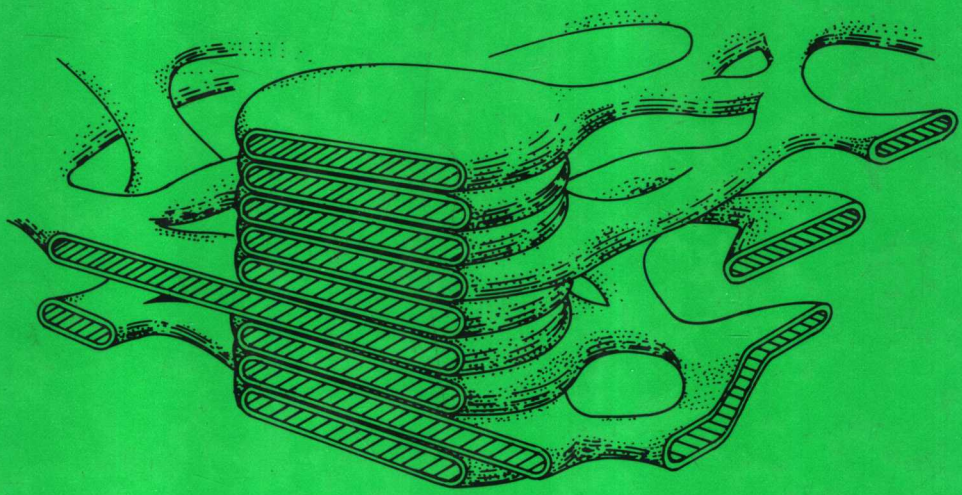
PHOTOSYNTHETIC SYSTEMS

Structure, Function and Assembly



Susan M. Danks,
E. Hilary Evans *and*
Peter A. Whittaker

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SUSAN M. DANKS
Biology Tutor
Open University

E. HILARY EVANS
Department of Biology
Preston Polytechnic

and

PETER A. WHITTAKER
Department of Biology
St. Patrick's College
Maynooth, County Kildare

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Preface

Photosynthesis is arguably the most important biological activity on this planet and has rightly received a considerable degree of attention from research workers. We feel that this research has led to a situation where the basic mechanistic principles of the light-dependent processes of photosynthesis (particularly phosphorylation of ADP) have now been established and a relatively full picture of the associated light-independent metabolic processes has been achieved. Recent discoveries have also begun to illuminate the problems of chloroplast assembly and development of the photosynthetic apparatus. This book is entitled *Photosynthetic systems: structure, function and assembly* because we have tried to examine the nature of photosynthetic processes in a whole range of organisms, including bacteria. Structure is discussed in chapter 1, function in chapters 2 and 3 and assembly in chapter 4. There is one inconsistency between chapters 2 and 3 which is deliberate. In chapter 2, which deals with photosynthetic phosphorylation ('light reactions'), photosynthetic bacteria are discussed first, since a great deal of research in this field has been carried out using these organisms, which possess 1 photosystem. Chloroplasts which have 2 photosystems are dealt with subsequently. However, in our experience the dark reactions of chloroplasts are more generally taught than the metabolism of photosynthetic bacteria. Chapter 3, therefore, has a bias towards chloroplast metabolism and not so much on that in photosynthetic bacteria.

The book is written primarily for undergraduate students taking biochemistry courses, e.g., biochemistry, biology and plant physiology students. Literature references are not included in the text, but instead there is a suggested reading list at the end of each chapter. This identifies publications by many authors prominant in the specific areas of research covered in this book and also reviews from journals which students may use to open up related fields of interest. We hope that this combination will provide interesting and useful further reading for more advanced students, i.e., third year students and research workers in photosynthesis.

Throughout the text, research techniques are mentioned wherever possible. Our aim is not to give definitive descriptions of these methods, but to encourage students to make the connection between experimental techniques and accepted knowledge, which they often study separately.

The use of 'di' rather than the alternative 'bis' nomenclature was chosen, being in line with the literature searched, and to avoid confusion to the student. In two cases however, those of ribulose bisphosphate carboxylase and its substrate, 'bis' seems the more generally accepted and so is used here.

We would like to thank Drs Philip Dix, Reg England, Jacqui Manwaring, Tony Moore, Andy Morgan, Mike Tribe and Professor Mike Evans for reading and making helpful comments on various parts of the manuscript and Malcolm Danks for the design of diagrams in chapters 1 and 3 and help with the index.

