Photochemistry of Proteins and Nucleic Acids

A D McLaren / D Shugar

PHOTOCHEMISTRY OF PROTEINS AND NUCLEIC ACIDS

BY

A. D. McLAREN

Professor, College of Agriculture,
University of California, Berkeley

AND

D. SHUGAR

Professor, Institute of Biochemistry and
Biophysics, and State Institute of Hygiene,
Academy of Sciences, Warsaw

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Preface

This book is offered as a contribution to the field of photobiology. The pronounced upsurge of interest in the biological effects of various types of radiation, which followed on the advent of nuclear fission, has undergone further stimulation in recent years. On the one hand the development of numerous new techniques, such as paper and column chromatography, has made it possible to isolate many of the small quantities of radiation products normally encountered in biological systems, while electron spin resonance, flash spectroscopy and nuclear magnetic resonance, amongst others, have furnished powerful tools for the investigation of excited states and photoproduct structures. On the other hand the phenomenal advances recorded in the field of molecular biology, in particular the remarkable progress achieved in the elucidation of the structure of proteins and nucleic acids and the role of the latter in genetics and protein biosynthesis, have made available a solid theoretical basis for an interpretation at the molecular level of the chemical modifications resulting from the action of radiation on these key cellular constituents.

At the time we initially considered the advisability of undertaking the preparation of a short monograph on the effects of ultraviolet and visible radiations on proteins and nucleic acids, we rather naively entertained the hope of being able to provide a reasonably complete coverage of the work done in this domain. We have been to some extent frustrated in the attainment of this goal, owing in large measure to the growing interest in the field and an increased rate of publication. In particular, phage photochemistry has now developed into a highly specialized field of its own, an adequate coverage of which would require a separate small volume. While these developments have made our task more difficult, they simultaneously testify to the need of a new review, insofar as proteins and nucleic acids are concerned, one which would at least partially fill the gap that has arisen since the publication during the last decade of Vols. II and III of Hollaender's comprehensive collective *Radiation Biology*.

An initial chapter, outlining briefly some of the principles of photochemistry, is followed by a survey of the absorption spectra of nucleoproteins and their constituents. Succeeding chapters deal with the physical and chemical effects of ultraviolet and visible radiation on proteins, nucleic acids, viruses and their primary building blocks. Some attention is devoted to such problems as energy transfer in macromolecules, photosensitized reactions, etc. An effort is made, where possible, to interpret these effects in terms of the theory, however spotty, of photochemical reactions.

Throughout, some of the implications of this study for the field of photobiology are incorporated.

We hope that the Appendix on some photochemical techniques will fulfil a practical need for many of those working in the photobiological field. If any apology is required for the section on Radiation Induced Syntheses in Chapter IX, it is only that it is not as comprehensive as might be desired; space research is now well out of the realm of fantasy and the role of ultraviolet and visible radiation both in space research and in the problem of the origin of life has attained such significance that an increasing number of research workers are now devoting their attention in whole or in part to this new domain.

It is a pleasure to acknowledge partial support to one of us (A. D. McL.) of some of the research reported in this book, by the U.S. Atomic Energy Commission.

Berkeley and Warsaw A. D. McLaren and D. Shugar

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CHAPTER I

Some Principles of Photochemistry

A. INTRODUCTION

1. *The Laws of Photochemistry*

Photochemistry as a branch of physical chemistry still suffers from a poverty of sweeping general rules which one can use as a guide for prediction. It is still largely an empirical branch. An absolute principle, sometimes called the first law, is that only light which is absorbed by a system can induce a chemical reaction; i.e. the mere passage of light through a system has no catalytic effect (Rollefson and Burton, 1942). It does not follow, however, that absorbed light will always induce a reaction, for the absorbed energy may be dissipated as heat via collisions of a second kind (whereby an excited energy-rich molecule collides with a normal molecule with an increase in translational energy of the molecules) or lost as fluorescence or both as consecutive steps. In considering the absorption of light by a reactant, the photochemist collides head-on with the eternal dilemma in the conceptual scheme of light (Raman, 1922). In keeping with N. Bohr's theory of complementariness in nature, the photochemist uses a *wavelength* λ in calculating relevant extinction coefficients and counts *quanta* in calculating the efficiency of a photochemical reaction!

