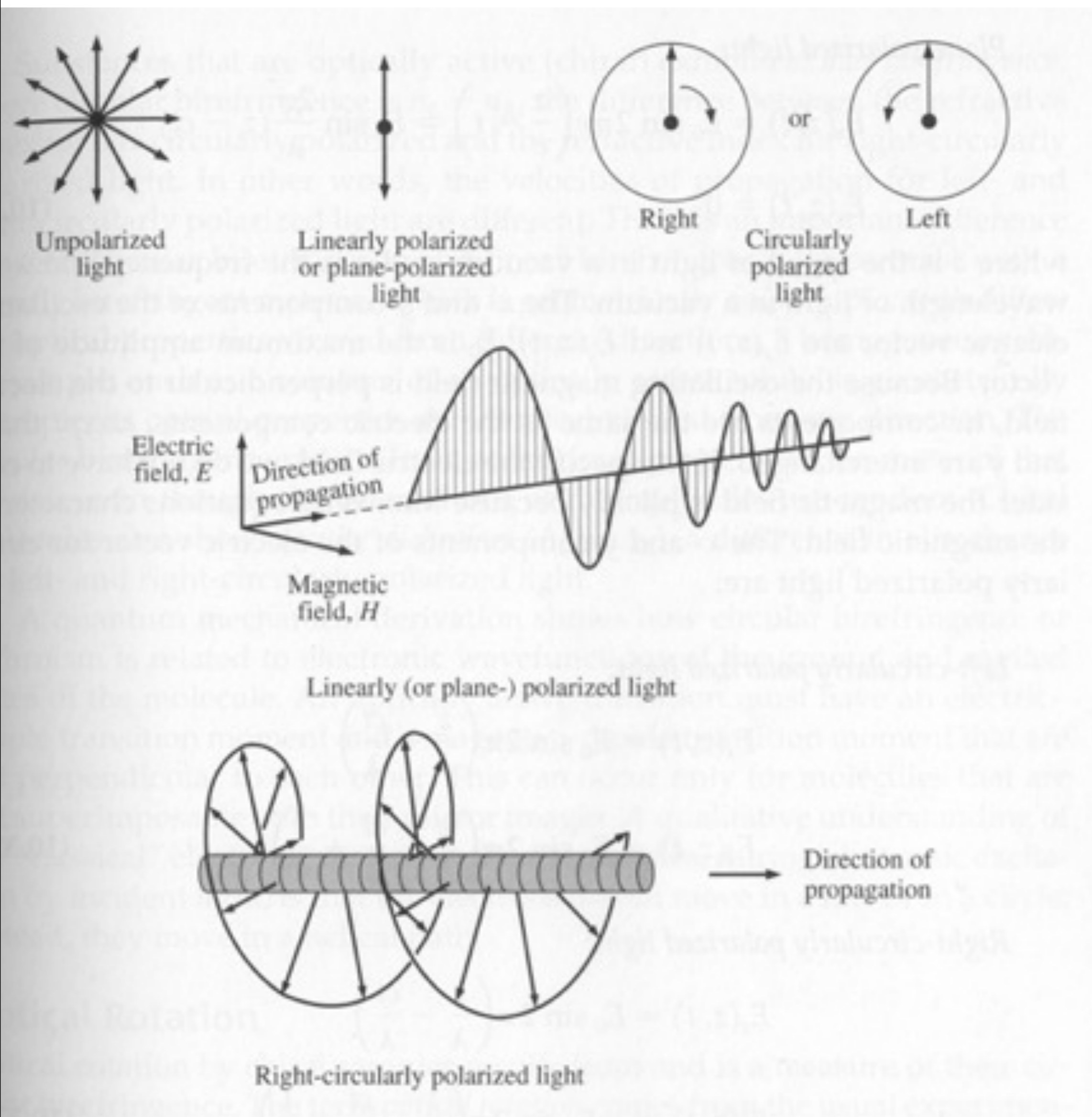
Optical Rotation and Circular dichroism

Background

- most biological molecules are asymmetrical or chiral
- Therefore molecules have a “handedness”
- higher order structures have a “hand” associated with it
- most DNA and protein helices are right handed
- there are exceptions due to the composition of bases or amino acids
- Chiral structures can be distinguished and characterized by polarized light

