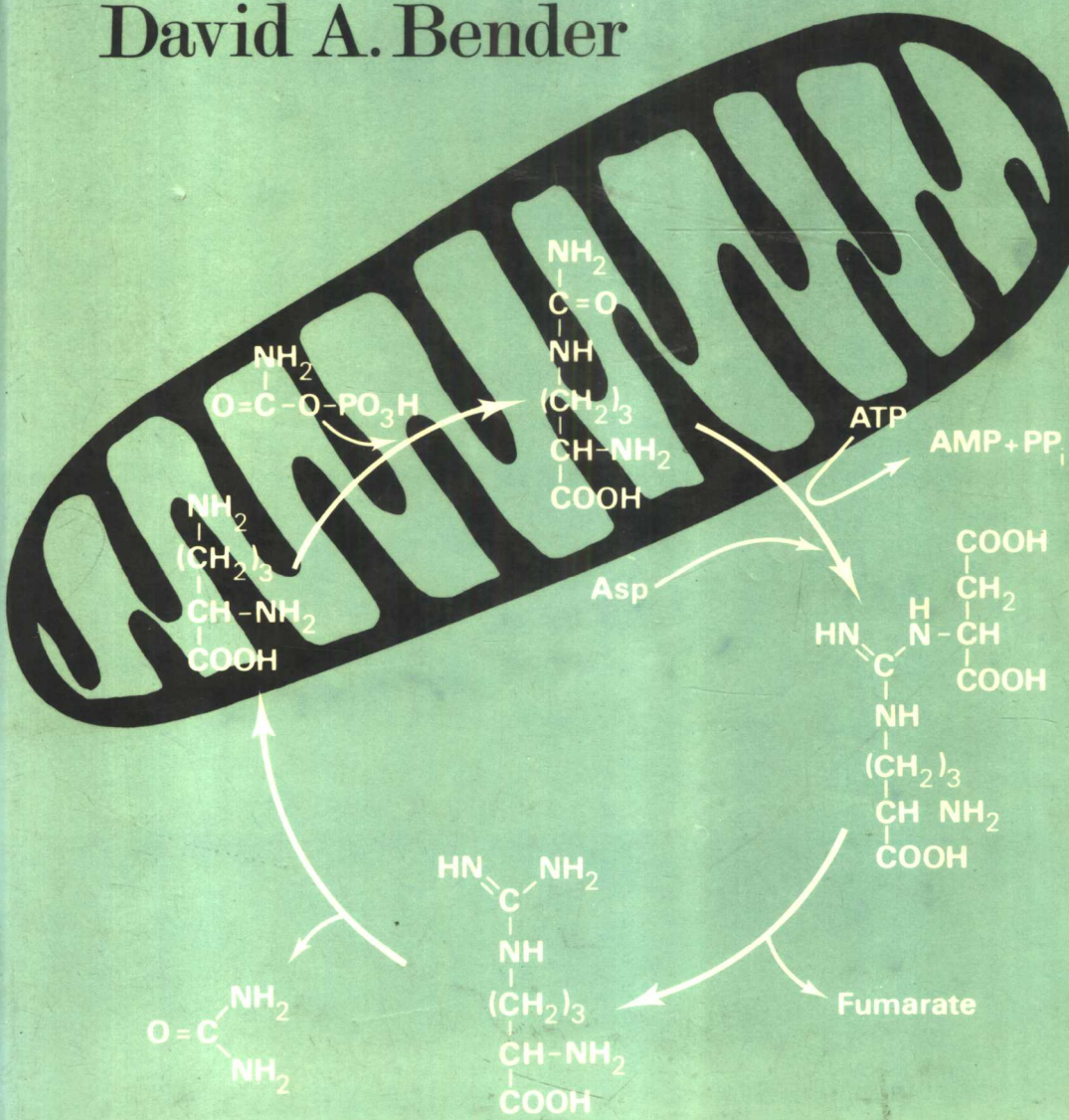
Second Edition

Amino Acid Metabolism

David A. Bender



Amino Acid Metabolism

SECOND EDITION

David A. Bender

Courtauld Institute of Biochemistry,
The Middlesex Hospital Medical School,
London

A Wiley–Interscience Publication

JOHN WILEY & SONS

Chichester · New York · Brisbane · Toronto · Singapore

Library of Congress Cataloging in Publication Data:

Bender, David A.
Amino acid metabolism.
(A Wiley-Interscience publication.)
Includes index.
1. Amino acids—Metabolism. I. Title.
[DNLM: 1. Amino Acids—metabolism. QU 60 B458a]
QP561.B46 1985 574.19'245 84-26941

ISBN 0 471 90668 9

British Library Cataloguing in Publication Data:

Bender, David A.
Amino acid metabolism.—2nd ed.–(A Wiley–Interscience publication)
1. Amino acids—Metabolism
I. Title
574.1'33 QP561

ISBN 0 471 90668 9

Printed in Great Britain

Preface

I am probably biased, because it is my specialist field of research, but I consider that the metabolism of amino acids is one of the more fascinating areas of biochemistry. While there are a number of specialist reference works that cover the field in an encyclopaedic manner, and reviews frequently appear in the various secondary journals, these are generally directed at the specialist research worker, and are more detailed than a student would want, or need, to read. At the same time, the coverage of amino acid metabolism in most undergraduate text-books of biochemistry is necessarily brief. I have written this book in the hope of filling the gap and providing a book that will be useful to both undergraduate and post-graduate students.

Because this is a book on a specialized aspect of biochemistry, it does not contain very much general biochemistry—I have assumed that readers will have a general understanding and knowledge of the principles of enzymology, metabolic biochemistry and molecular biology. An appropriate level of knowledge would be that of the final year undergraduate in a British Honours B.Sc. course, or a post-graduate student taking a Master's degree.

Comparative studies of biochemistry aid our understanding of evolution and the differentiation of species, but in general the major interest of biochemistry lies in its application to clinical and veterinary medicine, agriculture and, increasingly, to the industrial synthesis of a wide variety of compounds ranging from detergents and artificial sweeteners to drugs and pesticides. Through the history of biochemistry a number of different organisms have been studied for various reasons: yeasts have an obvious commercial importance; fungi such as *Neurospora crassa* are easy to maintain in culture; *Escherichia coli* is a convenient bacterium which is not particularly pathogenic; the rat is widely used as a conveniently small and friendly mammal; and more recently a number of human cell lines (mostly derived from tumours) have been used. We hope that the biochemistry we study in these different systems is sufficiently close to normal human biochemistry to permit us to make useful extrapolations from the laboratory to the human

situation. To a great extent this hope is justified. Where the biochemistry of bacteria, parasites and tumours differs from that of man and his crops and domestic animals, we have a key to controlling and treating disease.

I have attempted in this book not only to give an insight into the fascinating area of amino acid biochemistry, but also to indicate where the information is of relevance to the solution of human problems. I hope that I have shown that the study of basic biochemistry is not merely academic self-indulgence, but is justifiable in the terms of the most rigorous criteria of relevance and economic necessity.

I hope that I have steered the correct course between rigorous systematic nomenclature and common usage, and that the trivial names I have used are acceptable. I have avoided undefined abbreviations other than those which journals such as the *Biochemical Journal* permit without definition. The references I have cited are not intended to be exhaustive, but are to key papers and reviews that should give the reader ready access to the literature.

In the decade that has elapsed since the first edition of this book was written, the major advances in metabolic biochemistry have been in our understanding of the mechanisms of enzymic catalysis and regulation. I hope that I have done justice to these advances in this second edition, and that this book will prove useful to students and research workers alike.

August 1984 DAVID A. BENDER

Contents

CHAPTER 1

Nitrogen Metabolism

Antoine Lavoisier gave the name 'azote' to the gas nitrogen when he discovered it in 1787, meaning 'without life', because of both its lack of chemical reactivity and its inability to support life. Nevertheless, the metabolism of nitrogenous compounds is central to the metabolic processes of all living organisms.

Micro-organisms and plants are capable of using simple inorganic nitrogen compounds such as nitrogen gas, ammonia, nitrites and nitrates, and incorporating these into such organic compounds as amino acids, purine and pyrimidine nucleotides, amino sugars and a variety of co-factors and coenzymes that are vitamins for animals. Animals are not able to make use of inorganic nitrogen compounds to any significant extent, but must receive organic nitrogen compounds in the diet. These are taken in largely as proteins, although dietary nucleic acids and other organic nitrogenous compounds can also be used to a greater or lesser extent. Ruminants are able to make use of inorganic sources of nitrogen, indirectly, because of their large intestinal population of commensal bacteria. This is economically important, since chemically synthesized urea fed to ruminants releases large amounts of more expensive protein-rich oil-seed cake and single cell proteins for consumption by man and monogastric livestock. The waste products of nitrogen metabolism excreted by animals are generally relatively simple organic compounds (urea or purines), together with moderate amounts of ammonia and other inorganic salts.

