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The oxygen reactivity of bacterial respiratory haemoproteins: oxidases and globins

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1. Introduction

This summer marks the 70th anniversary of David Keilin's classic spectroscopic observations of the reversible oxido-reductions of 'cytochrome'. His conclusion that "cytochrome is not only widely distributed in nature ... and... much more important than haemoglobin" [1] was based on direct observations of yeast, insect tissues and cultures of *Bacillus subtilis* on which larvae of the fly *Gasterophilus* were fed. All these sources of respiring material displayed the same four-banded absorption spectrum, which responded similarly to anoxia and aeration. It is tempting to speculate whether this seminal conclusion on the universality of cytochrome would have been reached so early or so emphatically had the larvae been fed, not on *Bacillus*, but on *Escherichia coli* or *Azotobacter vinelandii*, the subjects of much recent work from these laboratories! These two Gram-negative bacteria exhibit features in the red region of the visible spectrum [2,3] quite distinct from that of *B. subtilis* (at least under most growth conditions). This spectral region is dominated by the characteristic absorbances of cytochrome *d*, its oxygen-bound intermediates, and its ligand-binding partner, cytochrome *b*-595. Indeed, the prize that eluded Keilin was the realization that cytochromes aa_3 and, in certain bacteria, the functionally equivalent haemoproteins, such as cytochrome *d*, were themselves oxygen-binding and -reducing proteins and synonymous with the elusive 'respiratory ferment' [1,4]. Subsequent measurements by Chance of the photochemical action and photodissociation spectra of animal and microbial materials resolved this issue and laid the foundations for almost half a century of work on the oxygen reactivity of terminal oxidases.

Keilin's contributions to the study of cytochromes (by definition, haem proteins that undergo reversible redox changes during normal function) are well-known. However, he also described for the first time in microorganisms [1] another class of haem protein, haemoglobin, on which research has lain fallow for many years. Here the most characteristic feature of the haem is not its reversible redox changes but the ability to bind oxygen reversibly. Studies of the cellular functions of microbial globins are in their infancy, but the diversity of protein types with which an oxygen-binding haem is associated suggests a rich diversity of function.

Oxygen is the most effective terminal oxidant for cell respiration. Its 'fitness' for respiration derives from its desirable combinations of kinetic and thermodynamic properties and a satisfactory solubility in water. However, characteristic of the remarkable metabolic flexibility of many bacteria is their ability to use, under appropriate anoxic conditions, other acceptors as terminal oxidants [5–7]. In anaerobic respiratory chains, the oxygen-reducing terminal oxidase is replaced by a cognate reductase and the respiratory apparatus augmented with soluble, frequently periplasmic, reduc-

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tases and cytochromes. Nevertheless, the supremacy of oxygen as oxidant is illustrated by the complexity of regulatory mechanisms exhibited by a facultative aerobe like *E. coli* to ensure that oxygen is used in preference to nitrate, fumarate or alternative acceptors [6,7]. The regulation of adaptation to the presence or absence of oxygen has been the subject of intensive study. In *E. coli*, numerous operons have been classified with respect to the global regulatory mechanisms to which they respond [6]. Thus, the *cyoABCDE* operon (encoding the cytochrome-*o*-containing oxidase) and the *cydAB* operon (encoding the cytochrome-*d*-containing oxidase) each belong to the ArcB/ArcA and Fnr modulons and the former genes are also regulated by Cya/Crp [6]. The consequence of this control is that cytochrome *o* predominates aerobically [8] and cytochrome *d* expression is maximal under microaerobic conditions [9]. However, regulation of gene expression, although essential, may not be sufficient for adequate control of electron flux (see below).

The diverse terminal oxidases may be classified according to (i) the nature of their immediate reductant, i.e., a quinol or cytochrome *c*, and (ii) the nature of the redox centres at which oxygen is bound and reduced. In the archetypal cytochrome *c* oxidase of mitochondria and many bacteria, one haem (a_3) and one copper atom (Cu_B) constitute a binuclear centre. Sharing this feature are other members of the haem-Cu super-family, notably the cytochrome *bo*-type oxidase of *E. coli* [1] [10]. Both are proton pumps and share many features of the oxygen-binding and -reducing reactions, including the initial, transient formation of an oxygenated species and, probably, successive two-electron donations to the bound oxygen yielding water. Interestingly, however, low temperature kinetic studies of the *E. coli bo*-type oxidase reveal a much slower 'on' rate for oxygen binding and a much smaller dissociation constant [12]. Both oxidases, however, have relatively high affinities for oxygen (in the μM range) [2]. Those features of the architecture or electron transport mechanisms in cytochromes aa_3 and *bo'* that are responsible for the kinetic differences have yet to be described. The alternative quinol oxidase of *E. coli* and its counterpart in *A. vinelandii* is the cytochrome *bd* complex. These have received more attention in our laboratories recently and are the main subject of this contribution.