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Abbreviations

ALA	5 aminolevulinic acid
AMP, ADP and ATP	Adenosine 5′ mono-, di- and triphosphate
ATPase	Adenosine 5′ triphosphatase
Chl	chlorophyll
CO_2	carbon dioxide
CoA	coenzyme A
ctDNA	chloroplast deoxyribonucleic acid
cyt	cytochrome
DHAP	dihydroxyacetone phosphate
DCMU	3-(3,4 dichlorophenyl)-1,1-dimethylurea
DCPIP	2,6-dichloroindophenol
DBMIB	2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone
EPR	electron paramagnetic resonance
Fd	ferredoxin
FCCP	*p*-trifluoromethoxycarbonylcyanide phenylhydrazone
K_m	Michaelis constant
m,r or tRNA	messenger, ribosomal or transfer ribonucleic acid
MW	molecular weight in Daltons
NAD^+/NADH	Nicotinamide adenine dinucleotide (oxidized/reduced)
$NADP^+$/NADPH	Nicotinamide adenine dinucleotide phosphate (oxidized/reduced)
PC	plastocyanin
PEP	phosphoenol pyruvate
Pi	inorganic phosphate
PQ/PQH_2	Plastoquinone (oxidized/reduced)
RBPC	ribulose bisphosphate carboxylase
S	Svedberg constant
SDS	sodium dodecyl sulphate

Contents

1
Introduction

1.1 Introduction to prokaryotic and eukaryotic cells

Living organisms can be divided into two groups, the prokaryotes and the eukaryotes. These two groups differ fundamentally in the structure and biochemistry of their cells (see table 1.1).

Prokaryotic cells (bacteria) generally have a cell wall and cell membrane enclosing the cytoplasm, but no internal cell membranes. The prokaryotic cell's DNA is not contained within a nuclear membrane, but may be attached to the cell membrane. Electron transfer reactions associated with ATP synthesis occur within the cell membrane. The ribosomes, which are involved in protein synthesis, are mostly suspended in the cytoplasm but may be attached to the cell membrane.

Eukaryotic cells, e.g., plant and animal cells, contain complex internal cell membranes which surround subcellular structures called organelles. The DNA is enclosed in the nucleus in the form of chromosomes. Electron transfer reactions occur primarily in the inner membranes of chloroplasts and mitochondria. Ribosomes, which have different sedimentation characteristics from those of prokaryotes, may be attached to the endoplasmic reticulum or in the cytosol (the soluble part of the cytoplasm). The other organelles present in eukaryotic cells are lysosomes, Golgi apparatus and vacuoles.

Photosynthesis is the process whereby light energy from the sun is converted to chemical energy and conserved in the form of ATP and NAD(P)H, which can be used to drive the biosynthesis of organic molecules such as glucose and amino acids. Photosynthesis can occur in both prokaryotes and eukaryotes, e.g., photosynthetic bacteria and green plants. (For convenience these are referred to collectively in this book as photosynthetic systems.)

1.1.1 Classification

This section gives a brief classification of prokaryotic and eukaryotic photosynthetic organisms. It is widely thought that photosynthesis is carried out only by eukaryotic plant cells, but about half of all photosynthetic reactions occur in prokaryotes.

A classification of prokaryotic photosynthetic organisms is shown in figure

Table 1.1 The major differences between prokaryotic and eukaryotic cells

	Eukaryotic cells	Prokaryotic cells
	Cell components divided between subcellular structures called organelles, e.g., nucleus, mitochondria, lysosomes,[1] Golgi apparatus[2] and endoplasmic reticulum. Plant cells also contain vacuoles[3] and plastids including chloroplasts.	Cell components contained within the cell membrane, no internal membrane systems, i.e., no organelles.
Cell membrane/ cell wall	Selectively permeable to ions. Contains about 50% protein and 50% lipid. Some animal cell membranes have a cell coat on the outer side, composed mainly of polysaccharide. Plant cells have a rigid cell wall consisting of cellulose polysaccharides and protein.	Selectively permeable to ions. Contains about 55% protein and 45% lipid – sometimes invaginated. Surrounded by a cell wall of rigid polysaccharide cross-linked with peptide chains which protects the cell from hypotonic solutions, i.e., swelling.
DNA	Surrounded by a membrane forming the nucleus. Combined with histone proteins and forming chromosomes. Mitochondria and chloroplasts contain some circular DNA.	Tightly coiled but not surrounded by a membrane, may be attached to the cell membrane.
Electron transport (ATP formation)	Occurs in mitochondria and chloroplasts. The proteins of the electron transport system are part of the inner mitochondrial membrane or thylakoid membranes in chloroplasts.	Proteins of the electron transport system are associated with the cell membrane. Chromatophores in photosynthetic bacteria are invaginations of the cell membrane.
Ribosomes	Attach to endoplasmic reticulum membrane system. Composed of 60S and 40S components.	Attach to cell membrane. Composed of 50S and 30S components.

