

**TERTIARY LEVEL BIOLOGY**

# **The Biochemistry of Energy Utilization in Plants**

**DAVID T. DENNIS, BSc, PhD**


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# Preface

Plants are not easy organisms with which to work. Their cells are surrounded by a tough cell wall that requires harsh measures to disrupt, and this process often leads to an inactivation of the molecules within the cell. Plants also produce large numbers of 'natural products', which will often denature essential cellular molecules. On top of all this, the amounts of proteins, nucleic acids, etc., present in plants are small relative to those in bacteria and animals.

Would it not, therefore, be better simply to leave the field to the animal and bacterial biochemists and trust that plants behave similarly, if not identically, to these organisms? Unfortunately, this is exactly what has happened. Until recently, plants have been regarded as nothing more, biochemically, than green animals, and the only aspect of their metabolism that has been treated independently is photosynthesis.

There is at present a surge of interest in plant biochemistry, as the gaps in our knowledge are seen as a major impediment to progress, especially in such areas as genetic engineering. Techniques for the transfer of genes in plants are well advanced, and the question has become not how to transfer the genes, but which genes should be moved. To be able to answer this question, it is necessary to know the pathways, and to have purified and characterized the enzymes that catalyse these pathways. In the cases that have been studied, fundamental differences between the biochemistry of plants and animals have been found. It is the aim of this book to discuss the subject of plant energetics as it is known now, and to compare our knowledge of plants with that of animals.

I have written this book for students who have some knowledge of biochemistry, such as is covered in the books by Lehninger or Stryer, and have attempted to bridge the gap between these books and the reviews on plant energetics that have appeared in publications such as *Annual Reviews of Plant Physiology*, which often assume a more complete knowledge than is found in elementary books.

I wish to acknowledge the help of Marjory Dennis and Florence Mansfield in drafting the diagrams, and to thank Dave Layzell and Kerry Walsh for reading the manuscript. I would like to thank Queen's University for providing pleasant and hospitable surroundings in which to work, and the Natural Sciences and Engineering Research Council for funding my research over the years.

Finally, I wish to apologize to my wife, Marjory, and to my sons, Roger and Bruce, for my neglect of them while I wrote, and to thank them for their support. It will not happen again—at least, not for two weeks.

DTD

*This book is dedicated to my father  
THOMAS RICHARD DENNIS  
who taught me more than any professor*

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## CHAPTER ONE

# THE NATURE OF ENERGETICS

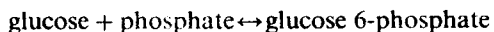
### 1.1 Introduction

The second law of thermodynamics states that in a closed system, that is, one isolated from its surroundings, the entropy or randomness of the components of a reaction must always increase. This simple law has led to all kinds of misconceptions about living things; it has even been used to argue that life has special properties that place it outside of normal chemical reactions, and furthermore to indicate that evolution is impossible. The fallacy of this argument lies in the fact that life is not a closed system but must at all times interact with its environment to obtain energy, not only to grow, but also to maintain its complex structure. Without this constant supply of energy in the form of light, reduced inorganic or organic food, an organism would clearly be seen to obey the second law and rapidly disintegrate into randomness.

The basic problem that leads to these misconceptions is the perception of the nature of energy itself, and the manner in which it is presented in textbooks. It is, in fact, much more useful to consider the role of energy in terms of what is important in living systems, namely the equilibrium position of the metabolic reactions. All organisms are ultimately derived from simple materials such as carbon dioxide, inorganic nitrogen and water. These may be obtained directly, as is the case with auxotrophs, or indirectly by heterotrophs through a complex series of interactions in the food chain. Without an input of energy, living organisms would disintegrate into these simple starting materials. In other words, the equilibrium positions of the reactions in the non-living state favour the starting materials. Energy is used to reverse the unfavourable equilibrium of the reactions that make up the pathways of the organism so that its structure can be synthesized and maintained.

## 1.2 Equilibrium positions of reactions

A metabolic pathway is made up of a series of reactions. For any of these reactions, it is important to determine the equilibrium position. As an example, one reaction that is central to all living organisms is the addition of phosphate to glucose:



An examination of this reaction in isolation tells us nothing about its equilibrium position. This is determined by a number of factors. The reaction will tend to move in the direction in which entropy increases and in which enthalpy or heat is lost. A prime consideration, however, is the concentration of the reactants, since any reaction can be made to favour the end products simply by increasing the concentration of the reactants.

Most biochemists are not interested in the details of the mechanism of a reaction, but are more concerned about its overall equilibrium. A measure of the equilibrium position is the free energy change ( $\Delta G$ ) that takes place when a reaction proceeds in either direction. It is related to the enthalpy and entropy changes by the equation

$$\Delta G = \Delta H - T \Delta S$$

where  $\Delta H$  is the enthalpy change,  $T$  is the absolute temperature and  $\Delta S$  is the entropy change.