Types of Polarized Light

- Light has a electronic and magnetic components that are perpendicular to each other
- Three types of polarized light
  - Plane-polarized light
  - Circularly polarized light
  - Elliptically polarized light - intermediate between plane and circular
- Plane and circularly polarized light can be produced from unpolarized light by passing it through an appropriate film or crystal
- Plane polarized light use a film that is geometrically anisotropic
  - refractive index is different for polarized planes. It allows only one plane of light through



◀ FIGURE 10.21
Different types of polarized light. At the top, the arrows represent the electric vector of the light as seen by an observer moving with the light. The light is moving into the page. At the middle and bottom, the light is seen by a stationary observer.

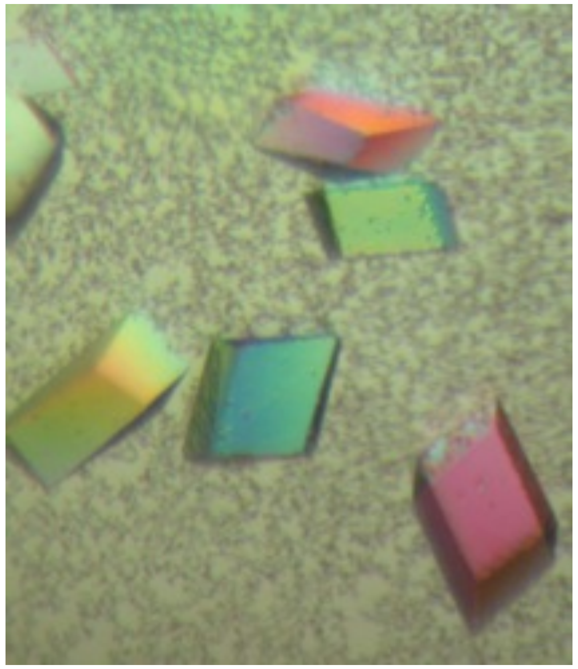
Linear Birefringence

- This is dependent on different refractive indexes.
- refractive index (n) is a ratio of velocity of light in a vacuum to the velocity in the medium of interest (v)
- $n=c/v$ $c=3.0\times10^8$ m/s
- difference in refractive index results in color
- refractive index is an intrinsic property of material and is related to composition

Refractive Index of Plane Polarized Light

- Objects, such as crystals and cells have different refractive indexes
- These materials are geometrically anisotropic - means different properties along different axis
- Linear birefringence (double refraction) occurs
  - refractive index is different for plane polarized light in different orientations of the material
- Linear dichorism occurs if the light is in the absorbance spectrum of the material
  - light will be absorbed based on orientation

Example of Linear Birefringence



Protein crystals seen under a microscope
with plane polarized light

Circularly Polarized Light

- Chiral molecules exhibit circular birefringence
- the refractive indexes are different for left- and right-circularly polarized light
- Difference between circular and linear birefringence
- Linear birefringence requires sample to be geometric anisotropic while circular birefringence does not.
- Circular birefringence - Optical properties are the same from any direction
- Circular dichroism an absorbance of left- and right- circularly polarized light.

Optical Rotation

- Optical rotation is a measure of a samples <u>circular birefringence</u>
- Plane-polarized light propagated through a chiral sample will emerge plane polarized but at different rotational angle.

Historical Significance

- Louis Pasteur took tartaric acid powder and grew crystals of it
- Examined the crystals under a light microscope with plane polarized light
- Noticed that the certain crystal rotated plane polarized light to the right and other to the left
- Dextrorotatory: A compound that rotates plane-polarized light to the right (clockwise) when viewed in the direction of the light source.
- Levorotatory: Describes compounds that rotate plane-polarized light to the left (counterclockwise) when viewed in the direction of the light source.