The absorption of light by a molecule is a quantized event; i.e. as a molecule absorbs light it receives energy in the form of discrete units called photons or quanta. The energy q of a quantum is $h\nu$ ergs where h is Planck's constant (6.61×10^{-27} erg sec) and ν is the frequency of radiation ($\nu = c/\lambda = 3.0 \times 10^{10}$ cm per sec/λ). With wavelength λ expressed in angstrom units Å (one Å = 0.1 mμ),

$$1 \text{ quantum} = h\nu = 1.98 \times 10^{-8}/\lambda \text{ ergs}$$

Since one does not ordinarily observe changes in single molecules of reactants, but rather in tangible quantities, another useful unit is the einstein, i.e.

$$\begin{aligned} 1 \text{ einstein} &= Nq = 6.02 \times 10^{23} \times 1.98 \times 10^{-8}/\lambda \text{ ergs} \\ &= 11.9 \times 10^{8}/\lambda \text{ joules} \\ &= 2.85 \times 10^{8}/\lambda \text{ calories} \end{aligned}$$

In other words, if Avogadro's number *N* of molecules each absorb one quantum at 2537 Å, the energy absorbed would be 112,000 calories per mole. The einstein is formally analogous to the faraday. At 6000 Å (red light) an einstein is 47,400 calories and at 2000 Å (for ultraviolet light) it is 142,000 calories. These energies may be compared with thermochemical bond strengths of typical bonds found in proteins, Fig. 1-1. Bond strengths vary somewhat from one kind of molecule to another. For a photochemical reaction to occur, the quantum absorbed must be equal to or greater than the energy required to dissociate or sufficiently activate the molecule, depending on the nature of the effect considered.

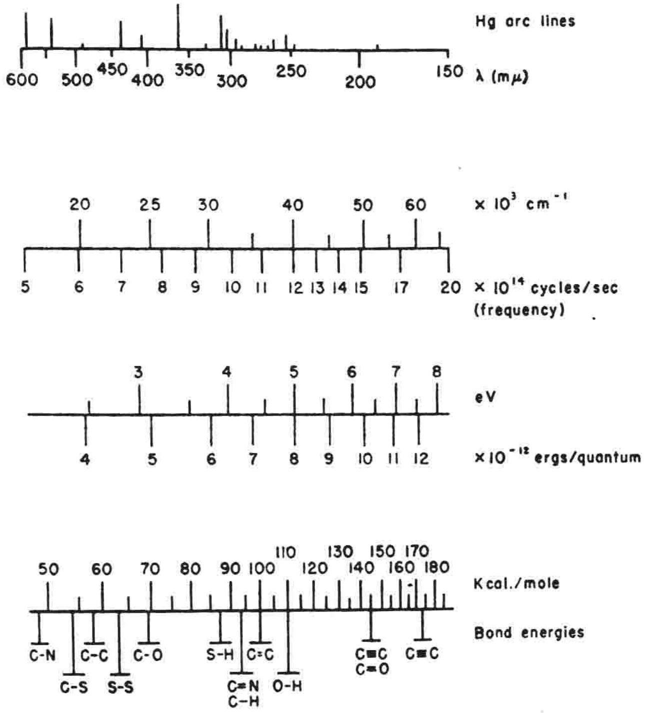


FIG. 1-1. Energy conversion chart (adapted from Bowen, 1946) and bond energies (from Pauling, 1948).

The Stark–Einstein law of photochemical equivalence, sometimes called the second law, states that in the primary process one quantum of light is absorbed per molecule of absorbing and reacting substance. By primary process is meant the immediate effect of the light on the absorbing molecule (Noyes and Leighton, 1941). It is possible for a single molecule to absorb more than one quantum in successive steps; however, the concentration of molecules which have absorbed one quantum is so low in a population that the absorption of further quanta by the excited molecule is highly improbable (the lifetime of excitation being short), and there is no positive evidence that such steps are ever of importance in photochemistry. An attempt to demonstrate this possibility with a very intense light source and a polychromophoric molecule (pepsin) failed (McLaren *et al.*, 1952).