[1] Lysosomes contain hydrolytic enzymes.
[2] Golgi apparatus is involved in secretion.
[3] Vacuoles act as stores of sugars, salts etc.

1.1, emphasizing the pigments present in them. Three groups of photosynthetic prokaryotes are known. Bacteria which have only a single photosystem, and use a reductant other than water (e.g., H_2S, reduced organic compounds or H_2) to provide the reducing equivalents for photosynthesis, are grouped into the Rhodospirillales.

The second major group of photosynthetic prokaryotes are the cyanobacteria, formerly known as Cyanophyta or blue-green algae. They have two photosystems, use water as a reductant and like plants and algae, evolve oxygen in the light. Recently, however, it has been decided to classify them as bacteria because of their prokaryotic characteristics. Their classification is complex and based mainly on morphology rather than pigmentation, which is the main feature used for classifying other photosynthetic microorganisms. Unfortunately, as further studies of cyanobacteria are revealing, morphological classification gives no guidance about metabolic complexity. Michael Herdman has recently identified three different sized genomes in cyanobacteria, 2a, 4a and 6a, compared with 2a, the genome size of the average bacterium. The genome size appears to be related to morphological and/or metabolic complexity and may provide the future basis of a more coherent classification. The majority of biochemical studies on cyanobacteria have used organisms of the following species: *Synechoccus, Phormidium, Anacystis, Aphanocapsa* (unicells); *Anabena* (filamentous); *Chlorogloepsis, Nostoc* (filamentous, aseriate mixed morphology).

The third group of photosynthetic prokaryotes, the Prochlorophyta, to date has only a single species called *Prochloron*, which contains chlorophyll *a* and *b* but lacks phycobilins. It is thought to be an evolutionary precursor of chloroplasts.

In recent years it has been found that a group of salt-tolerant bacteria, Halobacteria, are capable of ATP synthesis coupled to a light-driven reaction, totally unrelated to the photosynthetic electron transport chains described here and in chapter 2. Some more details of this unusual reaction will be given in section 2.13.

There is much dispute over the details of algal classification. In general, they are eukaryotic cells containing chloroplasts and some are classified as plants. However, like photosynthetic prokaryotes, their classification is based on the following characteristics:

(1) Pigments: their nature or chemical composition.
(2) Reserve food products or assimilatory products of photosynthesis.
(3) Flagella.
(4) Cell walls.
(5) Morphological characteristics of cells and thalli.
(6) Life history and reproduction.

To try to illustrate the variations in algae, table 1.2 shows some of the differences between six algal Divisions. All six have an oxygen-evolving, 2-photosystem photosynthetic apparatus, but differ markedly in their ultrastructure