“Do not put forward anything that you cannot prove by experimentation"

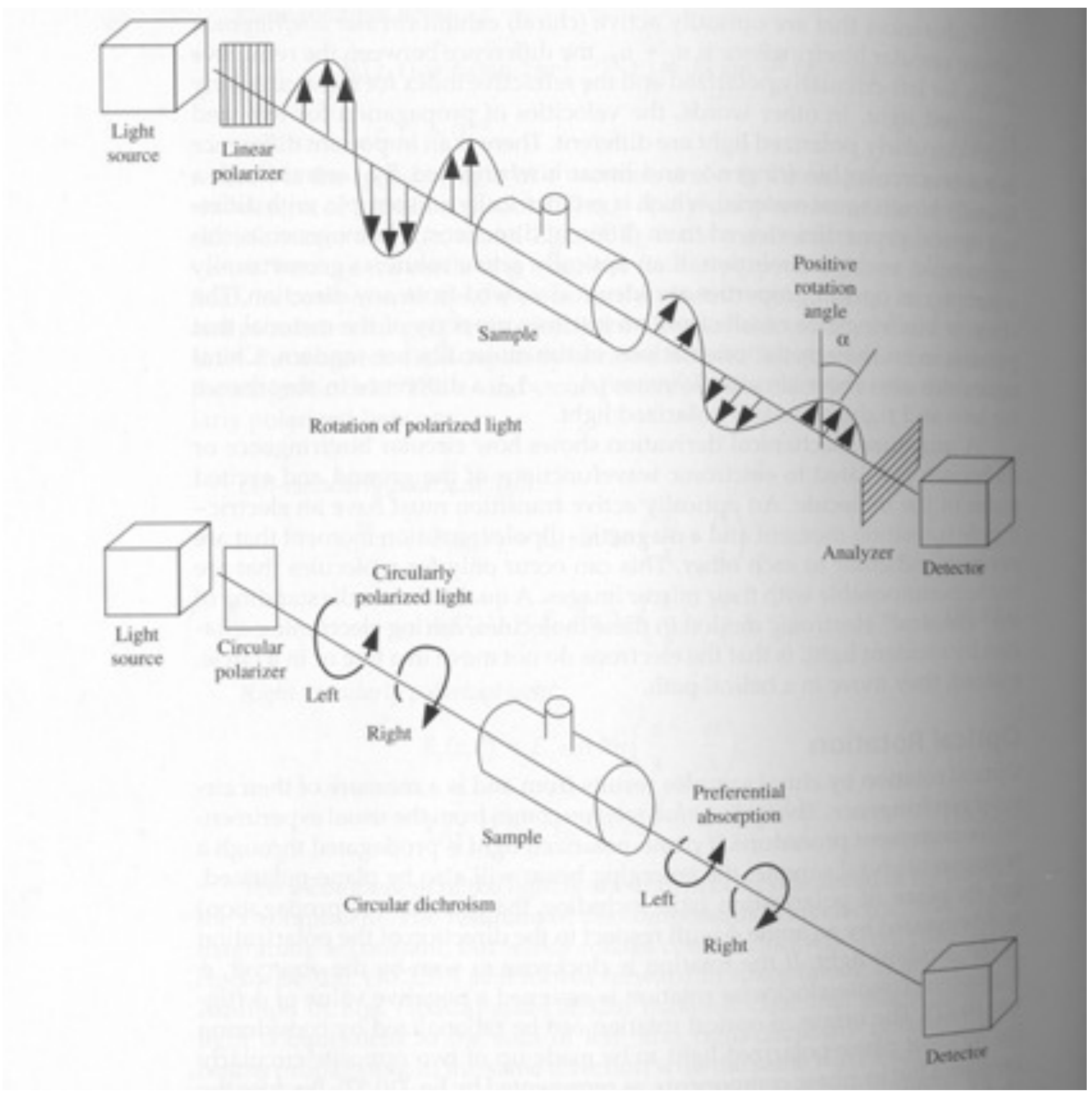
Circular Dichroism

- Circular dichroism (CD) is the differential absorption of right- and left-circularly polarized light
- Expressed as