Thus, nitrogen metabolism can be seen as a cyclic process, with the fixation of nitrogen gas into a usable form (ammonium ions) by micro-organisms, the assimilation of this into organic compounds by micro-organisms and plants, the interconversion of these primary nitrogenous compounds into the variety of metabolites required by micro-organisms, plants and animals, and finally the excretion of waste products of nitrogen metabolism, and their further catabolism to ammonia, nitrites and nitrates and even back to nitrogen gas, by chemo-autotrophic micro-organisms. This is the 'nitrogen cycle', shown in Figure 1.1.

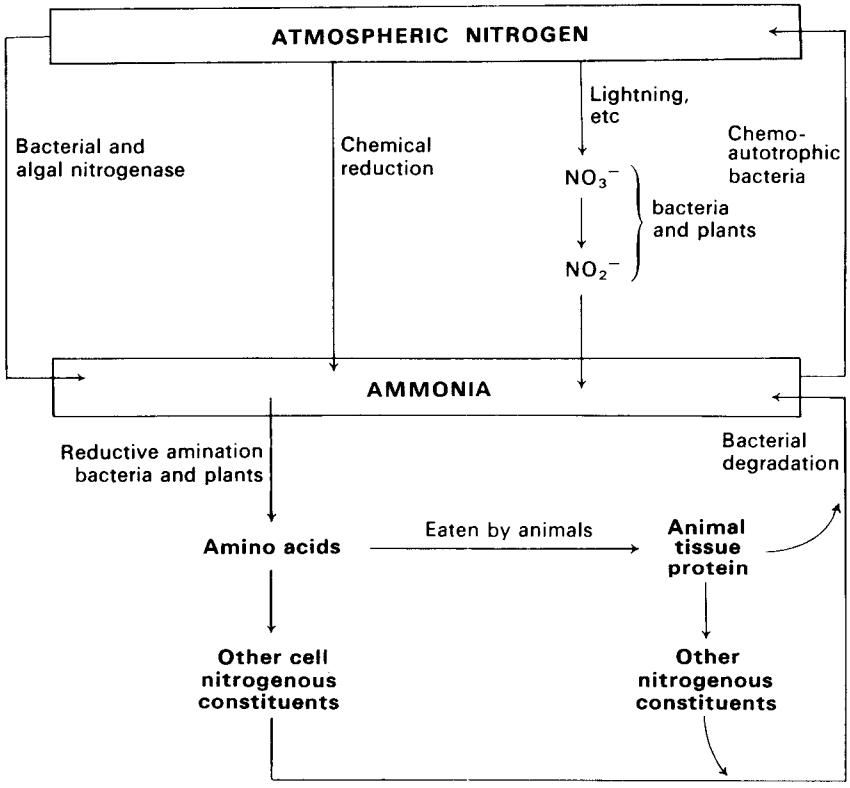


Figure 1.1. The nitrogen cycle

THE ASSIMILATION OF INORGANIC NITROGEN

Nitrogen fixation

The N ≡ N bond is extremely resistant to chemical attack, with a bond energy of 0.94 MJ (225 kcal) per mol. This stable bond must be broken before any metabolic use can be made of gaseous nitrogen, which makes up some 78% of the atmosphere. Chemically the bond can be broken for example by burning magnesium in air, when small amounts of magnesium nitride are formed, as well as the oxide, or by catalytic reduction with hydrogen at a temperature in excess of 300 °C and under several hundred atmospheres pressure—the Haber–Bosch chemical fixation of nitrogen which is the basis of the synthetic fertilizer industry.

The overall fixation of nitrogen into soluble ammonium salts, nitrites and nitrates, by all means, is of the order of 10^9–10^{10} tonnes per year. About 2×10^7 tonnes are accounted for by chemical reduction, and a further 5×10^7 to 50×10^7 tonnes by the formation in the atmosphere of oxides of nitrogen, which are washed into soil and water as nitrites and nitrates. While

most of this atmospheric oxidation is natural, the result of lightning, the contribution made by high compression internal combustion engines cannot be ignored. It has been estimated that in the United States as much as 3×10^6 tonnes of nitrogen may be fixed by automobiles, compared with about 8×10^6 tonnes from the American fertilizer industry. The remaining 9×10^7 tonnes of nitrogen fixed annually is the result of bacterial action.

