THE OCCURRENCE OF NITRATE ON THE EARLY EARTH AND ITS ROLE IN THE EVOLUTION OF THE PROKARYOTES

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Abstract. It has been suggested that the evolution of the respiratory system coupled to oxidative phosphorylation occurred under anaerobic conditions in which inorganic compounds, principally nitrate, served as electron acceptors. Such a hypothesis requires that nitrate be produced, consistently and at adequate concentrations for utilization in biological processes, at a time when much of the Earth's surface was still in a relatively reduced state. This paper is directed towards a consideration of the possible sources of nitrate under primeval conditions, its stability, and its concentration in sites favorable to the evolution of the bacteria.

1. The Biological Significance of Nitrate in Early Evolution

A hypothetical scheme for the evolution of the prokaryotes in terms of the development of increasingly efficient energy-generating mechanisms has been proposed (Hall, 1971). It was suggested that the utilization of nitrate as an electron acceptor played an important role in stimulating the appearance of the respiratory chain. Further investigation indicates that the role of nitrate may be even more significant than was originally envisioned. The ability to reduce nitrate is known to be very widespread among the bacteria and is used as a taxonomic trait in the classification of a variety of organisms among both the aerobes and the anaerobes (See Breed et al., 1957; Reed, 1942). Curiously, the physiological significance of this ability has been recognized only in the aerobes. To my knowledge, the only published studies of the physiological function of nitrate reduction for respiration rather than for assimilation in the anaerobes consist of work mentioned in Takahashi et al. (1963) and a recent abstract from Inderlied, et al. (1971).

The wide distribution of organisms which reduce nitrate in quantities convenient for routine assay, and therefore in quantities more likely to represent dissimilatory reduction than purely assimilatory processes, suggests that many anaerobes are able to utilize this acceptor in their energy metabolism (Sato, 1956). In evolutionary terms this implies that the use of nitrate as a sink for the discharge of excess reducing potential and possibly, the generation of energy via oxidative phosphorylation, is a very ancient process which appeared in ancestors common to many extant bacteria. Thus, nitrate may well be found to play a role in energy metabolism at many levels in the evolution of the energy generating mechanisms. Nitrate is highly suitable for such a role, being essentially equivalent to oxygen as an oxidizing agent. The oxidation of a mole of glucose accompanied by the reduction of nitrate to nitrite liberates 460 kcal of free energy compared to a free energy change of 700 kcal when oxygen is reduced as the electron acceptor. Most, if not all, organisms which utilize nitrate in respiration...
further reduce the nitrite produced in this initial step to nitrogen gas. The oxidation of a mole of glucose by nitrite leads to a free energy decrease of 730 kcal, or slightly more than that obtained with oxygen (Cooper, 1937). It is plain then, that the utilization of nitrate as a terminal electron acceptor will be limited by its availability and concentration and by the evolution of suitable biochemical mechanisms to process it, rather than by any deficiency in the oxidizing power of the compound.

Nitrate has the further advantage of being much less toxic to most organisms than oxygen. Although its redox potential is similar to that of oxygen, it does not react spontaneously nearly as readily, apparently because of a large activation energy barrier (Szabo and Bartha, 1957). This activation energy requirement can be circumvented by catalysis, permitting the development of enzyme systems which can use nitrate, but it inhibits the kind of spontaneous oxidation of cell components that makes oxygen so toxic to many anaerobes and has necessitated the evolution of special protective devices in aerobic organisms.

A previous publication (Hall, 1971) has discussed at some length the problems encountered by organisms growing under anaerobic conditions in producing energy efficiently from their organic food sources. The basic difficulty is the necessity of maintaining substrates and end products at the same net oxidation-reduction level, thus sacrificing the large amounts of energy obtainable by the net oxidation of the substrate. If nitrate can be reduced and thus utilized as an acceptor for electrons taken from the substrate, net oxidation of the latter is possible. At the most primitive level, simple fermentative bacteria may have used nitrate as an electron acceptor, and thus been able to oxidize a fraction of their organic substrates to yield additional sites of substrate-level phosphorylation. An organism utilizing the glycolytic pathway, for example, might ordinarily have to reduce the pyruvate formed to lactate in order to regenerate the oxidized form of the NAD cofactor which is reduced at an earlier stage in the sequence. If the electrons can be transferred to nitrate (or some other acceptor) from reduced NAD instead, however, the pyruvate can be further oxidized, yielding acetyl phosphate which can generate an additional ATP to satisfy the energy needs of the organism.

Slightly more sophisticated organisms which have developed the ability to produce ATP during electron transport, may possess the initial step in the oxidative phosphorylation sequence in which ATP is generated during the transfer of electrons from reduced NAD to a flavoprotein. The availability of nitrate would make this reaction a useful source of energy for any anaerobe which was able to reoxidize the flavoprotein by transferring the electrons from it to nitrate. Such microorganisms might provide useful model systems for the study of the mechanisms of oxidative phosphorylation, since their respiratory system would be much less complex than that found in mitochondria. The elaboration of a cytochrome chain would provide additional sites for ATP synthesis in still more advanced anaerobes. As mentioned previously, the electrode potentials of nitrate and its immediate reduction product nitrite, are high enough to permit the elaboration of a number of steps in the sequence, and would allow the development of the entire electron transport chain as we find it today in oxygen