THE LENGTH OF THE TRANSITION PERIOD FROM THE REDUCING TO THE NEUTRAL BIOSPHERE

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Abstract. The development of the complicated mechanisms for N_2 fixation, which in nature is an endergonic process and requires a great deal of ATP, must have taken a long time. During that time primeval NH_3 must still, albeit to a decreasing extent, have been available as a source of nitrogen. This is true, whether N_2 fixation originally arose in the primitive anaerobes, or, according to Postgate, in more advanced bacteria. As NH_3 resists UV radiation only in the presence of excess H_2 it follows that the disappearance of H_2 and the transition from the reducing to the neutral biosphere also took a long time, probably of the order of 10^9 yr. According to previous evidence, the transition from the neutral to the oxidizing biosphere likewise took long; this length enabled the organisms to adapt the N_2 fixing machinery to aerobic conditions.

On the basis of a consideration of the history of nitrogen metabolism it has been suggested that the transition to a neutral (non-reducing) from a reducing atmosphere must have taken a time long compared to the time needed for major innovations in bioenergetic processes (Broda, 1975b). This question will be discussed in more detail now. It will be argued that the necessarily slow evolution of N_2 assimilation required reducing conditions. Therefore, no rapid transition to neutrality can have taken place.

The secondary atmosphere, which formed on the early Earth by degassing, must, like the (lost) primary atmosphere, at first have been reducing (Haldane, 1929; Urey, 1962; Holland, 1964). To what extent carbon was present as hydrocarbons (Urey, 1962) or as CO and CO_2 (Rubey, 1955; Abelson, 1966) is not certain, but there is no doubt about the presence of H_2 and hydrides. In the laboratory, an appreciable production of biomolecules through action of UV radiation or of electric discharges has, beginning with Miller (1955), been demonstrated only in reducing conditions.

Gradually the free hydrogen was lost into space. Moreover, hydrides were partly decomposed by UV light, and the resulting H_2 was also lost. NH_3 was one of the most UV-sensitive hydrides (Abelson, 1966). But the life time of NH_3 during UV irradiation depends greatly on conditions (Ferris and Nikodem, 1972). H_2 protects NH_3; presumably NH_3 is reformed by UV from N_2 and H_2. Thus in presence of H_2 early NH_3 may have persisted. Rasool and McGovern (1966) have concluded from the composition of the early, reducing, exosphere that primeval H_2 did survive long after the formation of the Earth, maybe during a period of the order of a giga-year (10^9 yr). Moreover, the reducing gases, including H_2, in the atmosphere were for a long time supplemented from the mantle (Holland, 1964); the other gases, e.g. H_2S, provided, in their photolysis, more H_2.

Further protection was given to NH_3 by dissolution in the ocean as NH_4^+ and by adsorption of this ion on marine clays (Bada and Miller, 1968).

Sagan and Mullen (1972) have, to explain the fact that in the middle Precambrian, in spite of the lower luminosity of the Sun, temperatures were similar to those to-day, invoked an NH_3 content of the atmosphere of the order of a few ppm. On the biochemical side, Bada and Miller (1968) arrive, on consideration of the need to prevent deamination of aspartic acid, at a minimum NH_3 content of the atmosphere of 150 ppm for the period of chemical (prebiological) evolution, i.e. for the early Precambrian. The further argument for persistence of NH_3, to be put forward here, refers to the early part of biological evolution, before the advent of photosynthesis; roughly, to the period 4 to 3 giga-years ago.

As long as nitrogen could enter organisms and their precursors ('eobionts') in the form of NH_3, no complicated mechanisms were required to cover their nitrogen requirements. Only when NH_3 ran out, the organisms had to embark on the construction of mechanisms for the assimilation ('fixation') of N_2. Now fixation, as we know it, is a most difficult process (see Hardy and Havelka, 1975). A complicated system of enzymes, in spite of much research not yet fully elucidated, is needed. These enzymes may account for as much as 5% of the total cell protein in nitrogen fixers.

Moreover, a very large amount of energy, in the form of adenosine triphosphate (ATP), must be invested: about 20 molecules of ATP for each molecule of N_2 fixed (Yates and Jones, 1974; Hardy and Havelka, 1975; Hill, 1976). The enormity of this need is illustrated by a simple calculation. Bacteria and fungi supplied with reduced nitrogen need for about 10.5 g of biomass (Bauchop and Elsden, 1960), with $1 - 1.5$ g N, 1 mole of ATP. If this nitrogen, however, were all to be provided by fixation by the organisms themselves, about 1 additional mole of ATP would be required. Thus the organisms would have to double their energy supply.

