Electronic Absorption Spectroscopy

Classical View

According to the classical theory discussed previously, the dynamics of an electron in an atom are governed by its natural frequency $\omega_0 = \sqrt{(k/m_e)}$ and by dissipative processes—radiation and "viscous" damping. Monochromatic electromagnetic radiation at frequency ω will drive the electron in oscillatory motion, but the amplitude of the motion remains small until $\omega \rightarrow \omega_0$, a resonance condition. The extent to which the atom is polarized by an electric field, **E**, is determined by the polarizability, α, where

$$\boldsymbol{\alpha} = \mathbf{m/E}$$

(Note that $\boldsymbol{\alpha}$ is a tensor while **m,** the dipole moment, and **E**, the electric field, are vectors.)

As seen in the previous discussion, $\boldsymbol{\alpha}$ is small when $\omega >> \omega_0$ and $\omega << \omega_0$. The in-phase component is related to the index of refraction and therefore governs the scattering of light. The out-of-phase component of $\boldsymbol{\alpha}$ governs the absorption of light i.e., the processes by which, excluding fluorescence, etc., light energy is dissipated into heat.

According to this picture the electron can have a continuous range of energies. Thus the displacement of the electron in the harmonic (spring) potential is unrestricted. Hence the energy emitted by an electron relaxing to its resting state depends on the maximum amplitude of the displacement in the harmonic potential (x_0).

Quantum View

Quantum mechanics, which provides a more correct analysis o electronic motion and ato9mic structure, shows that these conclusions are incorrect. Accor5ding to quantum mechanics the electron in the atom must be in one of a set of defined *states*, each with a definite energy. Hence, an electron in state i has an energy E_i. When the electron passes from state i to state j, it emits (or absorbs) energy $E_j - E_i$, corresponding to emission or absorption of light with frequency:

$$\nu_{ij} = (E_j - E_i)/h$$

where h is Planck's constant. What causes the electron to undergo a transition from one state to another?

First, we must remember that the position of the electron in the atom is characterized by a probability density function, $P(r) = |\psi(r)|^2$, where $\psi(r)$ is a probability amplitude function or wave function obtained by solving the Schroedinger equation appropriate for the dynamic system under study. Solution of this equation also provides the E_i.

Quantum mechanics tells us how an electron can be caused to go from one state to another by perturbing its energy. In particular, for an atom exposed to light, it is the interaction of the atomic dipole moment $\boldsymbol{\mu}$ with the electric field vector of the light, **E**, provides the perturbation energy V(t), i.e.,

$$V(t) = \boldsymbol{\mu} \bullet \mathbf{E} \exp(i\omega t)$$

Then it can be shown that the rate at which an electron passes from state b to state a is

$$dP_b/dt = B_{ab}\, I(\nu)$$

where $B_{ab} = (2/3)(h^2/4\pi^3)^{-1} |\langle b|\boldsymbol{\mu}|a\rangle|^2$ and $\langle b|\boldsymbol{\mu}|a\rangle| = \int d^3r\, \psi_b(r)\, \boldsymbol{\mu}(r)\, \psi_a(r)$ and I(ν) is the incident intensity at frequency ν.

The transition dipole moment, $\langle b|\boldsymbol{\mu}|a\rangle$, measures the extent to which the electron in state a is polarized by the incident light so that its spatial distribution is similar to that of state b. More generally,

$$-dI(\nu)/dt = h\nu(N_aB_{ab} - N_bB_{ba})I(\nu)$$

Under ordinary conditions N_b is small, and so N_bB_{ba} can be neglected.

Incident light will promote a transition from a → b, and thereby absorption from the incident radiation field if the frequency of the light, $\nu = (E_e - E_a)/h$ and if $\langle b|\boldsymbol{\mu}|a\rangle \neq 0$. Hence, measuring the absorption of light while varying the orientation of its electric vector relative to the molecules axes provides information about the orientation of the transition dipole moments within the molecule.

The Molar Extinction Coefficient.