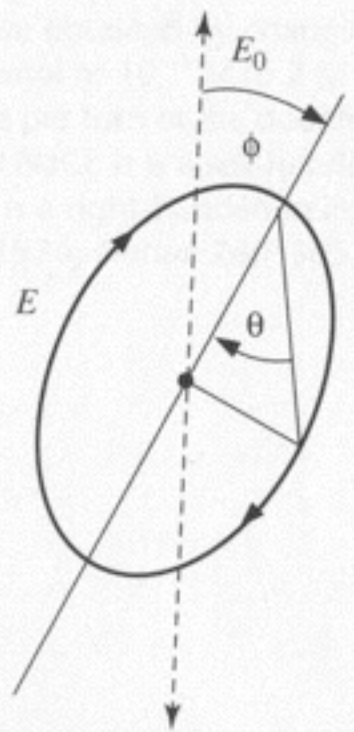
  $\Delta A = A_L - A_R$

- A_L and A_R is the absorbances for left and right circular polarized light

Measurement of optical Rotation and Circular Dichroism



Ellipticity

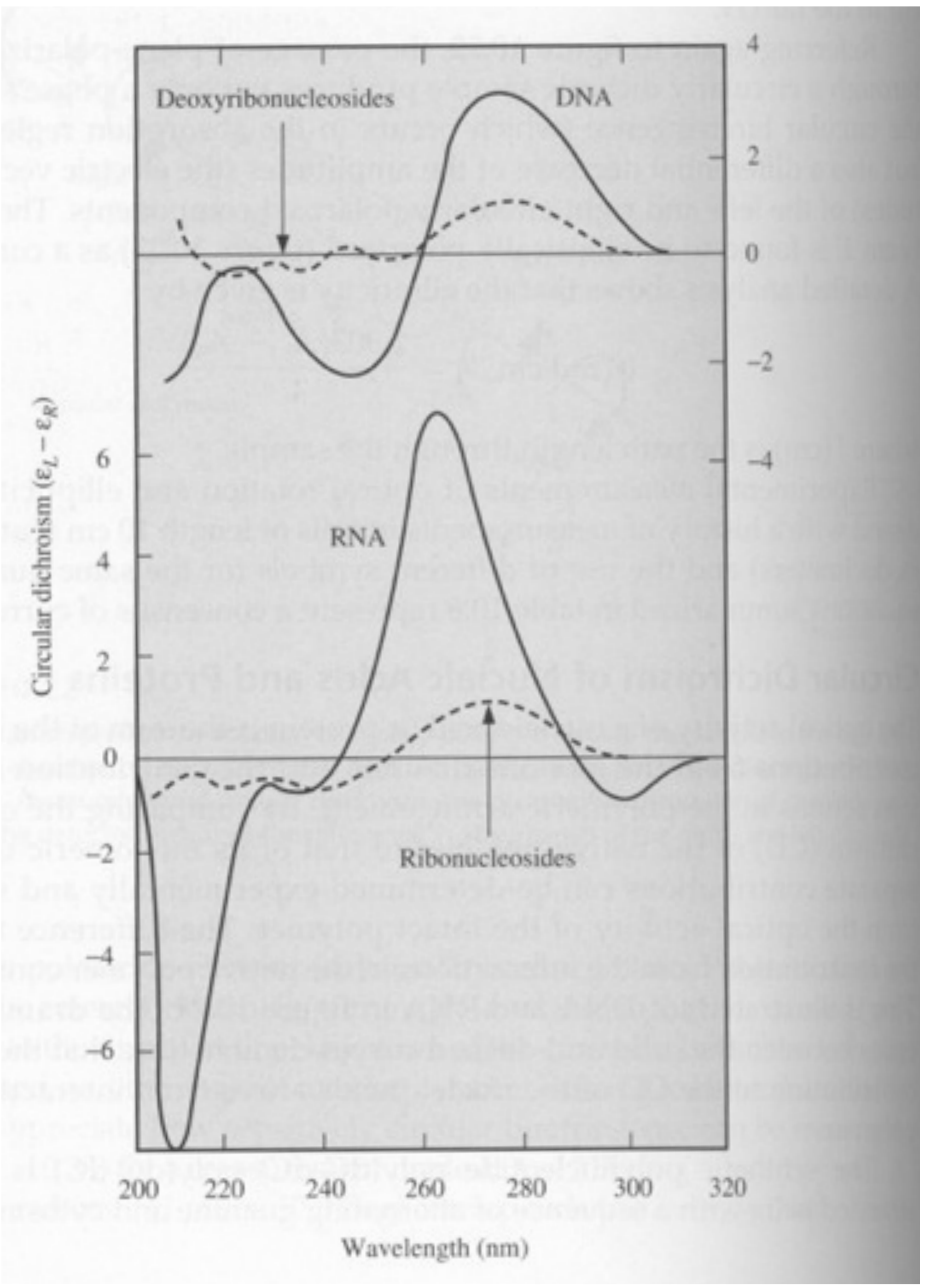


▲ FIGURE 10.23

Elliptically polarized light emerging toward the observer from a circularly dichroic sample. The sign convention is that ϕ is positive for clockwise rotation of the major axis and θ is positive for right-elliptically polarized light, as shown. The rotation angle is sometimes called α; the ellipticity angle is also called ψ.