Notwithstanding its tremendous difficulty, the process of N_2 fixation is mastered by many of the simplest extant organisms, the bacteria and the blue-green algae, i.e. prokaryotes. In fact, only prokaryotes fix N_2 while the eukaryotes appear to have lost this capacity (Millbank, 1969; for a discussion of possible reasons see Postgate, 1974).

Clearly the need for N_2 fixation, rather than mere NH_3 utilization, must have become pressing to some early ancestors of the present bacteria. Presumably the property was acquired just once, and the machinery has a common origin in the many groups of prokaryotes where it exists. The alternative possibility that the capacity was developed independently and in parallel by different groups, unlikely in any case, is refuted by the basic similarity of the mechanisms for N_2 fixation in all competent organisms (Hardy and Havelka, 1975). For an independent origin of fixation in the different groups, convergence would have to be invoked to an incredible extent.

The clostridia and methane formers may be considered as the most primitive extant bacteria (see Broda 1975a). These obligate anaerobes are incapable of photosynthesis or respiration, and obtain ATP exclusively by the simple processes of fermentation. Nevertheless some of the clostridia fix N_2. If the process arose in their ancestors, it must

have taken them an extraordinary effort and it must have happened in response to urgent necessity, i.e. under strong selection pressure. Moreover, a long time must have been needed to build up the required machinery.

For a process serving nitrogen supply to develop within a group of bacteria through mutation, genetic recombination and selection, every step must have brought some advantage, or else the step would not have been maintained. On the other hand, the initial machinery cannot have been very effective. Thus the organisms must during the early stages of the development of N_2 fixation have largely relied on another source of nitrogen, i.e. on preformed NH_3. As the build-up of the machinery for fixation must have taken long, NH_3 must also have been available for an extended period.

Postgate (1974) has likewise emphasized the problem presented by the emergence in evolution of the complicated machinery of N_2 fixation. However, Postgate gives no priority to clostridia. He argues that the gene cluster for N_2 fixation ('nif') may have arisen relatively late, in his view probably from a precursor structure with different functions, in a more advanced bacterial group, and has spread from there to other prokaryotic groups, including some of the clostridia, by genetic transfer. This radically new hypothesis would, however, like the view put forward here, presuppose that NH_3 remained available in the biosphere for a long time, namely until the advent of that more advanced group, and also for a sufficient time thereafter. Thus also with Postgate's hypothesis, long persistence of NH_3 in the atmosphere is required.

Because the development of the machinery for N_2 fixation could be brought to a successful conclusion only if NH_3, albeit to a decreasing extent, still remained available for a long period, NH_3 must have been backed by H_2 during that time. Therefore the disappearance of the latter from the biosphere and altogether the transition from reducing to neutral conditions must have been slow. In view of the complexity and difficulty of N_2 fixation, one arrives at an estimate of the order of magnitude of the length of the transition period that is consistent with that of Rasool and McGovern (1966), namely, 1 giga-year. This is 4–5 orders of magnitude more than the time given for the disappearance of NH_3 by Abelson (1966).

Geological evidence on the length of the transition period may not be expected to exist. While the oxidation state of early Precambrian rocks clearly indicates initial reducing conditions (see Rutten, 1971), this oxidation state cannot have changed in subsequent periods as a consequence of the mere disappearance of the reductants from the atmosphere, as long as this remained neutral, and there was no effective oxidant (free oxygen).

Be it added that the subsequent transition from the neutral to the oxidizing atmosphere is known to have taken a very long time (see Rutten, 1971). The time difference between the appearance of the first producers of O_2, blue-green algae (paleontological evidence from nanofossils), and the appearance of appreciable amounts of O_2 in the atmosphere (geological evidence from red beds) was more than 1 giga-year (see Broda, 1975a). The length of this time gave the N_2 fixers a chance to adapt from the neutral to the new oxidizing conditions, and to protect, often through complicated

physiological mechanisms, the O_2-sensitive nitrogenase (see Postgate, 1974; Yates and Jones, 1974; Hardy and Havelka, 1975). But once organisms mastered the difficulties of protection they could tap an abundant source of ATP for N_2 fixation:oxidative phosphorylation (respiration). N_2 fixers are now common not only among anaerobic, but also among aerobic prokaryotes. *Azotobacter* is famous for the enormous value of its ATP production, namely 7000 g/g biomass per day (see Broda, 1975a).

Acknowledgement

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Addition at the proof stage. According to C. R. Woese (personal communication), the methane formers are more ancient than the clostridia. Much less is known about nitrogen fixation by methane formers than about nitrogen fixation by clostridia (Postgate, 1974). The arguments put forward here are not affected thereby.