The only organisms capable of fixing nitrogen from the atmosphere into forms that can be used by other organisms are the relatively primitive prokaryotic bacteria and some of the blue-green algae. Most studies of nitrogen fixation have been carried out using free-living organisms, because their system is less complex, and more amenable to investigation, than those of the photosynthetic organisms or the plant—micro-organism symbionts. However, these latter two groups of organisms make a greater contribution to nitrogen ecology than do free-living nitrogen fixers.

Table 1.1. Some organisms capable of fixing nitrogen

Free-living organisms	
Obligate aerobic heterotrophs	*Azotobacter* and *Mycobacterium* spp.
Facultative anaerobic heterotrophs	*Klebsiella pneumoniae* *Bacillus polymyxa*
Obligate anaerobic heterotrophs	*Clostridium pasteurianum* *Clostridium butyricum*
Facultative anaerobic photo-autotrophs	*Rhodospirillum rubrum* *Rhodopseudomonas palustris*
Obligate anaerobic photo-autotrophs	*Chromatium* spp. *Chlorobium* spp.
Aerobic photo-autotrophs	Blue-green algae, including *Anabena, Nostoc* and *Plectonema*
Symbiotic associations	
Blue-green algae with	Fungi (the lichens) Liverworts *Azolla* and tropical grasses
Bacterial symbionts with higher plants	*Klebsiella* in leaf nodules *Azotobacter* in roots and leaves *Rhizobium* in legume root nodules *Frankia* in non-legume root nodules

Nitrogen fixation is found in organisms occupying a wide variety of ecological niches, as can be seen in the list in Table 1.1. Both obligate anaerobes (e.g. *Clostridium pasteurianum*) and obligate aerobes (e.g. *Azotobacter vinelandii*) as well as facultative anaerobes such as *Klebsiella pneumoniae* among the heterotrophic bacteria, and a number of autotrophic (photosynthetic) bacteria, including *Rhodospirillum rubrum* and *Chromatium* species, can be classed together as free-living nitrogen fixing organisms. By means

of bacterial mating and bacteriophage transduction it has also been possible to transfer the genetic information for nitrogen-fixation (the *nif* gene) from *Klebsiella pneumoniae* into the free-living *Escherichia coli* (Dixon and Postgate, 1972).

A number of plant–bacteroid symbiont pairs are also capable of fixing nitrogen. The best known is the association between leguminous plants and bacteroids of the genus *Rhizobium*, but a number of other organisms, which have been assigned the genus *Frankia*, form associations with non-leguminous plants. Organisms of these two genera are not capable of independent existence, but are obligate symbionts. By contrast, a number of other organisms (e.g. *Azotobacter* spp.) and blue-green algae, although they are capable of independent existence, frequently form symbiotic associations in leaf nodules of higher plants or around the roots of water plants. The association of *Azotobacter paspulum* with tropical grasses may contribute more than 100 kg of nitrogen per hectare per year, and the symbiotic blue-green algae in rice paddies may fix up to 20 kg per hectare per year. Legumes make a greater contribution to nitrogen ecology; the association of *Rhizobium* in the root nodules of alfalfa may fix up to 500 kg of nitrogen per hectare annually. One of the aims of much current plant research is to encourage the formation of nitrogen-fixing symbiotic associations between suitable organisms and temperate and sub-tropical cereal plants such as wheat, barley, millet, etc.; this would have obvious economic and agricultural advantages.

There are three essential components for nitrogen fixation: the enzyme nitrogenase, which reduces nitrogen to ammonia; a source of reductant for the reaction and an electron carrier to couple the reductant with the enzyme. A relatively large amount of ATP is also required. *In vitro* 15 mol of ATP are required per mol of nitrogen reduced, and *in vivo* the requirement may be higher. In *Clostridium* as much as 30% of the metabolic energy derived from fermentation may be used for nitrogen fixation.