[1] Nomenclature. The realization that many bacterial oxidases do not reoxidize a cytochrome (e.g., cytochrome *c*) but rather a quinol, implies that care is required in oxidase nomenclature. Thus 'cytochrome *bd* oxidase' is nonsensical: this oxidase does not oxidize cytochrome *bd*; rather it *is* the cytochrome and oxidizes quinol. It should be described, for example, as a cytochrome *bd*-type quinol oxidase. A primed cytochrome name implies that the haem is ligand-binding, as in *bo'* or *bb'd'*. This designation may be preferable to subscripts, as in o_3[11].

2. Results and discussion

2.1. Terminal oxidases of the cytochrome bd type

These oxidases have several distinguishing features. Foremost among these are (i) the ability to react with oxygen forming remarkably stable oxy [13] and ferryl [14] compounds, (ii) the absence of a copper centre analogous to Cu_B with the implication that the site(s) and mechanism for ligand binding [15] and oxygen reduction [3] must be fundamentally different from the haem-Cu super-family [3,10,12], (iii) relative insensitivity to respiratory inhibitors and divalent metal cations [16], and (iv) the remarkable catalytic capabilities (high affinity and/or high V_{max}) claimed for cytochrome *bd*-type oxidases in several bacteria [2].

2.1.1. Assembly of the E. coli cytochrome bd-type oxidase

This oxidase comprises two polypeptide subunits and three haem types, probably in a 1:1:1 stoichiometry (for references, see [15]). The structural genes *cydA* and *cydB* were cloned and sequenced by Gennis et al. and the predicted amino sequences used to propose a model for the transmembrane organization of the protein [17]. However, two other genes are necessary for synthesis of the oxidase complex. *cydD* mutants were isolated by direct spectroscopic screening for loss of the characteristic 'red' bands of cytochrome *d* and the mutation was mapped by three-point crosses to the 19.3 min region of the *E. coli* chromosome [16] close to the *cydC* locus [18]. Mapping of this region [19] has been claimed to result in the localization of another gene *'htrD'* apparently involved with cytochrome *bd*. However, cloning and sequencing of both *cydC* and *cydD* on a single 5.3 kb fragment and complementation analysis show that *'htrD'* is truncated *cydD* [20]. *cydD* and *cydC* encode proteins with predicted molecular masses of 65 and 63 kDa, respectively. The hydropathy profile of each reveals [20] an N-terminal hydrophobic domain and a C-terminal hydrophilic domain containing a putative ATP-binding site. It is probable that the *cydDC* operon encodes a membrane transporter in the ABC (ATP binding cassette) family [21]. Two separate integral membrane proteins are envisaged as being necessary for function as in the haemolysin exporter [22], but the CydDC system appears to be the first prokaryotic example of a *heterodimeric* ABC transporter. No such genes have been described or, to our knowledge, sought in *A. vinelandii*.

Analysis of the predicted protein sequences strongly suggests that the function of the transporter is export, as reported for a growing number of apparently related systems [22]. But what is the translocated substrate? Studies of *cydC* [18] and *cydD* mutants [23] reveal that the oxidase subunits are assembled into the membrane in the absence of haem. Furthermore, *cydD* is not

required for synthesis of haem D [23]. Our recent work shows that cytochromes other than the *bd*-type oxidase are affected in *cydD* mutants: periplasmic cytochrome(s) *c* [24] and cytochrome *b*-562 (Poole, R.K., unpublished data) are not assembled. Since other *b*-type cytochromes and the cytochrome *bo'* quinol oxidase *are* assembled in such mutants, a compelling hypothesis is that haems are exported to the periplasm. Indeed, a widely held view of cytochrome *c* assembly is that haem C is exported to the periplasm where cytochrome *c* haem lyase inserts haem into separately exported apoprotein. Transmembrane movement of haem may be slow [25], and so the discovery of a putative haem C transporter is not surprising. However, the finding that quinol oxidase assembly requires haem or some other assembly factor to be exported *is* unexpected and may have important consequences for understanding the topology of the subunits, the disposition of the haems and the site of oxygen reduction. Many questions remain unanswered. How many haem transporters are there in *E. coli*? Is the substrate perhaps not haem, but a protein required for cytochrome assembly? Why do *cydD* and *cydC* mutants exhibit growth defects at high temperatures, and on exit from stationary phase [19,26]?