The actual free energy change of a reaction is dependent upon the nature of the reaction, the temperature and the concentration of the reactants. In order to be able to compare reactions, the conditions are usually standardized so that the concentration of substrates is considered to be 1 M, the temperature 25°C and the pH 7.0. These exact conditions are never found in a cell, and this must always be kept in mind when discussing cellular reactions. However, under standard conditions reactions can be compared and the free energy change becomes the standard free energy change  $\Delta G^0$ . This is defined as the free energy change that occurs when 1 mole of substrate is converted to 1 mole of product, when all concentrations are at 1 M, except for the hydrogen ion concentration, which is at pH 7.0 ( $10^{-7}$  M).

The standard free energy change of the glucose  $\rightarrow$  glucose 6-phosphate reaction is + 13.8 kJ/mol, which means that 13.8 kJ of energy must be supplied to the reaction to convert 1 mole of glucose to glucose 6-phosphate. Or conversely, 13.8 kJ of energy will be liberated when 1 mole of glucose 6-phosphate is converted to inorganic phosphate and glucose.

Reactions will only proceed spontaneously in a direction in which free energy is liberated. Hence, the above reaction will not proceed spontaneously towards glucose 6-phosphate under standard conditions; in fact, under these conditions the reaction in the opposite direction, towards the formation of glucose, is favoured. This does not mean that glucose 6-phosphate cannot be formed under any conditions. For example, if the concentration of glucose and phosphate were exceptionally high, at zero concentration of products, then glucose 6-phosphate could be formed. However, the concentration of glucose and phosphate that would be required to maintain the concentration of glucose 6-phosphate that is normally found in a cell would be very high; outside the range that can be tolerated by a cell, and beyond the solubility of inorganic phosphate.

The main value of knowing  $\Delta G^{0'}$  is that it is related to the equilibrium constant of the reaction by the equation

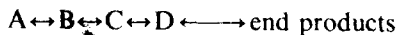
$$\Delta G^{0'} = 2.3 RT \log K_{eq}$$

where  $R$  is the universal gas constant,  $T$  the absolute temperature and  $K_{eq}$  the equilibrium constant for the reaction.

We can calculate that the equilibrium position of the above reaction is approximately 7000:1 in favour of glucose and phosphate. If the cell were to maintain glucose 6-phosphate at a concentration of 1 mM and glucose at 10 mM (concentrations that might be expected in animal cells) then the concentration of phosphate would have to be over 1000 M, an impossible value. Obviously, the cell must have a means of overcoming this problem: this forms the whole basis for the study of energetics in both plants and animals.

### 1.3 The solvent capacity of cells

The above discussion provides an example of the major problem facing living systems. In the cell, simple molecules are converted into complex molecules by a series of reactions known as a metabolic pathway. This can be shown schematically as



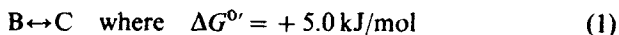
All the reactions are reversible, and the majority will have an equilibrium position that will enable the reaction to proceed in either direction. Although a few may favour the forward direction, many more will favour the starting materials. This overall unfavourable equilibrium of the pathway could be overcome by increasing the concentration of the starting

materials. However, enormous concentrations of these metabolites would be required, often beyond their normal solubilities or even the concentrations of the pure compounds, as was shown by the example of glucose 6-phosphate above. A further limitation is the solvent capacity of a cell. Within a cell there are thousands of small molecules in addition to the numerous complex polymers of carbohydrates, proteins and nucleic acids. All these molecules require water in which to dissolve. It has been calculated that, at least in a mitochondrion, there is practically no free water, since all of it is bound to the molecules within the organelle. It has been suggested by Atkinson (1977) that the lack of free water, or the problem associated with the limitation of solvent capacity, is a major hurdle for living systems.

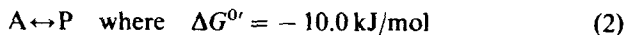
In practice, to overcome this problem of limited solvent capacity, a cell maintains the concentration of starting materials and the intermediates of pathways as low as possible. This means that the equilibrium position of the pathways must be made to favour the end products rather than the starting materials or intermediates. It is the mechanism of keeping these concentrations low that forms the basis of bioenergetics.

#### 1.4 Coupled reactions

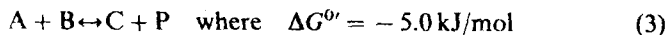
The equilibrium position of a reaction cannot be changed in itself, but the objective can be achieved by modifying the reaction by adding components to each side. Consider the following hypothetical reaction:



The equilibrium position of this reaction will be far to the left. If we now add the components of the reaction



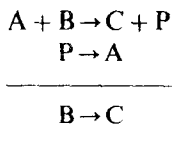
in which the equilibrium lies very far to the right, to each side, the equation becomes



The equilibrium remains in favour of the right-hand side, and the two reactions are said to be coupled. The highly favourable equilibrium of (2) enables the equilibrium of (3) to favour the end products, allowing the production of C from B.

