

Cambridge University Press

978-0-521-10531-6 - The Biochemistry of Natural Pigments

G. Britton

Excerpt

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SECTION I
CHEMICAL AND BIOCHEMICAL ASPECTS

1 Light and colour

1.1 Introduction

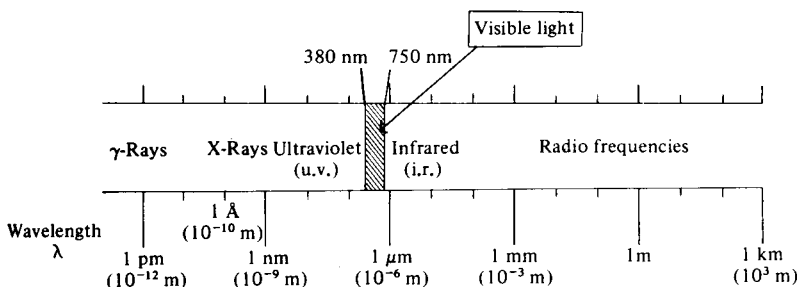
1.1.1 Solar electromagnetic radiation

All living processes on Earth ultimately depend upon that portion of the vast resources of the Sun's energy which eventually reaches the surface of our planet. The Sun emits a wide range of electromagnetic radiations, from long-wavelength infrared (i.r.) and radio frequencies to very short-wavelength ultraviolet (u.v.) and γ -rays (fig. 1.1). However, the Earth's atmosphere as it is today effectively and efficiently filters out much of this radiation, particularly the high-energy u.v., X-rays, and γ -rays that can have a disastrous effect on living tissues.

1.1.2 Visible light

Amongst the radiations that do reach the surface of the Earth, those with wavelengths between approximately 380 and 750 nanometers (nm, $\equiv 10^{-9}$ m) penetrate the atmosphere most readily, *i.e.* suffer least restriction on their passage. This wavelength range, 380–750 nm, is of fundamental importance in maintaining life. It is also the range which we recognise as 'visible light'. Animals, including ourselves, have developed very sophisticated photoreceptor systems for the detection of this light and also for accurate

Fig. 1.1. The electromagnetic spectrum.



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discrimination of different wavelengths within this region in the processes of colour vision. Colour, and the property of being coloured, thus become very important in the living world.

It is the same range of light energy which is harnessed by plants and microbes in the process of photosynthesis by which atmospheric carbon dioxide is fixed into a chemical form that is not only used by the plant but also provides the primary food source for the rest of the natural world. Variations in the amount of available visible light, for example variations in length of day and night, are also monitored by various photoreceptors. This provides the basis of extremely important mechanisms for regulating growth and development.

All these properties and processes – being coloured, detecting light and colour, photosynthesis, photoregulation – require mechanisms for detecting or absorbing light from the visible range. Molecules which have the special property of absorbing light of wavelengths in the 380–750 nm range are therefore of fundamental importance. Such compounds are the **natural pigments** or **biochromes**. It is the purpose of this book to review the main features of the chemistry and biochemistry of groups of natural pigments, and to describe, as far as possible, how these pigments function at the molecular level.

1.2 Colour and colour perception

1.2.1 *Colour*

Simultaneous perception of radiations over the entire range, 380–750 nm, produces (in man) the sensation that we recognise as white light. Other animals are able to perceive radiations of wavelengths outside this range, *e.g.* bees can ‘see’ u.v. wavelengths invisible to us.

The sensation of colour is given if radiations are received from only part of the visible range. ‘White light’ is a continuum of electromagnetic radiations covering the wavelength range 380–750 nm. When this continuum is separated by passage (refraction) through a prism, then a series of beams is obtained, each consisting of a much narrower range of wavelengths. We see these beams as a series of colours, the familiar red, orange, yellow, green, blue (indigo), violet of the rainbow, which is produced by prismatic effects of water droplets on sunlight. The sensation of each individual colour is associated with the wavelength on which a beam is centred, *e.g.* the sensation of yellow is produced by light of wavelength around 580 nm. The sensations that individuals with ‘normal’ colour vision identify with particular wavelengths are shown in fig. 1.2. It is also possible to achieve the sensation of a particular colour by mixing light of wavelengths associated with other colours, *e.g.* yellow can be produced by the addition of red and green light.