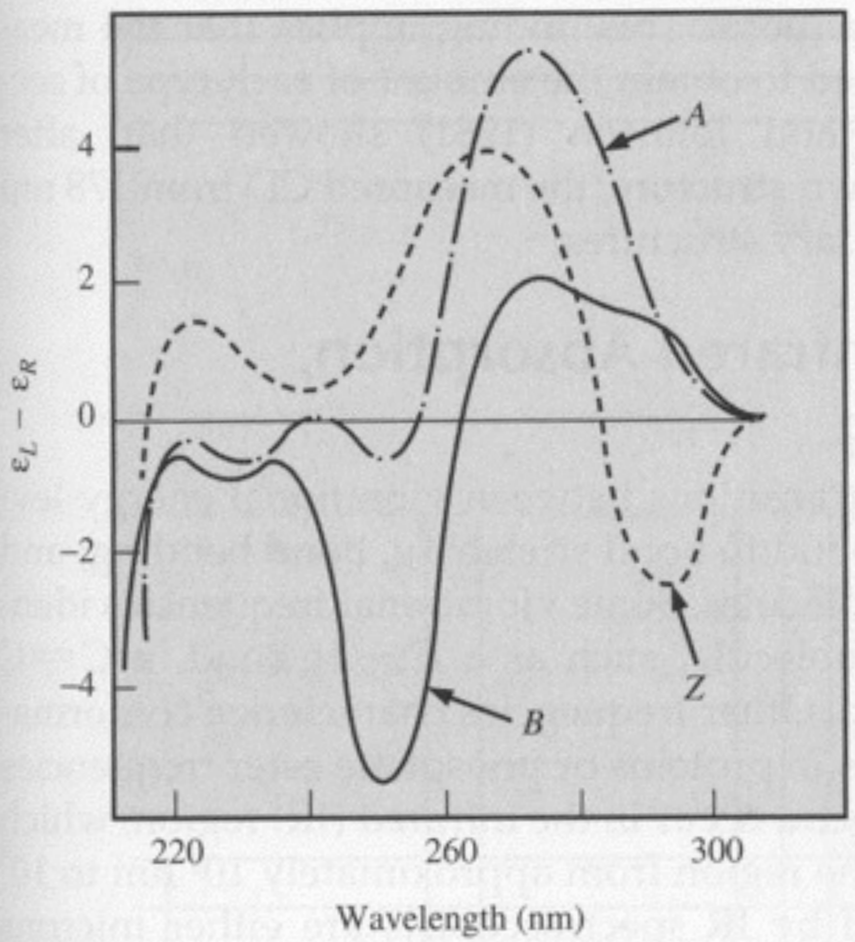
- The differential absorption of of circularized light results in the emergence of light that is elliptical
- Ellipticity (θ) is equal to the tangent of the major and minor axis of the ellipse
- It is usually given in degree or millidegree
- Need to normalize for number of residues and concentration

CD of Nucleic Acids



- CD of RNA and DNA compared to their component mononucleosides.
- The difference between the mononucleosides and polymer show the contribution from interactions within the polymer