If in turn A can be regenerated from P, we obtain the following sequence of reactions in which single arrows are used to indicate the equilibrium

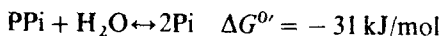
positions of the reactions:



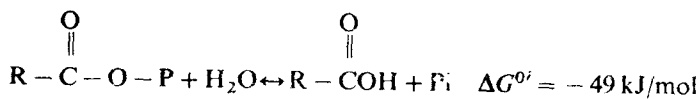
The equilibrium position of reaction (1) has now been made to favour the product C. Although reactions (1) and (2) are said to be coupled, they can only be separated in theory. In the cell they would occur as one reaction, as shown in (3). Many of the reactions in the cell are of this nature, so that the overall equilibrium position of the biosynthetic pathways can be made to favour the cellular products.

### 1.5 Phosphate as an intermediate

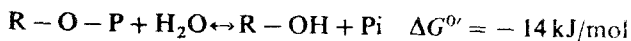
In all living organisms, phosphate is used as an intermediate in the majority of coupled reactions. There are many properties of phosphate that uniquely suit it for this role. In the first place, phosphoric acid will form an acid anhydride bond with itself to give pyrophosphate (PPi). This is unstable and will hydrolyse back to inorganic phosphate (Pi), the equilibrium position being in favour of inorganic phosphate:



Phosphoric acid will also form mixed acid anhydrides with other acids that are even more unstable than pyrophosphate itself:



In addition to forming acid anhydride bonds, phosphate can readily form esters with a whole range of alcohols that are more stable than the acid anhydrides:



Phosphate can also form compounds with sulphur and nitrogen. It is the versatility of phosphate in combining with a whole range of compounds that makes it of such great importance in living organisms. Of even greater relevance, however, are the great differences in the standard free energy

of hydrolysis of the various phosphate compounds that enable phosphate to act as an intermediate in many chemical reactions. It is difficult to imagine life without phosphate, just as it is impossible to visualize life without carbon. No other molecule has the versatile properties of phosphate for energy coupling.

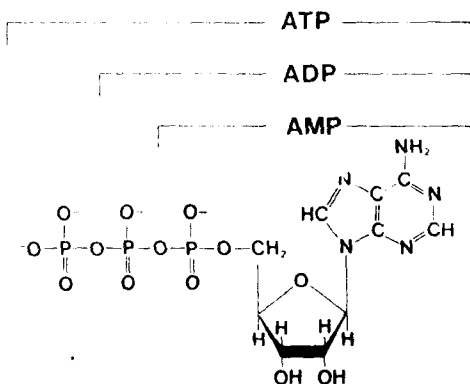
### 1.6 Adenosine triphosphate

Inorganic phosphate is present in all cells and plays a vital role in metabolism. Certain organisms, such as some bacteria and possibly all plants, also use inorganic pyrophosphate as an energy source. However, in most cases pyrophosphate is not used itself, in coupled reactions, but is linked to adenosine through a third phosphate to form adenosine triphosphate (ATP) (Figure 1.1). It must be recognized, however, that it is the terminal pyrophosphate group that is involved in the majority of reactions. The free energy of hydrolysis of the terminal acid anhydride bond is not significantly different from that of pyrophosphate itself.

In many reactions ATP acts as a phosphoryl donor, donating the terminal phosphate to a series of compounds and leaving as a product adenosine diphosphate (ADP). Hence, in the reaction discussed earlier ATP donates its terminal phosphate to glucose to form glucose 6-phosphate in a reaction that is catalysed by the enzyme hexokinase:

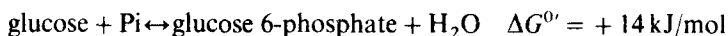


The  $\Delta G^{\circ}$  for this reaction is  $-17 \text{ kJ/mol}$  and can be thought of as being



**Figure 1.1** The structure of adenosine triphosphate (ATP). The structures of adenosine diphosphate (ADP) and adenosine monophosphate (AMP) are also indicated.

made up of two reactions:



It should be remembered, however, that the reaction occurs on the surface of the enzyme and the phosphate is transferred directly from the ATP to the glucose.

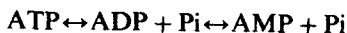
The second bond in ATP can also be hydrolysed with a similar free energy of hydrolysis to the first one to yield adenosine monophosphate (AMP) and pyrophosphate. In some reactions this acid anhydride bond is used. However, hydrolysis of the third bond with the formation of AMP is accompanied by a smaller free energy change since this is an ester bond.