The electron carrier for nitrogen reduction is frequently ferredoxin, the iron–sulphur protein that is found in all photosynthetic organisms. Some bacteria grown in iron-deficient media are capable of forming an alternative electron carrier protein, flavodoxin, which contains flavin mononucleotide rather than iron, and under similar conditions the blue-green algae form phytoflavin, and *Azotobacter* forms azotoflavin. In photosynthetic organisms the source of reductant for nitrogen fixation is the same photoreduction as is used in carbon dioxide fixation, and reduced ferredoxin from a variety of sources can be used as a reducing agent in cell-free preparations in the dark. Plant ferredoxins will react with nitrogenase from bacteria, although plant ferredoxin is a single electron carrier, while the bacterial protein carries two electrons per molecule. The flavoproteins azotoflavin and flavodoxin have also been shown to carry electrons between illuminated chloroplasts and nitrogenase *in vitro*. In non-photosynthetic (heterotrophic) nitrogen-fixing organisms, the reductant is frequently pyruvate, with oxidation linked to the

reduction of ferredoxin or a flavoprotein rather than through lipoamide to NAD, as occurs in other organisms.

Nitrogenase

Nitrogenase from various sources consists of two proteins, a small ferroprotein and a larger protein containing both iron and molybdenum, to which N_2 binds. Both proteins are essential for enzymic activity. They have been purified from a number of sources since the original demonstration of nitrogen fixation in a cell-free preparation from *Clostridium pasteurianum* by Carnahan and coworkers (1960). Although there are considerable species differences, the components of nitrogenase from different species will frequently combine to form active enzymes.

The reduction of N_2 to NH_3 is not the only reaction of nitrogenase. Indeed, it has been suggested that the reduction of nitrogen was not its function in the primitive organisms in which it evolved; in the ammonia-rich primaeval seas such a reaction would have been superfluous, and it is more likely that the most important reaction catalysed by nitrogenase was the reductive detoxication of cyanide.

Nitrogenase also catalyses the reduction of acetylene to ethylene. While the biological usefulness of the reaction is doubtful, it has been widely exploited as an extremely convenient and sensitive method of measuring the activity of nitrogenase, since acetylene can readily be measured by gas chromatography. It also provides a specific test for the presence of nitrogenase, since no other system capable of catalysing this reduction has been detected. It is mainly on the basis of ethylene reduction that the presence of small numbers of nitrogen-fixing organisms has been established among the human intestinal flora.

Nitrogenase is irreversibly inactivated by oxygen, and in all nitrogen-fixing organisms other than obligate anaerobes there are systems to regulate the activity of the enzyme in response to changes in P_{O_2}.

In the obligate aerobe *Azotobacter vinelandii*, the enzyme undergoes a conformational change to an inactive form which is also insensitive to oxygen, as the P_{O_2} rises. *Azotobacter* also has two distinct terminal cytochromes in its electron transport chain, a conventional cytochrome a_1/o, associated with phosphorylation site III, and an alternative, cytochrome a_2 which is not associated with the phosphorylation of ADP to ATP. When the organism is grown under conditions of high oxygen availability, the proportion of cytochrome a_2 increases. This is believed to represent an oxygen scavenging system, in that growth under conditions of high oxygen tension leads to increased use of the electron transport chain with a P : O ratio of 2 rather than 3 for the oxidation of NADH—a partial uncoupling of electon transport and phosphorylation to overcome respiratory control by the availability of ADP, and so maintain the intracellular concentration of oxygen below the level at which nitrogenase would be inactivated.

For the blue-green algae that fix nitrogen, the problem of oxygen inactivation of nitrogenase is more complex, since they release oxygen during photosynthesis. In the filamentous alga *Anabena* this has been overcome by the development of specialized heterocysts along the algal filament. Nitrogen fixation is limited to these heterocycts, which lack the oxygen-producing photosystem II, and have a highly reducing internal environment. The formation of these heterocysts is inversely related to the availability of ammonium salts in the culture medium.

The unicellular blue-green alga *Plectonema*, which can be either free-living or symbiotic in the leaf cavities of the water fern *Azolla*, fixes nitrogen only under conditions of low oxygen tension, and only when the level of illumination is so low that photolysis of water cannot occur. Nitrogenase in *Plectonema* undergoes a conformational change to a protected inactive form under conditions of high oxygen tension, as does that from *Azotobacter*. The symbiotic pair of *Plectonema* in the leaf cavities of *Azolla* can live entirely without an exogenous source of fixed nitrogen, and makes a considerable contribution to the fertility of rice paddies, and may add considerably to the fixed nitrogen content of ponds and canals in temperate regions.

