SODIUM AS AN ESSENTIAL ELEMENT FOR C₄ PLANTS

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SODIUM AS AN ESSENTIAL ELEMENT IN C₄ PLANTS

1. SUMMARY OF FINDINGS DESCRIBED IN THE PUBLICATIONS LISTED BELOW (pp 5-8)

1. Sodium was shown to be an essential micronutrient element for *Atriplex vesicaria* Howard ex Benth. This was the first report of sodium being essential for a higher plant (Publications 1 and 2).

2. Even with extremely careful exclusion of sodium from the plants’ environment (Publication 9), it was found that of thirty species examined, only ten Australian species of *Atriplex* required sodium. Other species including non-endemic species of *Atriplex* and some halophytes, grew normally without the addition of salts of sodium to the cultures (Publication 5).

3. Following the discovery of the C₄ photosynthetic pathway (Hatch and Slack 1966), we were able to demonstrate a clear correlation between the presence of the C₄ pathway and the requirement for sodium as a micronutrient (Publications 6 and 26).

4. Significant responses to sodium by the Crassulacean acid metabolism (CAM) plant, *Bryophyllum tubiflorum*, were obtained under conditions which favoured the CAM photosynthetic pathway but not under conditions favouring the C₃ photosynthetic pathway (Publications 7 and 26).

5. A rapid increase in the respiration rate of leaves of sodium-deficient *Atriplex nummularia*, a C₃ species, was obtained following the application of sodium salts to the culture solution. The response was sodium-specific; no other group
I element was effective (Publication 3).

6. The Cyanobacterium, Anabaena cylindrica, has a small but specific requirement for sodium (Allen and Arnon 1955) and the possibility was considered that the nutrient might have a similar function in the Cyanobacterium as in *Nitella*. Cyanobacteria, because of their shorter generation times and ease of manipulation, have advantages over higher plants as experimental material in studies of metabolism. Nitrate reductase activity increased markedly in sodium-deficient cells resulting in a dramatic increase in nitrite to levels which were toxic to the cells. Sodium appeared to have a role in controlling nitrate reductase activity. $^{15}$N$_2$ incorporation into cell protein suggested that sodium is required for the reduction of N$_2$ to NH$_3$ (Publication 4).

7. The discovery that sodium was required by plants having C₃, but not C₄ photosynthesis suggested that sodium was needed for the operation of the C₄ component which transports CO$_2$ to the bundle sheath cells where it is reduced to carbohydrates. This was substantiated by the observation that sodium-deficient plants grown under low CO$_2$ concentrations (15 µL CO$_2$ L$^{-1}$) had lower yields and accentuated deficiency symptoms; but those grown under conditions of high CO$_2$ (1500 µL CO$_2$ L$^{-1}$), gave increased yields and no deficiency symptoms. On the other hand, the yields of plants receiving sodium were unaffected by the CO$_2$ concentration (Publications 10, 13, 15).

8. The growth of sodium-deficient C₄ plants was significantly reduced when grown under elevated oxygen concentrations. The growth of sodium-sufficient plants was unaffected by oxygen concentration (Publication 18). This indicated
that under elevated oxygen concentrations, increased amounts of oxygen diffused into the bundle sheath cells causing a reduced ratio of CO$_2$ /O$_2$ within these cells and decreasing, in turn, the operation of the photosynthetic carbon reduction cycle relative to that of the photosynthetic carbon oxidation cycle. The overall effect of this would be to increase photorespiration and thus reduce the rate of photosynthesis. This would account for the reduced growth of plants in high concentrations of O$_2$.

9. A marked accumulation of alanine was observed in leaves of sodium-deficient plants. It was suggested that alanine which is in equilibrium with pyruvate builds up as a result of a block in the conversion of pyruvate to phosphoenolpyruvate (PEP) in the mesophyll chloroplasts in sodium-deficient plants (Publications 10 and 12). The pool sizes of the other key intermediates of the C$_4$ pathway provided further evidence for this lesion [Groat et al. (1985a); Publication 16; Johnston et al. (1988); Publication 21].

10. The major steps in the conversion of pyruvate to PEP involve the transport of pyruvate into the mesophyll chloroplast, its enzymatic conversion to PEP and the provision of energy required for the conversion. Sodium was without effect on the activity of pyruvate orthophosphate dikinase, the enzyme that catalyses the conversion of pyruvate to PEP (Bong 1981; Dorney 1985).

11. The light-harvesting system is the immediate source of energy for pyruvate transport and/or regeneration of PEP from pyruvate in the mesophyll chloroplasts. In sodium-deficient plants, there is evidence of damage to this system as indicated by lower chlorophyll a/b ratios (Publications 14, 15, 22) fluorescence yields (Publications 17 and 20) and photosystem II activity.
Furthermore, it has been established that the ultrastructure of mesophyll but not bundle sheath chloroplasts is altered in sodium-deficient plants of *Amaranthus tricolor* and *Kochia childii* (Publication 19).

12. It was considered possible that photosynthesis in sodium-deficient C4 plants might be limited by reduced activities of carbonic anhydrase. This enzyme catalyzes the interconversion of CO₂ and HCO₃⁻; the latter being the substrate for PEP carboxylase in C₄ and CAM plants.

It was found that carbonic anhydrase was about twice as active in sodium-deficient plants as in control plants when expressed on a fresh weight or protein basis and three times as active on a chlorophyll basis (Publication 25).

It appears unlikely, therefore, that the activity of this enzyme could be a limiting factor in sodium-deficient plants.