2.1.2. Studies of the ligand-binding haems in E. coli and A. vinelandii bd-type oxidases

Recent exploitation of the low-temperature techniques first devised by Chance [4] for the trapping of intermediates after photolysis of CO-ligated oxidase in the presence of oxygen has clearly shown that cytochrome *b*-595, in both the *E. coli* and the *A. vinelandii bd*-type oxidases, binds CO. This was demonstrated long ago [27] for *E. coli*, but recent studies (D'mello, R., Palmer, S., Hill, S., Poole, R.K., unpublished data) exploiting strains that over-produce this complex reveal more clearly the post-photolysis behaviour of the α-bands and demonstrate the clear Soret band of this component at about 436 nm (with the CO-bound form as reference). Cytochrome *d* makes only a small contribution to this spectral region as demonstrated previously (see Ref. [3]) and more recently with *A. vinelandii* using white light or the He-Ne laser as sources of actinic irradiation. Oxy-cytochrome *d* (648 nm) is formed on photolysis of the CO-ligated oxidase in the presence of oxygen and is followed by ligand recombination to cytochrome *b*-595; the data strongly suggest migration of oxygen from cytochrome *d* to cytochrome *b*-595 at a haem-haem binuclear centre during the oxidase reaction (D'mello, R., Palmer, S., Hill, S., Poole, R.K., unpublished data).

2.1.3. Roles of terminal oxidases in respiratory protection

The obligately aerobic diazotroph *A. vinelandii* has one of the highest known rates of respiration and the ability to adjust its oxygen consumption so as to match wide variations in oxygen supply. Rapid respiration is thought to provide 'respiratory protection' to the oxygen-labile nitrogenase during aerobic diazotrophy. Construction of a mutant lacking cytochrome *bd* allowed us to demonstrate directly that this oxidase, which closely resembles that of *E. coli* (based on predicted protein sequences), is responsible for the ability to grow aerobically while fixing nitrogen [28]. The question of whether the cytochrome *o*-type oxidase is also required for aerotolerant nitrogen fixation has now been addressed (Leung, D., Van der Oost, J., Kelly, M., Saraste, M., Hill, S. and Poole, R.K., unpublished data). A PCR fragment homologous to part of the *E. coli cyoB* gene, encoding subunit I of the cytochrome *bo*-type quinol oxidase, was mutated in vitro and introduced into the chromosome of *A. vinelandii* by homologous recombination. The mutant contained no spectrally detectable cytochrome *o*, but exhibited dramatically higher levels of cytochrome *bd* in stationary phase. Respiration of the mutant was insensitive to chlorpromazine, an inhibitor claimed to act specifically on cytochrome *o*. The mutant lacking only cytochrome *o* fixed nitrogen in air, clearly distinguishing the role of this oxidase from that of cytochrome *bd*.

To measure the oxygen affinities of *Azotobacter* terminal oxidases, we have employed a method that utilizes the kinetics of oxygen binding by haemoglobins [29]. This method avoids the oxygen gradients that are associated with membrane-covered sensors, and the sensitivity of the measurement is much greater (e.g., working range for leghaemoglobin is 0.003 to 0.1 μM). By using a time-sharing, multi-wavelength spectrophotometer, we have been able to monitor continuously two wavelengths that report the loss of absorbance of the oxygenated form and formation of the deoxy form of the globin during oxygen uptake. A second wavelength pair can be used to monitor the possibility of undesirable redox changes in the reporter globin. Over the range 0.003 to 10 μM (achieved using separately oxyleghaemoglobin and oxymyoglobin), three distinct affinities for oxygen were revealed in wild-type *A. vinelandii* [30]. The kinetic properties of each oxidase were distinguished by the use of mutants [28]. The deoxygenation kinetics of oxymyoglobin revealed the presence of two components with mean K_m values of about 0.33 and 4.5 μM. The latter is attributed to the cytochrome *bd*-type oxidase because it was lacking in intact cells and membranes of the cytochrome *bd*-deficient mutant strain. The deoxygenation kinetics of oxyleghaemoglobin revealed a high affinity oxidase in all three strains with K_m values for membrane preparations of 0.013 to 0.019 μM. In strains having the cytochrome *bd*-type oxidase, the K_m values of this component measured with intact cells were approx. 4-fold higher than in membranes. These results suggest