According to the Beer-Lambert law:

$$dI = -2.303\, \varepsilon\, C\, I\, dl$$

where ε = molar extinction coefficient
C = molar concentration of the absorbing species
I = incident intensity
l = pathlength through the solution.

Also, for a 1M solution the rate of energy absorption per cm^3 of solution is

$$-dI(\nu)/dt = [h\nu N_0B_{ab}/1000]I(\nu)$$

But $dI(\nu)/dt = [dI(\nu)/dl] \bullet dl/dt = cdI(\nu)/dl$.

Therefore, $-dI(\nu) = [h\nu N_0 B_{ab}/1000c]\, I(\nu)dl$, and so $B_{ab} = 2303\varepsilon c/(N_0 h\nu)$, or more generally,

$$B_{ab} = (2303c/N_0h) \int (\varepsilon(\nu)/\nu)\, d\nu$$

and so,

$$|\langle b|\boldsymbol{\mu}|a\rangle|^2 = 9.2x10^{-3} \int [(\varepsilon(\nu)/\nu] d\nu$$

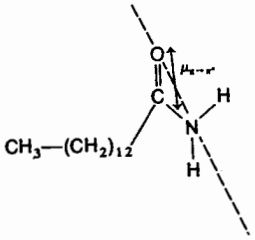
$|\langle b|\boldsymbol{\mu}|a\rangle|^2$ is called the "dipole strength".

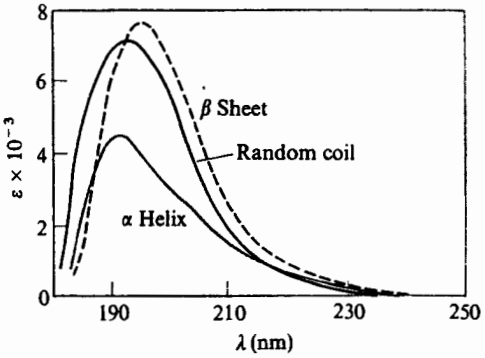
Linear Dichroism

The dichroic ratio is $(A_{\parallel} - A_{\perp})/(A_{\parallel} + A_{\perp})$. This parameter provides information about the orientation of the transition dipole moment(s) relative to the molecular axes.

Absorbance Properties of Proteins.

Peptide chromophore





π electrons are somewhat delocalized over the N, C, and O atoms. An electron in a nonbonding, n, orbital is concentrated near the O atom. The Lowest energy electronic transition from the peptide bond is an $n \rightarrow \pi^*$ transition in the range 210 to 220 nm and is very weak (because it is symmetry forbidden), $\varepsilon = 100\ (M\ cm)^{-1}$. The $\pi \rightarrow \pi^*$ at ≈190 nm is much more intense, $\varepsilon \approx 7000\ (M\ cm)^{-1}$ and is not polarized along any specific bond.

Aromatic Chromophores

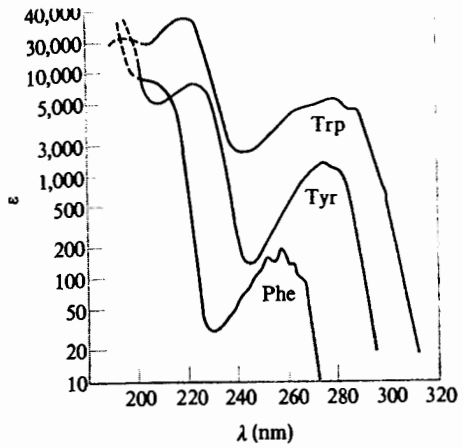


Figure 7-10

Absorption spectra of the three aromatic amino acids. A log scale has been used in order to display all three conveniently on one graph. [After D. B. Wetlaufer, *Adv. Protein Chem.* 17:303 (1962).]

Absorbance Properties of Nucleic Acids

The nucleotide bases dominate the near UV absorption in nucleic acids. There are many $\pi \rightarrow \pi^*$ nd $n \rightarrow \pi^*$ transitions between 200 and 300 nm. The transition dipoles are in the planes of the aromatic rings. On average $\varepsilon_{260} \approx 10{,}000$ $(M\ cm)^{-1}$.