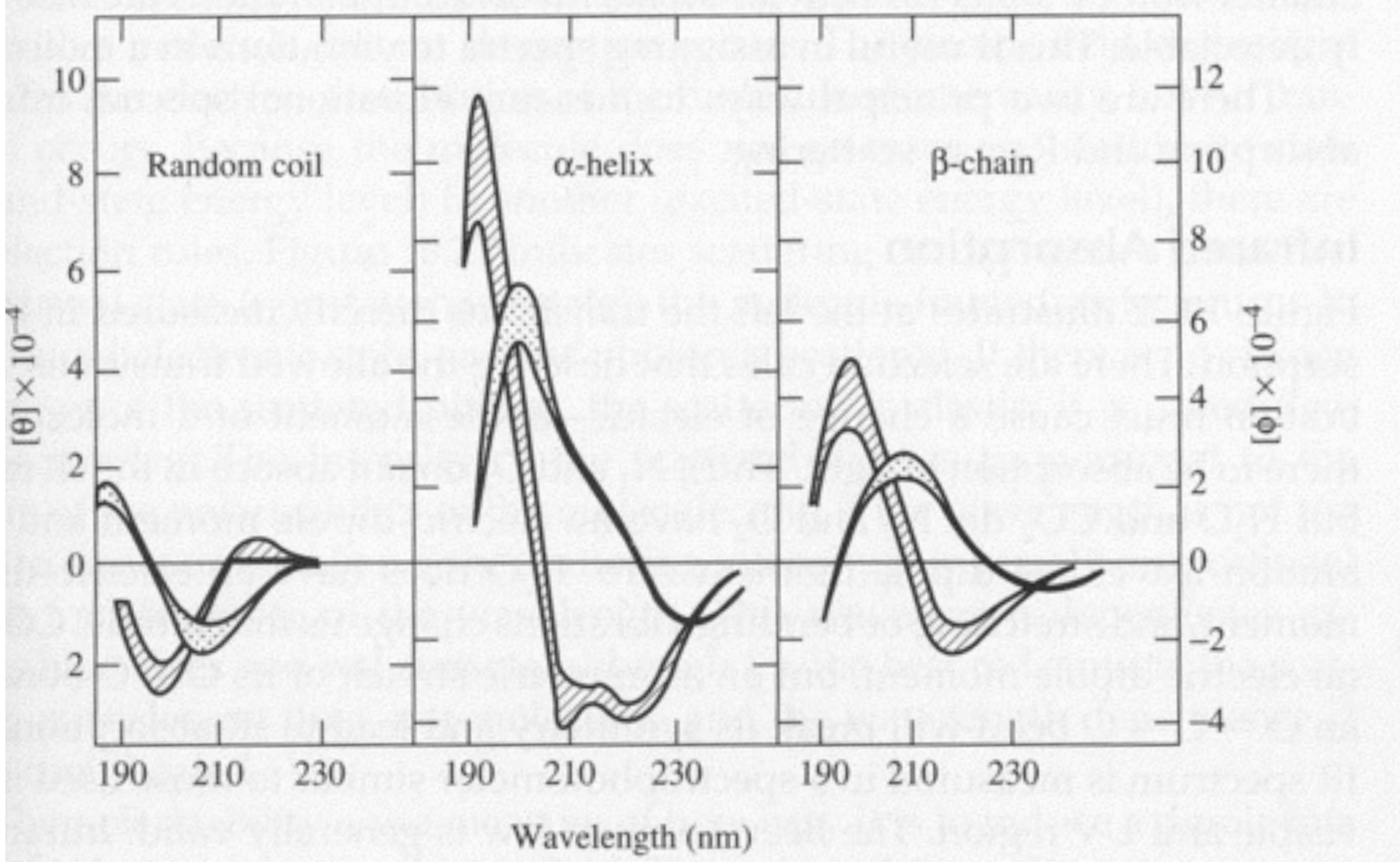
Different Conformations of Nucleic Acids



◀ FIGURE 10.25

Circular dichroism of the synthetic polynucleotide poly(dG-dC)·poly(dG-dC) in different conformations. The polynucleotide is a double-stranded helix; each strand has a sequence of alternating deoxyguanylic acid (dG) and deoxycytidilic acid (dC). Different conformations are obtained by changing the solvent. The B form is obtained in 0 to 40% ethanol or 10^{-3} *M* to 2 *M* NaCl; it is a right-handed helix with about 10 base pairs per turn of the double helix. The Z form is obtained in 56% ethanol or 3.9 *M* NaCl; it is a left-handed helix. The A form is obtained in 80% ethanol; it is a right-handed helix with about 11 base pairs per turn. (From F. M. Pohl, 1976, *Nature* 260:365. Copyright © 1976 Macmillan Journals Limited.)