a barrier to the transfer of oxygen to the high-affinity component by cytochrome *bd*, perhaps due to very fast oxygen binding or scavenging by cytochrome *bd* or to the location of the oxygen-consuming sites of these oxidases on different faces of the membrane. Two components, that with the highest affinity and that with a K_m of about 0.33 μM have not yet been assigned to particular oxidase(s). In accord with the high K_m for cytochrome *bd*, expression of this oxidase in *Azotobacter increases* in response to elevated O_2 supply (D'mello, R., Leung, D., Kelly, M.J.S., Hill, S., and Poole, R.K., unpublished), whereas in *E. coli* expression *decreases* when aeration rate increases [8,9].

2.1.4. Oxygen affinities of the E. coli cytochrome bd-type oxidase

Similar approaches have been used to measure the oxygen affinities of both quinol oxidases in *E. coli* and to avoid the problems of membrane-covered electrodes used in early studies. In striking contrast to the results with *A. vinelandii*, the *E. coli* cytochrome *bd*-type oxidase is found to have an astonishingly high affinity for oxygen: the K_m for intact cells of a *Δcyo* mutant is 3–5 nM (D'mello, R., Hill, S. and Poole, R.K., unpublished data). A further remarkable feature of the kinetics of this oxidase is the clear demonstration of substrate (i.e., oxygen) inhibition. Apparent respiration rates are maximal up to about 70 nM oxygen, but decline sharply at higher dissolved oxygen concentrations; the K_i is about 0.5 μM.

These results have interesting physiological implications for the control of respiratory electron flux in *E. coli* and provide a possible solution to an enigma. Recent work in other laboratories has demonstrated the proton pumping activity of the cytochrome *bo* complex and suggested that the cytochrome *bd* complex is responsible only for proton translocation via a chemiosmotic loop with $H^+/e^- = 1$ [31]. Nevertheless, studies using *cydAB-lacZ* fusions have shown that cytochrome *bd* expression is not shut down during aerobic growth. Calculations in Ref. [8] suggest that, anaerobically, the cytochrome *bd* content of *E. coli* is 600 molecules per cell and the cytochrome *bo* level is only 2 molecules per cell. During aerobic growth, cytochrome *o* levels increase about 150-fold but cytochrome *d* levels fall only 3-fold. What then restricts electron flow through the cytochrome *bd*-terminated branch and loss of the proton-pumping capacity of the cytochrome *o*-terminated branch? Inhibition of cytochrome *bd* activity at intracellular oxygen concentrations in the micromolar range might achieve this control. The structural basis for substrate inhibition in the cytochrome *bd* complex remains a matter for further study. However, the findings that (i) both high-spin haems bind CO (see section 2.1.2), (ii) in *E. coli*, CO migrates from haem *d* to haem *b*-595 [15] and (iii) in *A. vinelandii*, there is evidence for oxygen migration from cytochrome *d* to cytochrome *b*-595 (see section 2.1.2) all suggest that the haem-haem binuclear centre in this class of oxidases may be a site of fascinating control mechanisms as well as oxygen reduction.

2.2. Bacterial haemoglobin-like proteins: a third oxidase activity in E. coli

Until recently, the existence of globin-like proteins in microorganisms and the roles of these proteins in microbial physiology had not been well established. The most comprehensively understood example is leghaemoglobin, which serves to deliver oxygen to respiring, nitrogen-fixing bacteroids at very low and controlled oxygen concentrations. In *Vitreoscilla*, a soluble protein originally referred to as 'cytochrome *o*' was subsequently shown to be 26% identical to lupin leghaemoglobin [32]. The function of *Vitreoscilla* haemoglobin (VHb) is not known, but the elevated levels observed in hypoxic conditions and the finding that multi-copy expression of the cloned *vhb* gene in *Escherichia coli* enhances growth under oxygen-limited conditions have suggested that the globin has an important function in delivering, or perhaps storing, oxygen.