Effects of Conformation

Hypochromism

Linear Dichroism of DNA

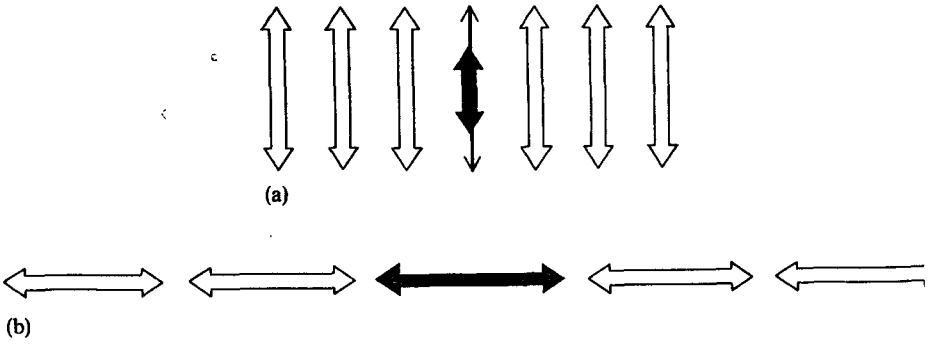


Figure 7-21

Schematic diagram showing origin of hypochromism and hyperchromism. **(a)** This alignment of induce dipoles (*unshaded*) and transition dipole (*black*) produces hypochromism (*shaded*). **(b)** This alignmer of induced dipoles and transition dipole produces hyperchromism.

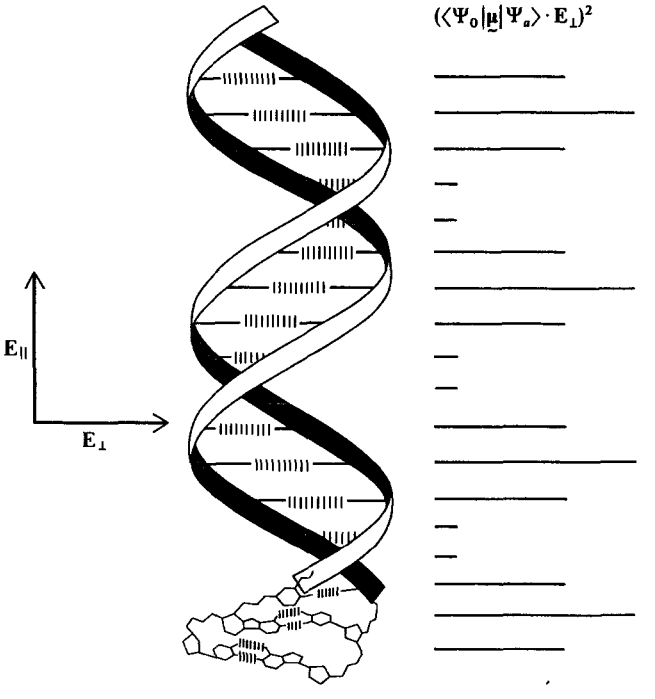


Figure 7-22

Linear dichroism expected for the B-form DNA double helix, when aligned as shown relative to polarized incident light. Because $\langle \Psi_0 | \underset{\sim}{\mu} | \Psi_a \rangle$ is in the plane of the base pairs, it is always perpendicular to $\mathbf{E}_\parallel$. The intensity of absorption will be periodic along the helix because the angle between $\langle \Psi_0 | \underset{\sim}{\mu} | \Psi_a \rangle$ and $\mathbf{E}$ varies with each 36° rotation of successive base pairs. [DNA structure after A. Kornberg, *DNA Synthesis* (San Francisco: W. H. Freeman and Company, 1974).]