Legume root nodules containing symbiotic *Rhizobium* are aerobic, and the nitrogenase, which is contained entirely in the bacteroids, must be protected from oxygen, while ensuring adequate penetration of oxygen to the actively metabolizing tissue of the root. Leghaemogobin is a haemoglobin-like protein in the root nodules; it both stimulates the nitrogenase activity of the bacteroids and increases oxygen uptake by the nodules. In intact nodules, nitrogenase is inhibited by carbon monoxide, while the enzyme in isolated bacteroids is not; carbon monoxide pretreated leghaemoglobin will not enhance the activity of isolated bacteroids as does the native protein. Therefore Bergerson *et al.* (1973) have suggested that the function of leghaemoglobin in root nodules is to maintain a sufficiently low oxygen tension around the bacteroids to allow nitrogen reduction, while ensuring the transport of adequate amounts of oxygen to the remainder of the tissue for normal metabolism to continue. The kinetics of the association of leghaemoglobin with oxygen support this hypothesis.

The assimilation of nitrate and nitrite

Nitrates applied to the soil as fertilizer, or washed into the soil together with nitrites formed by the atmospheric oxidation of nitrogen, are reduced to ammonia before they are used by plants for amino acid synthesis. The two enzymes involved, nitrate reductase (which forms nitrite) and nitrite reductase (which forms ammonia), are widely distributed in plants and microorganisms.

The intermediate formation of nitrite from nitrates has given rise to concern about pollution of drinking water supplies by nitrates used as fertilizers. Nitrite, either in the water or formed by intestinal bacteria, is capable

of combining irreversibly with haemoglobin to give the biologically inactive methaemoglobin. Fetal haemoglobin (HbF) is especially sensitive to nitrite, and methaemoglobinaemia resulting from nitrate in the drinking water and some foods is a problem in some parts of the world. The presence of nitrites in foods is also potentially hazardous, since under the acid conditions of the stomach small amounts of carcinogenic nitrosamines may be formed by reaction between nitrite and dietary amines. Nitrates do not present the same hazard, since the reduction of nitrate to nitrite occurs by bacterial action in the large intestine.

Nitrate reductase in plants is a molybdeno-flavoprotein which uses ferredoxin as the intermediate electron carrier from the photosynthetic light reaction, which is the ultimate reductant for the reaction, coupled through NAD or NADP.

The six-electron reduction of nitrite to ammonia occurs without any detectable intermediates in algae and green tissues of higher plants. Nitrite reductase is a ferro-protein in the chloroplasts; the photosynthetic light reaction is again the source of reductant, and the reaction uses ferredoxin as the electron carrier.

As well as using the photosynthetic light reaction as a source of reductant for both nitrate and nitrite reductases, some plants show a requirement for light for the synthesis of the enzymes. Etiolated rice seedlings require about 3 h after exposure to light before there is any detectable activity of either enzyme. By contrast, seedlings that have been raised in the light and then kept in the dark for up to 36 h show no such lag in the activation of the reductases after exposure to light.

While photosynthetic organisms generally reduce nitrate and nitrite at the expense of photosynthetically generated reductant to allow the incorporation of inorganic nitrogen into tissue components, fungi and heterotrophic bacteria use nitrate and nitrite as terminal electron acceptors under conditions of low oxygen availability. The production of usable nitrogenous compounds is coincidental.

In *Klebsiella aerogenes*, the same nitrate reductase can function either aerobically for nitrogen assimilation, when the nitrite produced is further reduced to ammonia, or anaerobically as a terminal electron acceptor, when the nitrite accumulates. In anaerobic respiration cytochrome *b* is used as the electron carrier, as in normal oxygen-terminated respiration in this organism, while in nitrate assimilation the cytochrome is not used, and the reaction requires metabolic energy. Under aerobic conditions there is competition between oxygen and nitrate for cytochrome *b* and the electrons generated by substrate oxidation (van't Riet *et al.*, 1972).

In the mould *Neurospora crassa*, nitrate reductase is again an alternative to oxygen as the termination of electron transport. The enzyme consists of two proteins, a particulate molybdenum-containing protein which is constitutive and a nitrate-inducible protein which solubilizes the particulate enzyme and has NADPH-cytochrome *c* reductase activity. The inducible protein will