A molecule which has absorbed a quantum is said to be in an excited state. Aside from the afore-mentioned fluorescence (or phosphorescence) or degradation of excitation energy to heat, the energy-rich molecule may dissociate to yield atoms or free radicals, or rearrange, eventually or immediately, into final products. These atoms, radicals, or isomers may be regarded as having been formed in the *primary process*. The time interval between activation and dissociation or rearrangement may be shorter than the time for an intramolecular vibration (*ca.* 10^{-13} sec) or as long as some minutes (as seems to be the case with some rearranged forms of irradiated chymotrypsin molecules). Via secondary reactions, excited molecules may lose energy by reactive collision with other molecules, thereby initiating chemical reactions. Such molecules are said to perform the role of photosensitizers. Thus excited acriflavine can inactivate tobacco mosaic virus and excited methylene blue can inactivate chymotrypsin.

A consideration of the photochemical equivalence law has led to a definition of great practical importance. The quantum yield is defined as

$$\Phi = \frac{\text{number of molecules reacting per unit volume}}{\text{number of quanta absorbed by the reactant per unit volume}} \qquad (1\text{-}1\text{a})$$

or

$$\Phi = \frac{\text{number of moles reacted per unit volume}}{\text{number of einsteins absorbed by the reactant per unit volume}} \qquad (1\text{-}1\text{b})$$

Sometimes the experimentally determined quantity is the number of moles of product formed, and one can also usefully define a yield Φ_p as

$$\Phi_p = \frac{\text{moles product formed}}{\text{einsteins absorbed}} \qquad (1\text{-}2)$$

Φ_p can be more or less than Φ, depending on whether more than one mole of product is formed per mole of reactant changed, whether polymerization is initiated by excited molecules, and depending on whether all products are identified and summed.

The observed rate of reaction may be expressed as ΦI_{abs}, in which Φ may be a constant or a function of concentration of reactants in a system and I_{abs} is the energy absorbed as einsteins per unit volume and time, i.e. the intensity.

2. *Absorption Spectroscopy*

Light of intensity I_0 impinging on a solution with a path-length l is partly absorbed (I_{abs}) and partly transmitted with an intensity of I. For any given substance, pure or in an optically transparent solvent with a molar concentration C, these quantities are related by the Beer–Lambert equation, $I = I_0 10^{-\varepsilon Cl}$. The proportionality constant, ε, is called the molecular extinction coefficient. The variation in the extinction coefficient with wavelength is called an absorption spectrum. A complete theoretical understanding of absorption spectra is available for only the most simple molecules; we shall summarize some of the aspects which apply to these molecules and point out a few of the complications which render difficult their detailed application to complex molecules.

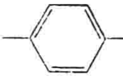
Just as with atoms, the absorption of energy $q = \Delta E$ by a molecule leads to an increase in energy $\Delta E = h\nu = E_1 - E_0$, where E_0 is the energy of the entity before, and E_1 that following absorption of a light quantum, viz.

$$\mathrm{A} + h\nu \underset{k''}{\overset{k'}{\rightleftharpoons}} \mathrm{A}^*$$

where A* is an "electronic isomer" (Bowen, 1947) of the normal molecule, i.e. the energy-rich, excited state of a molecule A, and k' is the specific reaction rate of the forward reaction—the fraction converted per quantum absorbed—(Rollefson and Burton, 1942). The extinction coefficient is dependent on λ because the excitations possible are peculiar to the kinds of chemical linkages found in a molecule, such as individual bonds:

e.g. —CONH—, —S—S— etc.

or groups of bonds,

e.g. —OH, —C=C—C=O, etc.

and are related by the Bohr–Planck relationship† $\Delta E = hc/\lambda$. ΔE must not only be sufficient for the transition but the latter must also be permissible.