Vibrational Spectroscopies

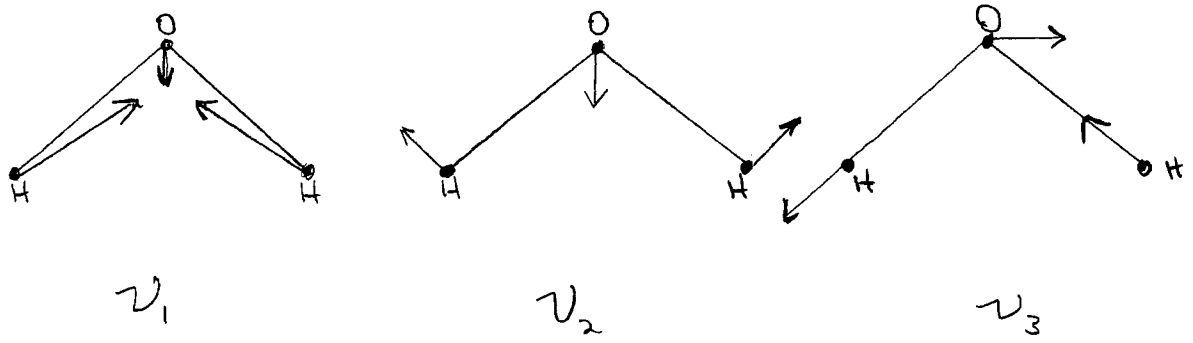
Molecular Vibrations

Infrared (IR) and Raman spectroscopies measure changes in the vibrational energy levels of nuclei in molecules. We begin by reviewing the classical simple harmonic oscillator. A particle of mass m is connected by a spring of stiffness constant k to an immovable site. The spring is at equilibrium (no net force) at length x_0. When the spring is stretched or compressed, it exerts a force to restore its length to x_0 : $F = -k(x-x_0)$. For simplicity let x_0 be the origin. Then $F = -kx$. The potential energy stored in the spring is $U = kx^2/2$. The classical equation of motion is $m\, d^2x/dt^2 = -kx$, which yields solutions of the form: $X = A \sin\omega t$, where A is an amplitude of oscillation and $\omega = \sqrt{(k/m)}$.

The quantum mechanical analysis of the simple harmonic oscillator is discussed at length in elementary textbooks. As expected, the system is quantized. The energies of the system are characterized by $E_n = (n + \frac{1}{2})h\nu_0$ where n is a positive integer and $\nu_0 = [\sqrt{(k/m)}]/2\pi$. Note that the quantum mechanical and classical mechanical analyses give the same fundamental frequency. Whereas a classical oscillator can have any energy, the quantum oscillator is constrained to discrete energies. Even when $n = 0$, $E_0 = h\nu_0$, the "zero point" energy.

As in electronic spectroscopy, light is absorbed due to molecular vibration when $\nu = (E_2 - E_1)/h$, where E_1 and E_2 are vibrational energy levels. A selection rule specifies that $\Delta n = \pm 1$. Hence, $\Delta E = h\nu_0$.

The analysis of the vibrations of polyatomic molecules can be quite complex. Equations of the form $m_i\, d^2x_i/dt^2 + \sum_i f_{ij}\, x_j = 0$ must be solved. This is a system of simultaneous liner second order differential equations. There are 3N-6 equations where N = the number of nuclei (for nonlinear molecules). Due to coupling of each nucleus, in principle, to all the other nuclei, the solutions are in the form of normal modes. For example, water has 3 x 3 - 6 = 3 normal modes.



For a specified normal model all the atoms move in phase at the frequency defined for that mode. Note that it is possible to find a coordinate system $\xi_s = \sum_s \alpha_{is} x_i$ such that the equations of motion in the new coordinate system are uncoupled. That is, $\mu_I d^2\xi_I/dt^2 + \phi_I \xi_I = 0$. The ξ_I are the "normal coordinates" which define a normal mode of vibration. In the ξ_I mode the system oscillates in phase at the frequency $\nu_I = \sqrt{(\phi_I/\mu_I)}$. Similarly, the Schroedinger equation can be expressed in terms of normal modes to yield a system of uncoupled harmonic oscillator Schroedinger equations, each of which has solutions in the form of a simple uncoupled oscillator.