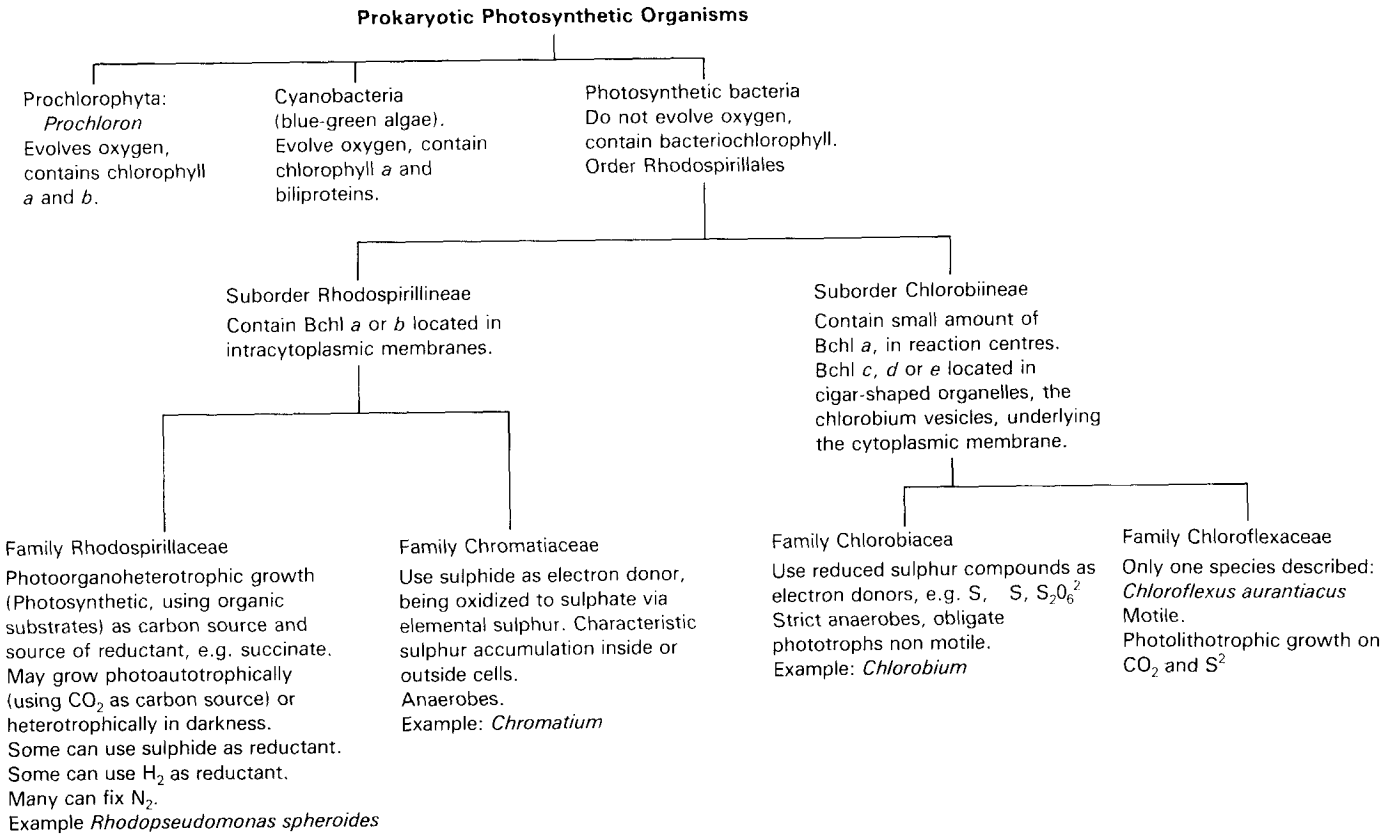

Figure 1.1 Classification of prokaryotic photosynthetic organisms

Table 1.2 Differences between six algal Divisions

Division	*Chrysophyta* (golden algae) (diatoms)	*Pyrrophyta* (Dinoflagellates)	*Euglenophyta* (euglenoids)	*Chlorophyta* (green algae)	*Phaeophyta* (brown algae)	*Rhodophyta* (red algae)
Chlorophyll *a*	+	+	+	+	+	+
Chlorophyll *b*	–	–	+	+	–	–
Chlorophyll *c*	+	+	–	–	+	–
Chlorophyll *d*	–	–	–	–	–	sometimes
Carotenoids						
Carotenes	+	+	+	+	+	+
Fucoxanthin	+	+	–	–	+	–
Peridinium	–	+	–	–	+	–
Others	–	–	–	lycopene, lutein	–	zeaxanthin
Phycobilins						
Phycocyanin	–	+	–	–	–	+
Phycoerythrin	–	+	–	–	–	+
Storage material	chrysolaminarin, oils	starch, oils	paramylon, oils	starch	laminarin, oils mannitol	floridean starch, oils
Flagella	1 or 2	2 lateral 1 trailing 1 girding	1, 2 or 3 equal	1, 2, 4 to many	2	none
Notes			has a gullet			
Number of thylakoids grouped together in chloroplast	3	3	3	2 or more	3	1
Example	*Ochromonas* (*Chromulina*)	*Amphidinium*	*Euglena*	*Chlamydomonas*		*Prophyridium*

and the pigmentation of their chloroplasts. Table 1.2 shows the major differences in pigmentation, storage material, thylakoid grouping and flagella.

