electron volts) will supply soft x-rays, ultraviolet, visible and infrared radiation to 16 additional ports. The radiation from many of these 44 ports will be divided between two, three and sometimes even four experiments. Thus, in a few years, there may be 100 or more different experiments in operation. We expect five experiments to be designed primarily for biophysical research, specifically 1) UVISIR spectroscopy, 2) x-ray spectroscopy, 3) x-ray crystallography,

4) small angle x-ray scattering and 5) x-ray microscopy. Roughly an equal number of experiments designed primarily for some other purpose will occasionally be used for biophysical research. To put these data in perspective, I must also point out that the NSLS represents about 50% of the presently planned synchrotron radiation capabilities of the United States, indeed of the western hemisphere. In Europe, more ports will be available at more research centers in a smaller geographical area. Even here, and certainly in the rest of the world, many scientists who use synchrotron radiation will have to travel some distance from their home institution. If we are to realize the fullest potential of synchrotron radiation, experimental apparatus and support facilities and personnel must be available at the synchrotron radiation facility. The need for support facilities and personnel is even more important for biological research than in the physical sciences. The inconvenience of performing one's experiments at a central facility is, however, a small price to pay for the advantages inherent in the use of synchrotron radiation.

SUMMARY

The advantages of synchrotron radiation in several types of spectroscopy, microscopy and diffraction studies are clear. The availability of synchrotron radiation will expand rapidly in the early 1980's as experimental programs start at the new generation of dedicated storage rings.

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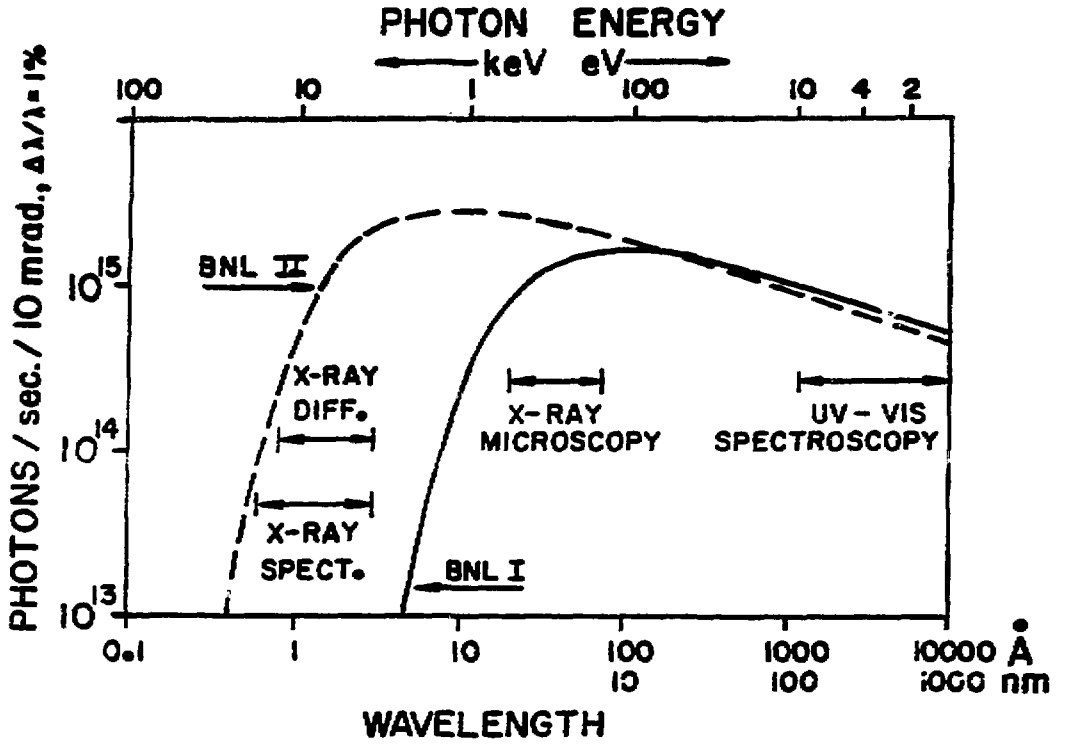

Fig. 1 The spectra anticipated to be emitted by the two synchrotron storage rings of the National Synchrotron Light Source. The smaller ring (BNL I) will be used for ultraviolet, visible and infrared spectroscopy while the larger ring (BNL II), which will circulate higher energy electrons, will mainly be used for x-ray research. The spectra were calculated assuming a horizontal acceptance of 10 mrad, although some experiments will use three to five times this amount.

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