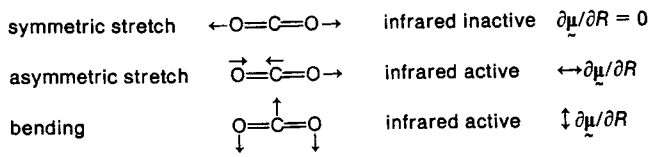
Infrared and Raman Spectroscopies

Transitions between vibrational energy levels occur at much lower frequencies than do electronic transitions: vibrational—$2\ \mu m \leq \lambda \leq 50\ \mu m$; visible (green light) $\lambda = 0.5\ \mu m$. For a molecule to absorb an infrared photon due to a molecular vibration two conditions must be satisfied:

(1) $\nu = E_0/h$ and

(2) The permanent dipole moment of the molecule must change due to the molecular vibration. Thus $d\mu/dR \neq 0$ where μ = permanent dipole moment and R = normal coordinate

Example, CO_2:



Hence, one of the three vibrational modes of CO_2 will not show up in an IR spectrum. Note that of the other two modes, one is polarized parallel, the other, perpendicular to the molecular axis.

Raman spectra result from scattering rather than absorbance as in IR. In contrast to IR the incident light, typically in the visible range, is far from the frequencies of molecular vibrations. Raman complements IR; the polarizability rather than the dipole moment must change with vibration.

When the incident light approaches an electronic absorption, there is a large increase in the polarizability and consequently, a large increase in the amplitude of Raman scattering. This is known as "resonance Raman" scattering.

Vibrational Spectroscopy of Proteins

In polypeptides three peptide backbone infrared bands are of greatest importance. One is dominated by an N-H stretch at approximately 3300 cm^{-1}, one by a C=O stretch at

1630-1660 cm^{-1} (amide I band), and the third by a N-H deformation at 1520-1550 cm^{-1} (amide II). Characteristics of these bands are shown in the following table.

Table 8-5
Characteristics of principal infrared absorption bands of the peptide group

		Hydrogen-bonded forms				Non-hydrogen-bonded
		α Helix		β Sheet		
Vibration	$\partial\underline{\mu}/\partial R$	Frequency (cm^{-1})	Dichroism	Frequency (cm^{-1})	Dichroism	Frequency (cm^{-1})
N—H stretch	←N—H→ ↔	3,290–3,300	∥	3,280–3,300	⊥	~3,400
Amide I (C=O stretch)	←C=O→ ↔	1,650–1,660	∥	1,630	⊥	1,680–1,700
Amide II	↑ H | ←C—N→ ↙ ↓	1,540–1,550	⊥	1,520–1,525	∥	<1,520?

SOURCE: Adapted from J. A. Schellman and C. Schellman, in *The Proteins*, 2d ed., vol. 2, ed. H. Neurath (New York: Academic Press, 1962), p. 1.

Infrared absorption dichroism is similar to electronic absorption dichroism. In the α-helix N-H···O=C peptide hydrogen bonds are oriented parallel to the long axis of the molecule. Then, for oriented samples, the N-H stretch and amide I bands should preferentially absorb IR light when the polarization is parallel to the helix axis. The amide II band should have orthogonal polarization.

In an actual protein or polypeptide the situation is more complex because of interactions which couple the peptide vibrations to each other and to those of other structural elements. In simple α-helices, however, the situation is simplified by the symmetry of the structure. Due to the helical symmetry there are only three bands: an intense polymer absorption at $\nu_{\parallel}$ polarized parallel to the helix and two degenerate bands at $\nu_{\perp}$ polarized perpendicular to the helix axis. For anti-parallel β sheets there are four vibration bands corresponding to normal vibrational modes of the fundamental asymmetric unit (involving 4 peptides. One amide band is IR inactive, $\nu(\pi, \pi)$; two other bands are polarized perpendicular, and the fourth parallel to the helix axis as summarized in the following table.