A 44 kDa protein with spectral characteristics similar to that of VHb has been identified in *E. coli* [33]. This haemoprotein (Hmp), product of the *hmp* gene, has a haem domain that is 46% identical to VHb, with conservation of the putative haem ligands and the globin fold characteristic of animal haemoglobins. In addition to the ligand-binding haem (see below), the protein contains FAD, consistent with the identification of a C-terminal domain in the sequence, homologous to that in members of a family of flavoprotein oxidoreductases, and the flavin domain of yeast haemoglobin [34]. Thus, the *E. coli* Hmp protein, and its homologues in other bacteria and in yeast, may be designated as flavohaemoglobins. The presence of FAD in the haemoglobins of *E. coli* and yeast (but not of *Vitreoscilla*) appears superfluous if the role of these proteins is simply that of an oxygen carrier. However, since only ferrous haems bind dioxygen, FAD may serve to ensure maintenance of the haem in the Fe(II) state by electron transfer.

Hmp has oxidase activity, capable of depleting either reductant (NADH) or oxygen under appropriate conditions. Recent studies of the reactions of purified Hmp with oxygen and NADH using rapid scan spectroscopy and stopped-flow techniques have demonstrated that Hmp forms very rapidly an oxygenated species [35–37]. This form spectrally resembles the CO compound of Hmp [35] and is very similar to the oxygenated forms of animal globins, Vhb, leghaemoglobin, and the *Alcaligenes eutrophus* and yeast flavohaemoglobins.

A remarkable feature of Hmp is that the reduction level of FAD in the aerobic steady state is very low when oxygen is bound to the haem [37]. Thus, an effect of oxygen binding is to cause or maintain net oxidation of FAD perhaps by conformational gating or by diverting electron flow from flavin to haem. These data suggest that Hmp could serve to couple the presence or absence of oxygen to an oxidoreduction reaction and thus serve as an oxygen sensor. However, if Hmp is cytoplasmic, as it appears to be, it presumably must compete for oxygen with the terminal oxidases on the inner face of the cytoplasmic membrane. The affinity for oxygen during NADH oxidation, determined by using the deoxygenation of oxymyoglobin as a sensitive probe of oxygen concentration (Ioannidis, N. and Poole, R.K., unpublished), gives a K_m of about 2 μM. The affinity of Hmp for oxygen is thus lower than that of either of the two quinol oxidases, implying that Hmp could sense intracellular oxygen concentrations above those required for function of the terminal oxidases. Such a mechanism might be involved in the activation of transcriptional regulators implicated in adaptation to anaerobiosis or perhaps in triggering responses to oxidative stress.

3. Conclusions

Recent studies of the reactions with CO and oxygen of cytochrome *bd*-type oxidases of *E. coli* and *A. vinelandii* suggest that, despite their probable similarity at the amino acid level, their reactivities with oxygen are strikingly different. The respiratory protection afforded to the obligately aerobic diazotroph *A. vinelandii* by the cytochrome *bd* complex appears to be accompanied by, and may be the result of, a low affinity for oxygen and a high V_{max}. The poorly characterized cytochrome *o*-containing oxidase in this bacterium appears not to be required for respiratory protection. In *E. coli*, the cytochrome *bd*-type oxidase has a remarkably high affinity for oxygen, consistent with the view that this is an oxygen-scavenging oxidase utilized under microaerobic conditions. The demonstration of substrate inhibition in this complex suggests a mechanism whereby wasteful electron flux through a non-proton-pumping oxidase is avoided at higher dissolved oxygen tensions. The demonstration of two ligand-binding sites in oxidases of this type suggests plausible mechanisms for this phenomenon. There is at least one additional oxygen-consuming protein in *E. coli* – the flavohaemoglobin encoded by the *hmp* gene. The function of this NAD(P)H oxidase is unknown; one of several possibilities is that its low affinity for oxygen could enable it to function as a sensor of falling (or rising) cytoplasmic oxygen concentrations.