Alternatively the sensation of colour may be produced by subtraction of what can be a fairly narrow band (20–30 nm wavelength range) from the

white-light continuum. In this case what is 'seen' is the colour complementary to that of the missing waveband. Thus, if white light is passed through a filter or substance which absorbs blue light, i.e. 480 ± 30 nm, the emergent beam is seen as the colour complementary to blue, i.e. yellow. The complementary or subtraction colours observed when light of a particular colour or wavelength range is subtracted from the white-light continuum are also shown in fig. 1.2.

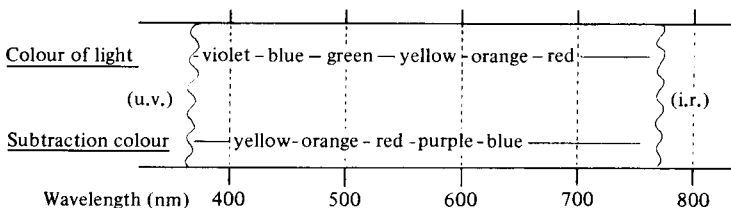
1.2.2 Light perception and colour discrimination

The above brief introduction to light and colour has frequently alluded to our ability to 'see colours'. Although the identification and description of colours is to a considerable degree subjective, there must be an underlying fundamental physiological mechanism which is not only capable of detecting electromagnetic radiations in the wavelength range 380–750 nm, but is also able to produce different sensations in response to radiations of different wavelengths within this range. Plants and microbes do not have vision as we know it, although they are able to use the energy of light of specific wavelengths in, for example, photosynthesis (chapter 10), and they may have the ability to move or grow towards or away from a light source (chapter 11). It is only in the animal kingdom that the mechanisms of light detection and colour discrimination have developed into the accurate and sensitive powers of vision that we know and enjoy.

1.2.3 The eye and colour vision

The processes of light detection and colour recognition by animals take place in the eye. In man and many other animals there are specific rod and cone cells in the retina of the eye; these cells contain the photoreceptors, or visual pigments. Mammalian retinal rod cells are responsible for the detection of low-intensity light. They contain pigments, **scotopsins**, which are sensitive to very low levels of light. The sensitivity maximum of the human scotopsin, **rhodopsin**, is at about 520 nm, although light of quite a wide

Fig. 1.2. The visible spectrum, showing the colours which individuals with 'normal' colour vision identify with particular wavelengths and also the complementary or subtraction colours observed when light of a particular colour or wavelength range is subtracted from the white light continuum.



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range of wavelengths around this value can be detected. In the visual process, light of the appropriate wavelength is absorbed by the visual pigment. This leads in turn to the generation of an electrical stimulus and a neural impulse. The same response is produced by light of all wavelengths that can be absorbed by the scotopsin, *i.e.* there is no differential response to light of different wavelengths.

The retinal cone cells, on the other hand, contain a set of visual pigments, **photopsins**, that are sensitive to different wavelength ranges. In man there are three such pigments sensitive to blue, green and red light, respectively. These three photoreceptors cover almost the entire range of the visible spectrum and provide a colour-discrimination mechanism sensitive enough to distinguish very subtle variations in colour, shade or hue. This trichromatic system and the pigments involved are described in more detail in chapter 9. Modern colour television also employs a trichromatic system to produce any colour, shade or hue by mixing red, green and blue light.

