Silicate in the Metabolism of *Azotobacter vinelandii*

MOLYBDENUM complexed as silico-molybdate has been shown to be very active in the re-activation of aldehyde oxidase from which the molybdenum has been removed by dialysis[1]. Other reports have implicated silicate, phosphate and other anions as agents which increase the activity of molybdate-dependent enzymes[2,3]. Jacobs and Sanadi[4] suggested that a silico-molybdate complex was involved in a terminal electron transport system that was coupled with phosphorylation. It was shown by Glenn and Crane[5], however, that the *in vivo* functioning of aldehyde oxidase was probably not dependent on silico-molybdate even though it did greatly enhance the activity of the *in vitro* system.

It has already been shown that *A. vinelandii* is capable of incorporating into molybdo-protein as much as 100 times more molybdate than is required for optimum rate of growth[6].

We were led to consider the possible relationship of silicate and molybdate in the metabolism of *Azotobacter* by the observation that 100 p.p.m. germanium (as germanate) inhibits the growth of *Azotobacter* by 30–50 per cent. Because germanium is in the same chemical group as silicon it seemed possible that germanium was acting as an inhibitor of a silicate function much as tungstate inhibits molybdate function[6].

We therefore measured the uptake of silicate directly by the use of silicon-31 prepared from reagent grade silica gel (Mallinckrodt) by neutron bombardment. (Reactor facilities were provided by the Battelle Memorial Institute.) The irradiated silicon-31 was fused with sodium carbonate in a platinum crucible according to standard methods[7,8]. Half-life determinations showed the material to be about 99·9 per cent radiochemically pure.

Under culture conditions (in Burk's[9] nitrogen-free medium) such that *A. vinelandii* cells would take up nearly all the 1 p.p.m. molybdenum added to the culture, only 0·2 p.p.m. silicon-31 was taken up and only one-fourth of this was retained when the whole cells were washed thoroughly. When the molybdate-level of the medium was decreased so that the quantity of molybdate taken up decreased by a factor of 100, there was no change in the uptake of silicon-31. Furthermore, germanium at a level of 100 p.p.m. had no influence on the uptake of silicon-31, or molybdenum-99. Silicate concentrations also had no effect on the uptake of molybdenum-99.

The distribution of the silicon-31 retained by the cells in centrifugal fractions of these cells after sonic disruption was examined. In contrast to the published[10] distribution of molybdenum, in which both the highest total (more than 65 per cent) and highest specific activity of molybdenum-99 are found in a fraction sedimentable at 144,000*g*, only 5 per cent of the retained silicon-31 was found in the corresponding sedimentable fraction. The supernatant fraction contained 85 per cent of the total silicon-31, and the specific activity was 3½ times greater in the supernatant fraction than in any other.

As a result of the lack of correlation between the behaviour of silicon-31 and molybdenum-99 in these uptake and distribution studies in *Azotobacter vinelandii*, it is concluded that silicate is not involved in the molybdate metabolism of these cells as a silico-molybdate complex anion. Further, even though germanate does inhibit the growth of *Azotobacter*, it has no effect on the uptake of silicate.

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Absorption and Distribution in Rats of Radioactive Phosphorus biologically incorporated in Food

IN this investigation absorption and distribution of phosphorus-32, biologically incorporated into fish muscle and bean leaves, was compared with its absorption after feeding as inorganic phosphate. Within limits of error, no differences were detected.

The experimental animals were 179 young adult female Sprague–Dawley rats (wt.-range 190–304 gm. : mean wt. 238 gm.) maintained *ad lib.* upon a Purina Lab Chow diet. The following solutions and suspensions containing radioactive phosphorus were administered orally to these animals :

(1) *Inorganic phosphorus*: 100 rats received 1 ml. of a *p*H 2 solution containing 25 μc. phosphorus-32 ($Na_2H^{32}PO_4$) and less than 0·6 μgm. of non-radioactive sodium hydrogen phosphate.

(2) *Fish protein phosphorus*: 51 rats received 2 ml. of a water/whitefish muscle homogenate (1/1, w/w) which had a concentration of a 0·5 μc. phosphorus-32 per ml. The muscles were derived from a fish which 72 hr. previously had received an intraperitoneal injection of 10 mc. of inorganic phosphorus-32.

(3) *Plant leaf phosphorus*: 28 rats received 1 ml. of a water/bean leaf homogenate (1/1, w/w), which contained 3·3 μc. phosphorus-32 per ml. It was prepared from leaves of young red kidney bean plants grown in an appropriate nutrient culture medium containing radioactive phosphorus-32.

Excreta were collected individually from each of eight animals of the inorganic phosphorus-32 group over a period of 40 days following the feeding. In the other two experimental groups, excreta were collected from each of eight animals up to 20 days following feeding.

The experimental animals were killed in groups of four at time-intervals ranging from 3 hr. to 40 days following radioisotope administration. The concentrations of phosphorus-32 in aliquots of blood, femur, liver, and excreta were determined. In order to measure the overall distribution of phosphorus-32 and total inert phosphorus[2] in the entire skeleton and teeth, a $Na_2H^{32}PO_4$ solution was orally administered to 17 additional young female rats. Three of these

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