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References

[1] Keilin, D. (1966) The History of Cell Respiration and Cytochrome, Cambridge University Press, Cambridge.
[2] Poole, R.K. (1983) Biochim. Biophys. Acta 726, 205–283.
[3] Poole, R.K. (1988) in Bacterial Energy Transduction (Anthony, C., ed.), pp.231–291. Academic Press, London.
[4] Chance, B. (1989) Biochim. Biophys. Acta 1000, 345–347..
[5] Poole, R.K. and Ingledew, W.J. (1987) in *Escherichia coli* and *Salmonella typhimurium* Cellular and Molecular Biology, Vol. 1 (Ingraham, J.L., Low, K.B., Magasanick, B., Schaechter, M. and Umbarger, H.E., eds.), pp. 170–200. American Society for Microbiology, Washington, DC.
[6] Iuchi, S. and Lin, E.C.C. (1993) Mol. Microbiol. 9, 9–15.
[7] Stewart, V. (1993) Mol. Microbiol. 9, 425–434.
[8] Cotter, P.A., Chepuri, V., Gennis, R.B. and Gunsalus, R.P. (1990) J. Bacteriol. 172, 6333–6338..
[9] Fu, H.A., Iuchi, S. and Lin, E.C.C. (1991) Mol. Gen. Genet. 226, 209–213.
[10] Saraste, M., Holm, L., Lemieux, L., Lubben, M. and Vanderoost, J. (1991) Biochem. Soc. Trans. 19, 608–612.
[11] Puustinen, A. and Wikstrom, M. (1991) Proc. Natl. Acad. Sci. USA 88, 6122–6126.
[12] Poole, R.K., Salmon, I. and Chance, B. (1994) Microbiology 140, 1027–1034.
[13] Poole, R.K., Kumar, C., Salmon, I. and Chance, B. (1983) J. Gen. Microbiol. 129, 1335–1344.
[14] Kahlow, M.A., Zuberi, T.M., Gennis, R.B. and Loehr, T. M. (1991) Biochemistry 30, 11485–11489.
[15] Hill, J., Alben, J.O. and Gennis, R.B. (1993) Proc. Natl. Acad. Sci. USA 90, 5863–5867.
[16] Poole, R.K., Williams, H.D., Downie, J.A. and Gibson, J.A. (1989) J. Gen. Microbiol. 135, 1865–1874.
[17] Fang, H., Lin, R.-J. and Gennis, R.B. (1989) J. Biol. Chem. 264, 8026–8032..
[18] Georgiou, C.D., Fang, H. and Gennis, R.B. (1987) J. Bacteriol. 169, 2107–2112..
[19] Delaney, J.M., Wall, D. and Georgopoulos, C. (1993) J. Bacteriol. 175, 166–175.
[20] Poole, R.K., Hatch, L., Cleeter, M.W.J., Gibson, F., Cox, G.B. and Wu, G. (1993) Mol. Microbiol. 10, 421–430.
[21] Higgins, C.F. (1992) Annu. Rev. Cell Biol. 8, 67–113.
[22] Fath, M.J. and Kolter, R. (1993) Microbiol. Rev. 57, 995–1017.
[23] Bebbington, K.J. and Williams, H.D. (1993) FEMS Microbiol. Lett. 112, 19–24.
[24] Poole, R.K., Gibson, F. and Wu, G. (1994) FEMS Microbiol. Lett. 117, 217–224.
[25] Light, W.R. and Olson, J.S. (1990) J. Biol. Chem. 265, 15623–15631.
[26] Siegele, D.A. and Kolter, R. (1993) Genes Dev. 7, 2629–2640.
[27] Poole, R.K., Scott, R.I. and Chance, B. (1981) J. Gen. Microbiol. 125, 431–438.

[28] Kelly, M.J.S., Poole, R.K., Yates, M.G. and Kennedy, C. (1990) J. Bacteriol. 172, 6010–6019..
[29] Bergerson, F.J. and Turner, G.L. (1979) Anal. Biochem. 96, 165–174.
[30] D'mello, R., Hill, S. and Poole, R.K. (1994) Microbiology 140, 1395–1402.
[31] Puustinen, A., Finel, M., Haltia, T., Gennis, R.B. and Wikstrom, M. (1991) Biochemistry 30, 3936–3942.
[32] Webster, D.A. (1987) Adv. Inorg. Chem. 7, 245–265.
[33] Vasudevan, S.G., Armarego, W.C.F., Shaw, D.C., Lilley, P.E., Dixon, N.E. and Poole, R.K. (1991) Mol. Gen. Genet. 226, 49–58.
[34] Andrews, S.C., Shipley, D., Keen, J.N., Findlay, J.B.C., Harrison, P.M. and Guest, J.R. (1992) FEBS Lett. 302, 247–252.
[35] Ioannidis, N., Cooper, C.E. and Poole, R.K. (1992) Biochem. J. 288, 649–655..
[36] Orii, Y., Ioannidis, N. and Poole, R.K. (1992) Biochem. Biophys. Res. Commun. 187, 94–100.
[37] Poole, R.K., Ioannidis, N. and Orii, Y. (1994) Proc. Roy. Soc. Ser. B 255, 251–258.