An upper value for ε may be obtained by the following consideration (Braude, 1945). Consider an intensity I falling on a slice of thickness dl of a cell of unit area filled with a dilute absorbing solute in a transparent solvent, at a concentration C moles/l. Let the average effective absorbing area ("chromophore area") of an absorbing molecule in the plane perpendicular to the incident light be σ. Then the illumination absorbed is

$$-\mathrm{d}I = \frac{\text{area of absorbing molecules}}{\text{total area}} \times I \times F = \frac{FCN\,\mathrm{d}l\,I\sigma}{1000}$$

---

† A diagram showing the approximate position of the absorption bands of some commonly occurring chromophores has been published by Scott (1955).

where F represents the fraction of the light falling on the absorbing molecules which is absorbed. Upon integration

$$\varepsilon = \frac{1}{Cl}\log\frac{I_0}{I} = \frac{FN\sigma}{2300}$$

If $F = 1$ and $\sigma = [\sigma]$, the cross section of an entire molecule, taken as 10 $Å^2$ for illustration, $\varepsilon \cong 10^5$ as a maximum value for a simple molecule. Clearly ε is proportional to the absorption probability k' (Rollefson and Burton, 1942). ε is generally less than 10^5 because $\sigma < [\sigma]$, because orientation of a molecule with respect to the beam can influence absorption, and because $F < 1$ since the transition between energy states E_1 and E_0 does not always occur when a quantum, conceivably of the right magnitude, falls on the molecule in the optimum orientation.

Transitions are governed by certain selection rules and the Franck–Condon principle (Livingston, 1955). These principles are best discussed by reference to Fig. 1-2, a potential energy diagram for a diatomic molecule.

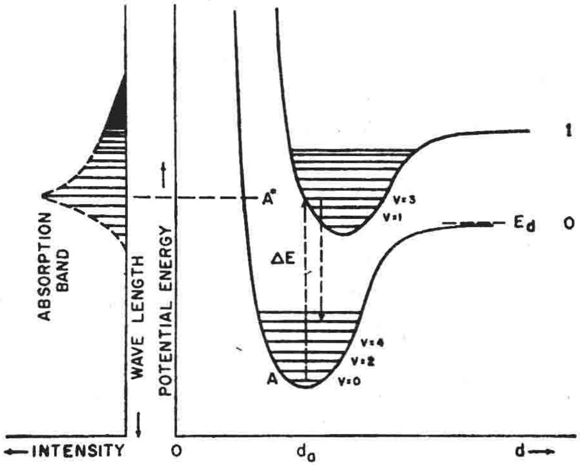


FIG. 1-2. Potential energy diagram for a diatomic molecule. One atom is considered fixed at the vertical axis and vibration due to thermal energy can be represented by movement of the other atom through a distance d along the horizontal axis. E_d is the energy of dissociation for the ground state, 0. Note the similarity of these curves to those describing linear harmonic oscillators. The curves for molecules are quantized and the lowest vibrational energy level extant is $\frac{1}{2}\nu_0 = E_v$. Only the first few vibrational energy levels are identified by quantum numbers and rotational energy levels are not shown (after Bowen, 1947).

Curve 0 represents the parabolic potential energy curve which describes the energy of attraction as a function of distance d between two neutral atoms which have been brought together to form a molecule. The equilibrium distance d_a is the distance at which the magnetic attraction of a single valence pair of unlike spinning electrons just balances the repulsion of the two atomic nuclei. Curve 1, the upper curve, is a similar vibration curve for the molecule A*, one of whose valency electrons has "jumped" by light absorption to a higher level, causing the molecular link to lengthen. The horizontal lines inside each parabola represent the possible discrete, vibrational energy levels of both the excited state and the unexcited or ground state.