1.3 **Colour in living organisms**

When most living organisms or tissues receive white light, *e.g.* from the sun, they pass on to the eye of the observer light of only part of the visible range. In other words they appear to possess colour. This may be **structural colour**, produced as a consequence of the physical nature of the surface of the tissue. Alternatively the colour may be due to the presence of chemical compounds (**pigments** or **biochromes**) which absorb specifically some of the wavelengths of visible light.

1.3.1 *Structural colours*

In the animal kingdom there are many examples in which the observed colour is the result of optical phenomena such as light scattering, interference or diffraction by microscopic structures present in the tissues. Colours produced in this way are known as structural colours. The subject of structural colours is a large and important one, but detailed descriptions of the characteristics of structural colours and of the optical phenomena that produce them are not really within the scope of this book. Only a very brief account will be given.

1.3.2 *Light scattering - Tyndall blue*

Very small particles, smaller in diameter than the wavelength of red or yellow light, will reflect or scatter more of the short-wave than of the long-wave components of white light. The most familiar example of this effect is the blue of the sky. Minute particles of dust, *etc.*, in the atmosphere scatter incident white light so that the light reflected to the surface of the Earth contains a greater proportion of short-wave (blue and violet) than of longer-wave (red-yellow) light, and is thus seen as the familiar sky-blue

colour. This process is often called Rayleigh or Tyndall scattering, and the colour produced is known as Tyndall blue.

Most non-iridescent blue colours in animals are Tyndall blues. Thus the blue colour of human eyes is due to the scattering of white light by minute protein particles in the iris. In the blue feathers of many birds, *e.g.* blue tit, budgerigar, parrot, light-scattering particles in the form of minute air-filled lamellae are present within the keratin of the feather barbs.

Tyndall blues are identified as structural colours by virtue of the fact that no blue pigment can be isolated from the tissues and because the blue colour is not evident when the tissues are viewed by transmitted white light. They are characterised by a matt, non-iridescent appearance, and by exhibiting the same colour when viewed from almost all angles.

Green colour, especially in feathers, is often due to superimposition of structural blue and a yellow pigment.

1.3.3 *Iridescent colours*

Among the most striking visual effects produced in Nature are the glittering, iridescent structural colours frequently encountered in the animal kingdom, particularly in birds, insects and fishes. It is a characteristic of iridescent colours that the observed hues change according to the angle of viewing. Two optical phenomena are involved, interference and diffraction.

Interference. The property of interference is perhaps known best from the example of a thin film of oil on the surface of water. Light reflected from the lower surface (oil-water interface) of the film travels a small but finite distance farther than light reflected from the upper, oil-air, surface. When the difference in distance travelled is equivalent to half the wavelength of the light, then the two light rays reflected from the upper and lower surfaces will effectively be 'out of phase' and will cancel each other out. Thus light of this particular wavelength will not be present in the reflected light observed. The reflected beam will therefore appear coloured. With a more acute viewing angle the travelling distance between the upper and lower surfaces is greater. The interference will therefore occur in a different part of the spectrum (longer wavelength) and hence a different colour will be seen.

There are many examples of interference colours in animals. The transparent wing structure of many insects serves as a thin film producing a range of interference colours when seen from different angles. Many butterflies have in the surface of their wing scales laminae with minute air spaces between them. The intralamellar distance is approximately constant so that an almost constant colour may be given over a reasonably wide range of viewing angles.

Interference colours are commonly found in birds, *e.g.* the peacock. The flattened feather barbules that contain the laminar structures which constitute

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the interference film are twisted so that their flat surface faces the viewer. In many cases the brilliance of the iridescent colours is enhanced by the presence of an underlying black surface (melanin) which absorbs all other light. A metallic lustre often results.

Diffraction. Rather similar iridescent colour effects may be produced by diffraction, although this is much less common than interference in natural tissues. The artificial diffraction grating used in certain optical instruments consists of a series of very close, equidistant, parallel lines scratched on to a polished surface. There are some examples of iridescent colours being produced by natural lamellar structures that behave as diffraction gratings, e.g. mother of pearl.