Another group of eukaryotic photosynthetic organisms are the higher orders of plants: mosses, liverworts, ferns, gymnosperms and flowering plants. They all contain chloroplasts which have a similar pigment composition to the Chlorophyta (table 1.2), i.e., they contain chlorophyll *a* and *b* and some other pigments such as carotenoids. Their chloroplasts carry out oxygen-evolving, 2-photosystem photosynthesis.

1.2 Structure and ultrastructure

This section does not pretend to cover the structure of photosynthetic organisms in great detail, but tries to show the main features that enable them to carry out their unique reactions. The main structures shown here are therefore chloroplasts from eukaryotic cells and chromatophores in prokaryotic cells.

To observe the detailed structure of such small particles as chloroplasts, which are invisible to the naked eye and many of whose components are impossible to resolve under optical microscopes, the electron microscope is used. Essentially this works on the same principle as the light microscope, except that a beam of electrons, instead of light, 'illuminates' the sample, and a series of electric coils act as electron lenses bending the electron beam in the same manner that optical lenses bend a light beam.

Two methods of preparing samples for the electron microscope are widely used. The more common, as in optical microscopy, is to fix and stain the material under investigation. Fixing, a measure intended to preserve the ultrastructural detail in its natural state, is done by immersion in an aqueous solution of osmium tetroxide (OsO_4) or potassium permanganate ($KMnO_4$) or in glutaraldehyde. The sample is then dehydrated using ethanol and then embedded in a synthetic resin such as Araldite. Then the sample is sliced to obtain a specimen for the electron microscope of 50–100 nm thickness. Solutions of lead hydroxide, lead citrate or uranyl acetate can then be used to stain the specimen if necessary.

The second technique, particularly useful for studying membrane ultrastructure, is called freeze-etching. The sample is rapidly frozen to a temperature near that of liquid nitrogen (−195°C) and then kept cold in a vacuum chamber. Slicing the sample under these conditions exposes regions from which ice sublimes, leaving membrane edges clearly exposed. A replica is then made by shadowing with platinum and carbon. It is the replica that is observed under the electron microscope after the remains of the sample have been removed.

In green plants and photosynthetic algae, photosynthesis occurs in subcellular organelles called chloroplasts. Chloroplasts are one of a group of organelles called plastids, all of which comprise a double membrane surrounding an internal membrane system. Some plastids act mainly as storage organelles,

e.g., amyloplasts contain starch and elaioplasts contain lipids. Chloroplasts are the most complex of the plastids and are thought to develop from precursors called proplastids, which are 1–3 μm long with only a few internal membranes. Chloroplast development from proplastids involves an increase in volume of the plastid, rapid chlorophyll biosynthesis and an increase in internal membranes. This development will be discussed in more detail in chapter 4.

Figure 1.2 shows an electron micrograph of a chloroplast in the cell of the halophyte *Suaeda maritima* (which grows in salt marshes): the sample has been

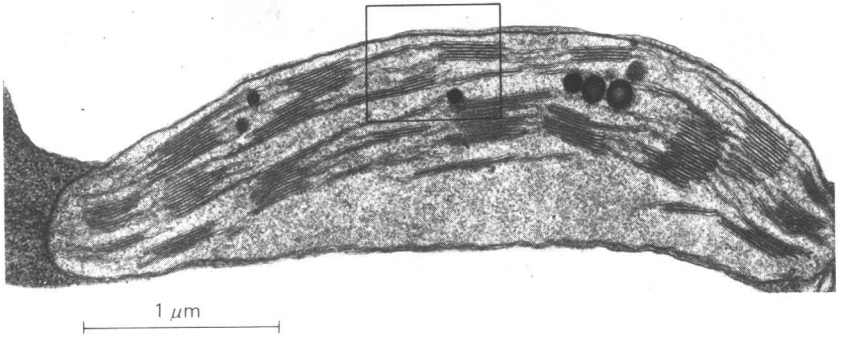

Figure 1.2 Electron micrograph of chloroplast from *Suaeda maritina*, fixed and stained. (Courtesy of Professor J. L. Hall)

fixed and stained. Figure 1.3 is a schematic representation of part of the same chloroplast enlarged two-fold to show all the features. Chloroplasts are generally ellipsoidal in shape and vary in length from 4 to 10 μm. There can be 1 to 100 chloroplasts per cell. The chloroplast in figure 1.2 is surrounded by a thin layer of cytosol and a vacuole can also be seen. The chloroplast has a double boundary membrane referred to as the chloroplast envelope. Each of these two membranes is 6–8 nm thick and a gap of 10–20 nm exists between them.