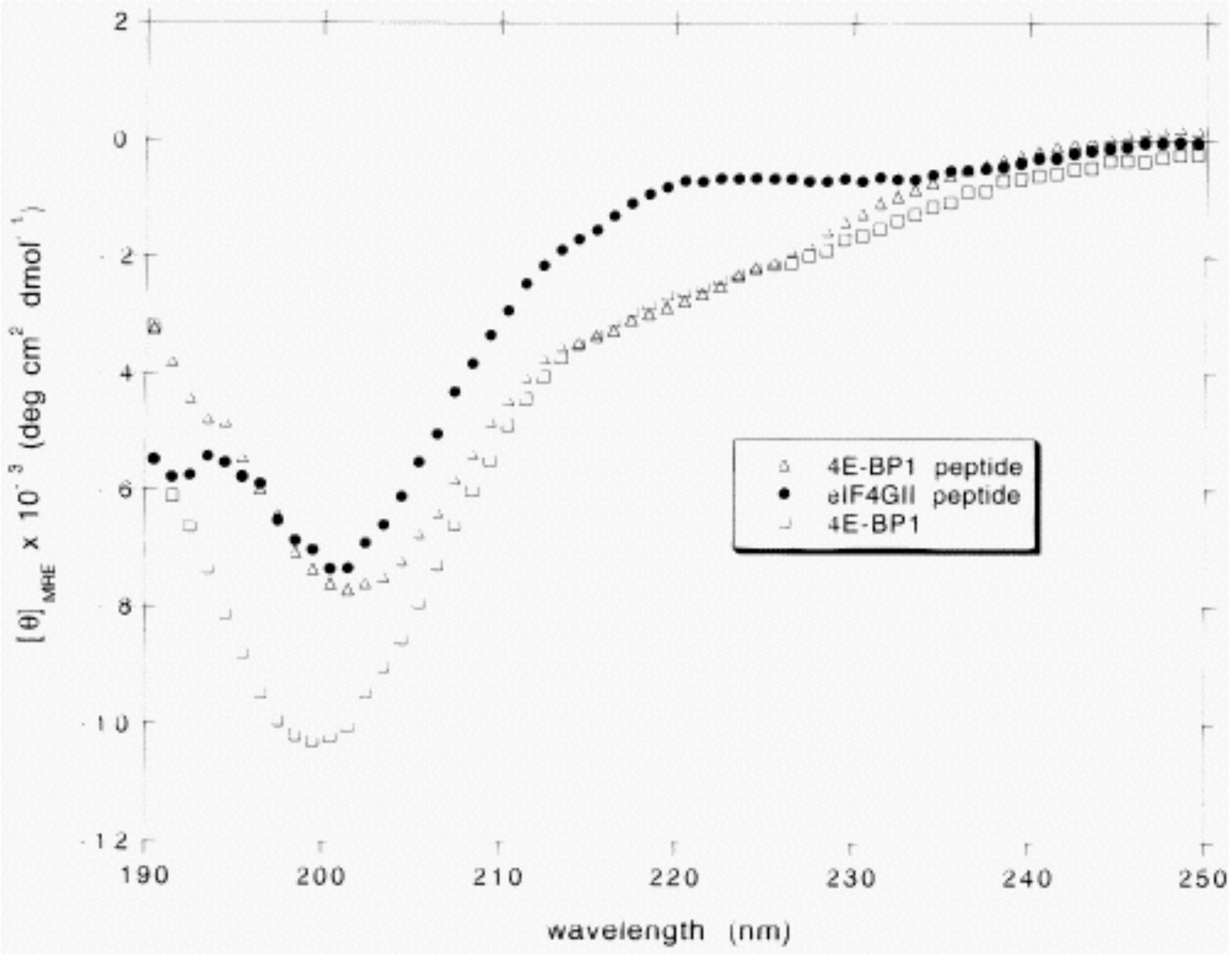
Different Conformations of Proteins



◀ FIGURE 10.26

CD (crosshatched) and ORD (dotted) of homopolypeptides in the random coil, α-helical, and β conformations. The shaded areas indicate the range of values. (From W. B. Gratzer and D. A. Cowburn, 1969, *Nature* 222:426. Copyright © Macmillan Journals Limited.)

Experimental CD Data



CD Spectroscopy of Recombinant 4E-BP1 and eIF4GII and 4E-BP1 PeptidesCircular dichroism spectra of recombinant 4E-BP1 (°) and eIF4GII (·) and 4E-BP1 (Δ) peptides in solution, plotted as versus mean residue ellipticity (deg cm^2 $dmol^{-1}$) versus wavelength (nm).