1.3.4 *Structural white*

The concept of structural white may be illustrated by the example of snow, which owes its brilliant white appearance to the reflection of incident white light from countless small crystal surfaces. Similar effects are given by reflection of white light by other solid or liquid particles or surfaces maintained in media of different refractive index. The particles must not be so small as to effect differential scattering of different wavelengths, *i.e.* Tyndall scattering. Some examples of structural whites in Nature are white hair (reflection from air bubbles trapped in a translucent solid), white feathers (reflection from numerous small colourless barbules), milk (reflection from droplets in an emulsion of two liquids of different refractive indices), white moths and butterflies (reflection from ribbed and reticulated scale surfaces) and white and silver fish (reflection from guanine crystals).

1.3.5 *Chemical colour - natural pigments*

Most natural colour results from the preferential absorption of some of the wavelengths of visible light by chemical substances present in the tissue. The chemical compounds responsible for this light absorption are the **natural pigments**. It is with the biochemistry of these natural pigments or **biochromes** that this book is concerned. The natural pigments are, almost without exception, organic molecules and differ greatly from the industrial pigments widely used in paints and dyes. Before details of the various groups of natural pigments are presented, however, it is necessary to consider in general terms those molecular properties which are responsible for the absorption of visible light.

1.4 **Light absorption**

1.4.1 *Energy and wavelength*

When an atom or molecule absorbs a quantum of electromagnetic radiation energy, the absorption results in a transition from one energy state

to another. Any particle may occupy only certain discrete energy levels, and absorption is possible only when the radiation energy E is equal to the energy difference between two of these energy states. However, according to 'selection rules' not all these transitions are 'allowed'; some are 'forbidden'. The absorption depends strictly on the energy of the quanta, and hence on the wavelength of the radiation, since

$$E = h\nu$$

where E is the energy of the quantum, h is Planck's constant and ν is the frequency of the radiation, which is related to the wavelength, λ , by the expression

$$\nu = c/\lambda$$

where c is the velocity of light. There is thus an inverse relationship between energy and wavelength, in other words the greater the quantum energy difference between the two energy states, then the shorter will be the wavelength of the radiation required to bring about the transition. Light in the u.v. and visible regions of the spectrum is of sufficiently high energy to bring about electronic transitions, *i.e.* to promote an electron from a lower to a higher energy state. In addition, each electronic energy state of even the simplest diatomic molecule may occupy various vibrational and rotational energy levels. The vibrational and rotational energy differences are very much smaller and correspond to i.r. radiation quanta.

1.4.2 *Radiation absorption by atoms*

Absorption of radiation by an atom is relatively simple, and causes an electron to be raised from its normal lowest energy level (ground state) to a higher-energy excited state by a quantum whose energy is exactly equal to the energy difference between the two electronic energy levels. Because of the relationship between energy, frequency and wavelength, it follows that for a simple electronic transition such as this the radiation or light absorbed can only be of a single wavelength, and a single absorption line will be observed in the absorption spectrum.

1.4.3 *Radiation absorption by molecules*

In the case of molecules things are not so simple. Even diatomic molecules are quite large compared with atoms, and can no longer be considered as rigid particles. Molecular rotation and vibration of the nuclei occur, and the rotational and vibrational energy levels are again quantified (fig. 1.3). Thus for any electron in a molecule the electronic ground state may exist in several vibrational energy levels; for each of these in turn several rotational energy levels are possible. The same is true of the electronically excited state. Although the vibrational and rotational energy differences are small compared