The acid anhydride bond in ATP is often referred to as a high-energy bond. This is inaccurate usage since, in chemistry, a high-energy bond is a stable bond, quite the opposite from the bond in ATP. Similarly, ATP is often called a high-energy compound. This is more acceptable, but it still can lead to some confusion. To be effective as an intermediate in energy reactions, not only must ATP be able to donate a phosphate to other compounds, but also it must be possible to reform ATP from ADP and Pi. The free energy of hydrolysis must, therefore, occupy an intermediate position. It must be high enough for it to act as a phosphoryl donor but not too high to prevent ADP from accepting a phosphate and reforming ATP.

The high free energy of hydrolysis of ATP results from a number of factors. The first, and most important, is the stabilization of the products, especially Pi, by resonance. Resonance in ATP is reduced because of the limitation imposed by the acid anhydride bond. When the terminal phosphate is released the potential for resonance is greatly increased, making the products much more stable. The second factor contributing to the instability is the proximity of similar charges within the ATP molecule. Likewise, the repulsion of the charges on ADP and Pi inhibit their combination. Finally, on hydrolysis a proton is released which at pH 7.0 tends to pull the reaction in favour of hydrolysis.

### 1.7 The phosphate potential or energy charge

The two terminal acid anhydride bonds in ATP have a high free energy of hydrolysis. The system can be described by the following equation:



It can be compared with a battery that can be either fully charged (all the adenylate as ATP) or fully discharged (all the adenylate as AMP). Atkinson (1977) has suggested that when all the adenylate is present as ATP the energy charge should have a value of 1. When it is all present as AMP then the charge should be 0. The importance of ATP in maintaining the integrity of a cell can be judged from the fact that, in a healthy cell, the energy charge is always controlled at a value of between 0.8 and 0.9; in other words, most of the adenylates are present as ATP. At values significantly below this the cell dies.

A discussion of the energetics of living systems is in reality a description of coupled reactions. Energy is supplied by sunlight and, through a series of coupled reactions, is ultimately used in biosynthetic pathways in both plants and animals to reverse reactions with unfavourable equilibria so that the overall equilibrium of a pathway favours the end product. The most commonly used molecule involved in the transfer of energy in these coupled reactions is ATP, so that energetics can be reduced essentially to two components. In the first place, it is necessary to describe the mechanism by which ATP is involved in the reaction of enzymes. Secondly, it is important to investigate the means by which ATP can be resynthesized from ADP and inorganic phosphate.

The synthesis of ATP in turn can be considered to be composed of two parts. In the first place, ATP is synthesized directly, utilizing the energy derived from sunlight in the process of photosynthesis which occurs in plants and some prokaryotes. Secondly, in animals, in plants in the dark, and in non-photosynthetic prokaryotes, ATP synthesis is coupled to the degradation of either ingested or stored food material. In this latter process, the complex molecules of the food material are degraded to simple molecules, carbon dioxide and water. Therefore, even though the complex molecule, ATP, is formed and subsequently used to synthesize other complex molecules, the overall entropy of the system increases.

In all the processes of energetics, some of the most fundamental reactions involve the transfer of electrons in what are referred to as oxidation-reduction reactions. The initial reactions of photosynthesis result in the synthesis of highly reduced compounds. Hence, the understanding of energetics requires a knowledge of oxidation-reduction reactions and these are discussed in the next chapter.

More detailed information on the topics covered in this chapter can be found in, for example, Goodwin and Mercer (1983), Lehninger (1971), Morowitz (1978), Morris (1974), Smith *et al.* (1983) and Stryer (1981).



## CHAPTER TWO

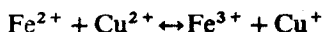
# OXIDATION-REDUCTION REACTIONS

### 2.1 Introduction

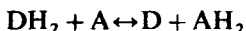
Reactions involving the transfer of electrons from one compound to another are of the most fundamental importance in all living systems. Photosynthesis generates compounds that are powerful reducing agents, and from them electrons are transferred through a series of compounds ultimately to oxygen in either animal or plant mitochondria.

Oxidation-reduction reactions involve the transfer of electrons from the reductant, which is oxidized in the process, to the oxidant which is reduced. It is the transfer of the electron or electrons that is the essential part of the reaction, even though hydrogen atoms or hydride ions may in fact be the actual unit that is moved. Oxidation-reduction reaction may take the following forms in living systems:

- (i) The transfer of an electron between two metal ions that may be bound into a biological molecule, for example



- (ii) The transfer of hydrogen atoms from the reductant to the oxidant, for example



where D is a donor molecule and A is an acceptor.

- (iii) The transfer of a hydride ion ( $\text{H}^-$ , equivalent to  $\text{H}^+$  plus  $2\text{e}^-$ ), for example