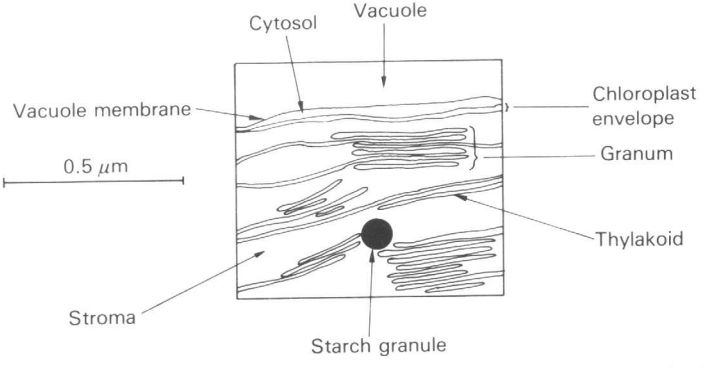

Figure 1.3 Diagrammatic representation of the structure contained within the square drawn on figure 1.2, magnified two-fold

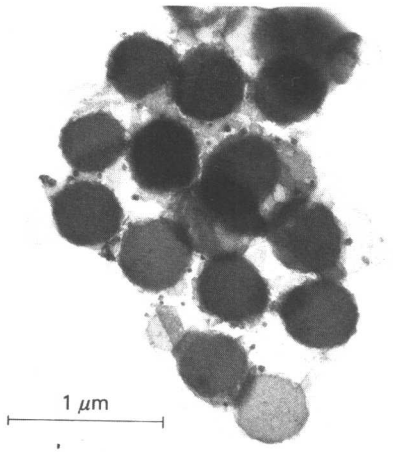

Figure 1.4 Electron micrograph of a segment of the internal membrane system of chloroplasts from Swiss Chard, fixed and stained, showing various stages of thylakoid growth. (Reproduced from J. Heslop-Harrison, *Science Progress*, 1966, **54**, 538, 539 by permission of Blackwell Scientific)

Chloroplast membrane structure will be discussed in section 1.2.1. Inside the envelope is the liquid part of the chloroplast, the stroma, which appears granular under the electron microscope and is rich in enzymes. Within the stroma is an inner membrane system consisting of flattened sacs or discs called thylakoids. These can often be seen arranged in stacks known as grana, which are linked by hollow membranous bridges, the lamellae (sometimes referred to as frets or stroma thylakoids). The whole system of interconnected chambers is believed to enclose a single continuous space, the thylakoid space. Figure 1.4 shows an isolated segment of a thylakoid membrane system. Various stages of thylakoid growth can be seen: small, presumably immature granum thylakoids appear superimposed upon fully extended ones. Figure 1.5 gives a representa-

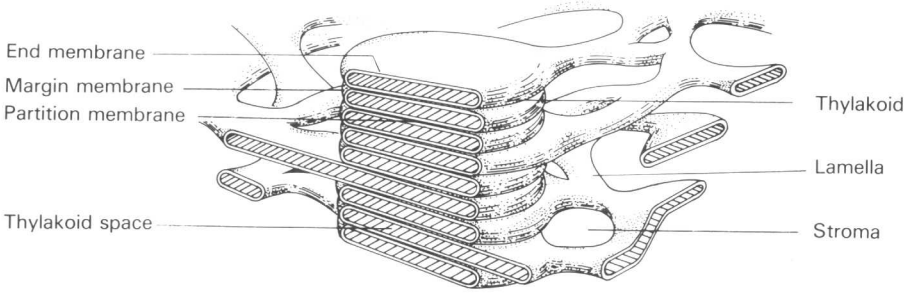

Figure 1.5 Diagrammatic representation of thylakoids, grana and lamellae, indicating how the space within them may be continuous.