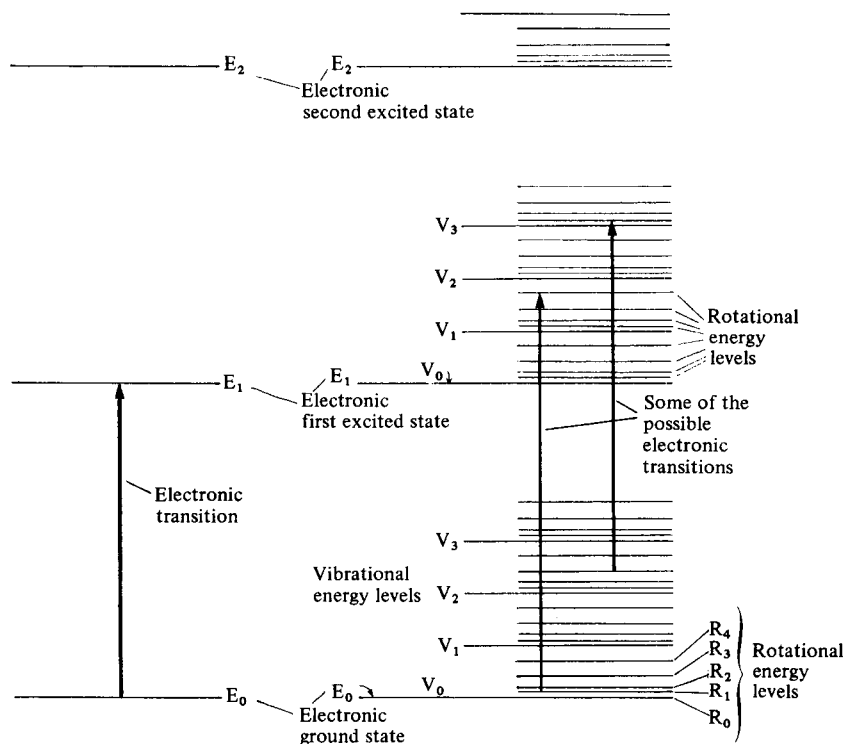
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to differences in electronic energy levels they must be taken into account in a discussion of electronic transitions. An electronic transition therefore does not have a single, sharply defined, energy requirement, but may be brought about by a range of energy quanta corresponding to the energy differences between the electronic ground state and excited state in the various vibrational and rotational energy levels. Electronic excitation may also be accompanied by vibrational and rotational excitation. The overall effect is for electronic absorption to occur over a range of radiation energies or wavelengths, so that for molecules the spectroscopic absorption lines are broadened into absorption bands, centred on the wavelength of maximal absorption (λ_{max}), and usually with a band-width of 50–100 nm. Normally it is not possible to resolve the absorption bands well enough to see the vibrational and rotational fine structure.

Fig. 1.3. Diagram illustrating electronic (and vibrational and rotational) energy levels and some possible electronic transitions for (a) an atom and (b) a diatomic or polyatomic molecule.

(a) An atom

(b) A di- or polyatomic molecule



The excitation of an electron to a higher energy level takes place extremely rapidly (*ca* 10^{-15} s), and the much heavier atomic nuclei cannot move appreciably in this time. Electronic transitions therefore take place with essentially no change in the nuclear positions or internuclear distances (Franck-Condon principle).

Molecular vibrations are of a more or less harmonic form, so the vibrating nuclei will spend the greatest time at the extreme positions where the motion is slowest. Electronic transitions are therefore most likely to occur when internuclear distances are at their extreme maxima and minima, so quanta of the corresponding energy are those most likely to be absorbed, and light absorption at the corresponding wavelength will be the most intense.

Overall, the *position* of an absorption band λ_{\max} is determined by the energy required to bring about the most probable electronic transition, and the *intensity* of the absorption reflects the probability of that transition taking place.

1.4.4 Absorption properties of some simple molecules

Any electron in a simple molecule is capable of excitation. The energy required will depend primarily on the type of orbital occupied (fig. 1.4). Thus excitation of an electron in a σ -orbital requires the highest energy. Saturated hydrocarbons are therefore the most difficult simple organic molecules to excite electronically. The only accessible electrons are those in

Fig. 1.4. The five electronic energy levels normally encountered with organic molecules, and the most common electronic transitions that can be brought about by absorption of u.v. and visible light.