Whereas electron jumps in atoms correspond to light absorption only at certain narrow lines in the spectrum, such as the familiar 2537 Å line of mercury, in molecules broad regions of line absorption, or bands, are observed because simultaneous changes in both electronic and vibrational, and usually rotational energies take place. For each line of a band the Bohr frequency condition obtains

$$\nu_{v,r,e} = \frac{\Delta E}{h} = (\Delta E_{\text{electronic}} + \Delta E_{\text{vibrational}} + \Delta E_{\text{rotational}})/h$$

Let us consider each of these changes of energy. Changes in rotational energy alone are small in magnitude and give rise to lines in the long wavelength region of a spectrum. For molecules with a dipole moment

$$\nu_{J+1,J} = \frac{2(J+1)h}{8\pi^2 i}$$

where i is the moment of inertia and $J = 0, 1, 2, 3, \ldots$ For absorption, ν corresponds to a change from J to $J + 1$ or to $J - 1$, (a selection rule), and the lines compose a set of equal spacing. These lines are of no importance photochemically. The next simplest spectra are those corresponding to a simultaneous change of vibrational and rotational energies. Each horizontal line of the curves of Fig. 1-2 represents to a first approximation the energy of a harmonic oscillator

$$E_v = (v + \tfrac{1}{2})h\nu_0$$

where ν_0 is the fundamental frequency of the lowest energy state and v is the vibrational quantum number. In a strictly harmonic oscillator Δv is limited to one (a selection rule) and

$$\nu_{v,r} = (v'' - v')\nu_0 \pm \frac{h}{4\pi^2 i}(J' + 1)$$

The double primes refer to the higher energy states, the single primes to the lower. Since the energy contribution of the first term is much greater than the second, it determines where on the spectrum ν will lie. The second term, allowing for changes in rotational energies along with a change in vibrational energy, gives the fine structure about $(v'' - v')\nu_0$, thereby forming a band in the near infrared.

In visible and ultraviolet portions of the spectrum, in the region of interest to the photochemist,

$$\nu_{e,v,r} = \nu_e + \nu_v + \nu_r$$

and the corresponding energy change is indicated in Fig. 1-2 by the vertical line connecting the two oscillator curves. Since ν_0 is characteristic of a particular electronic state, a change to a new state involves a new set of vibrational states; there is no selection rule which limits the change in vibrational quantum number in transitions of this type. There are, however, other selection rules which apply, based on the following spectral designations.

Spectral designations for molecular states. It is convenient for many purposes to have a method of defining the electronic state of a molecule and one similar to that describing atoms is employed. It will be recalled that the motion of extranuclear electrons in Bohr orbits may be described in terms of four quantum numbers. For a single electron, one particular orbit corresponds to each set of numbers. In terms of wave mechanics, to each set of quantum numbers there corresponds a particular probability of finding the electron at any given point in space (and in Fig. 1-2, the potential energy curves would be represented by broad smears of probable values of energy for a given separation of nuclei). The quantum number $\underline{n}$ represents the *size* of the orbit, the number $\underline{l}$ represents the angular momentum or *shape* of the orbit, and the number $\underline{s}$ stands for the *spin* of the electron. Associated with the orbital motion of the electron there is a magnetic moment designated by a number $\underline{m}$. According to Pauli, for a given atom no two electrons can have identical sets of the four numbers. Heitler and London found with hydrogen that when the electrons are in their lowest states, two hydrogen atoms will attract each other as pictured in Fig. 1-2 provided the spins of the electrons are opposite. In other words a stable molecule will form if the two electrons are paired off. Such a pair is equivalent to a valence of one.

The resultant azimuthal quantum number for the molecule, which is analogous to $\underline{l}$ for an atom, is represented by $\underline{\lambda}$, and the symbols Σ, π, and $\underline{\Delta}$ are used as term symbols for $\underline{\lambda} = 0, 1, 2$ respectively. $\underline{\lambda}$ specifies the component of the total resultant angular momentum of the electrons in the direction of the axis of the molecule. A selection rule states that $\underline{\lambda}$ may change by $0, \pm 1$, i.e. transitions between two Σ or two π or between a Σ and a π or between a π and a $\underline{\Delta}$ term are permissible. By further analogy to atomic spectra the multiplicity of the Σ, π, $\underline{\Delta}$ energy terms are designated by superscripts, such as $^3\Sigma$. The multiplicity is equal to twice the vector sum of the electron spins plus one, where each electron is spinning with an angular momentum represented by $\frac{1}{2}$. The multiplicity is therefore odd for an even