Table 8-6
Observed and calculated infrared spectra of polypeptides and proteins

		Amide I		Amide II	
Conformation	Mode	Calculated	Typical observed	Calculated	Typical observed
α Helix	$\nu_{\parallel}(0)$	(1,650)	1,650	(1,516)	1,516
	$\nu_{\perp}(2\pi/n)$	1,647	1,652	1,540	1,546
Antiparallel	$\nu_{\parallel}(0, \pi)$	(1,685)	1,685	(1,530)	1,530
β sheet	$\nu_{\perp}(\pi, 0)$	(1,632)	1,632	1,540	——
	$\nu_{\perp}(\pi, \pi)$	1,668	——	1,550	——
Parallel	$\nu_{\parallel}(0, 0)$	1,648	1,645	1,530	1,530
β sheet	$\nu_{\perp}(\pi, 0)$	1,632	1,630	1,550	1,550
Random coil	ν_0	1,658	1,656	1,535	1,535

NOTE: Frequency values are given in cm^{-1}. Calculated values shown in parentheses were adjusted to equal the corresponding observed values by the choice of parameters.
SOURCE: Adapted from J. A. Schellman and C. Schellman, in *The Proteins*, 2d ed., vol. 2, ed. H. Neurath (New York: Academic Press, 1962), p. 1.

A schematic representation of the vibrational modes of the antiparallel β-sheet is shown below.

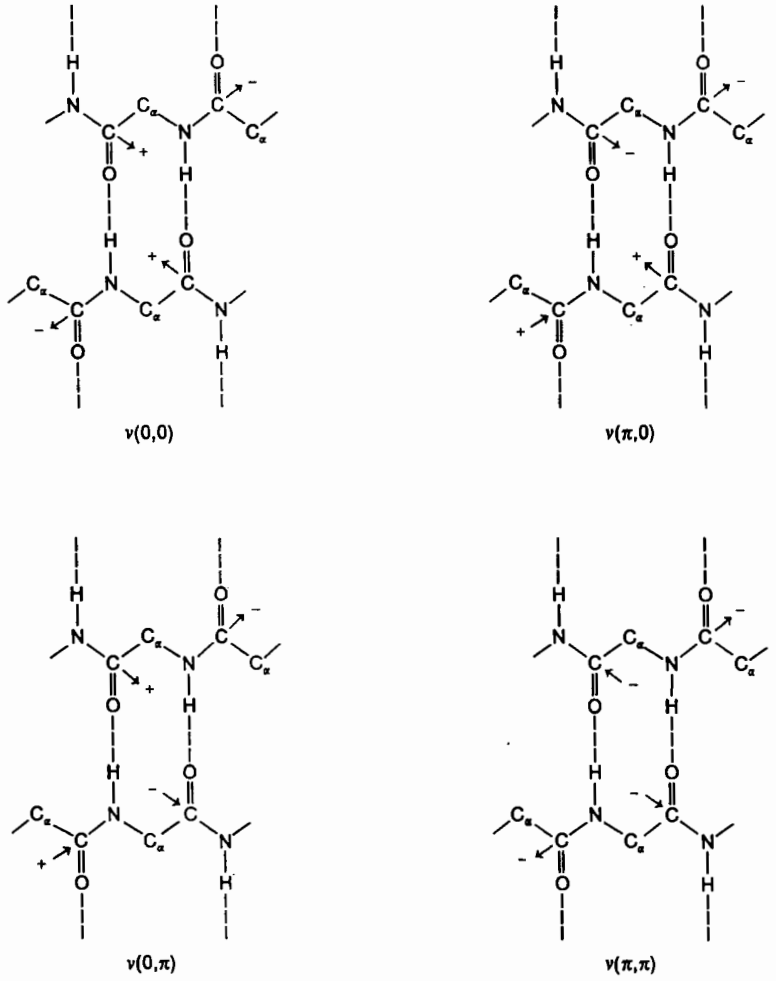


Figure 8-27

Schematic representation of the vibrational modes of the antiparallel β sheet. Arrows represent components of transition moments in the plane of the paper; plus and minus signs represent out-of-plane components. [After T. Miyazawa, *J. Chem. Phys.* 32:1647 (1960).]