METAPHOSPHATE AND AEROBIC CO_2 IN CHLORELLA

Otto WARBURG, Günter KRIPPAHL and Arnold LEHMANN
Max Planck Institute of Cell-Physiology, Berlin-Dahlem

Received 1 July 1968

1. Introduction

A striking parellelism between the metaphosphate and the "aerobic CO_2" of Chlorella has been observed.

If the photolyte [1] of photosynthesis is built up in Chlorella, the first intermediate is the "aerobic CO_2". It is called "aerobic", because the fixation of this CO_2 requires not only CO_2 but also O_2. The equilibrium of the fixation of the aerobic CO_2 is reached at 20° in 30 minutes. At the same rate the aerobic CO_2 dissociates if the CO_2 or the O_2 is removed from the gas mixture in the presence of which the aerobic CO_2 was formed.

We have discovered that the highly polymeric metaphosphate of Chlorella increases, when the "aerobic CO_2" is formed and that the metaphosphate decreases, when the "aerobic CO_2" dissociates on the removal of the CO_2 or the O_2.

2. Isolation of the metaphosphate

Chlorella suspensions are acidified at room temperature with acetic acid to a final concentration of 0.2 N. The supernatant is removed on the centrifuge and the sediment is extracted with methanol until the residue is white. The white residue is extracted 3 times with 0.1 N aqueous NH_3. The extract contains the metaphosphate.

The extract is acidified with acetic acid to pH 4. A considerable precipitate is removed on the centrifuge. To the supernatant HCl is added to pH 2. A small precipitate is removed on the centrifuge. To the supernatant a great excess of $BaCl_2$ is added, which precipitates the metaphosphate as Ba-salt. This is redissolved in NaOH and re-precipitated at pH 2 with a great excess of $BaCl_2$. After drying in high vacuum at 60° it contained 41.1% Ba and 18.7% P, as expeced for $Ba/2\ PO_3 + H_2O$.

The Ba-salt was free of organic substances and of nitrogen. Chromatography according to Rossel [2] showed, that it contained no $Na_5P_2O_{10}$, no pyrophosphate, only traces of orthophosphate and only non-migrating phosphate. All the phosphate of the Ba-salt was split to orthophosphate by heating 30 minutes in 2 N HCl. After the removal of the Ba with H_2SO_4 the supernatant gave the toluidine-blue test according to Wiame [3] and precipitated dialysed serum proteins in diluted acetic acid at room temperature according to Mann [4]. Thus the metaphosphate extracted by 0.1 N NH_3 from Chlorella was highly polymeric.

3. Yield of metaphosphate

From 15000 µl Chlorella 38.5 mg dried Ba-salt were obtained, containing 232 micromoles P when the cells had been pretreated at 20° with 20 Vol % CO_2 in air. This means a yield of 1.55 micromoles metaphosphate P from 100 µl cells saturated with the CO_2-air mixture. When, on the other hand, the cells were pretreated with CO_2 but without O_2 and therefore were free of "aerobic CO_2", the yield of metaphosphate was only 1.1 micromoles P from 100 µl Chlorella. No pre-treatment of cells could raise the yield of the metaphosphate above 1.1 micromoles of P from 100 µl cells except the saturation with $CO_2 + O_2$, that is except the synthesis of the "aerobic CO_2".

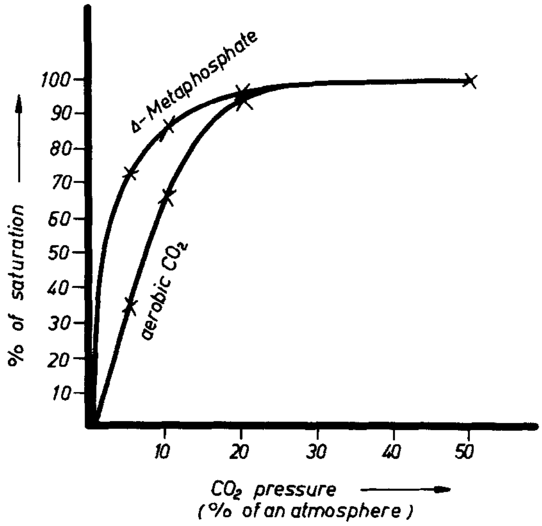

Fig. 1. 400 μl of Chlorella, suspended in 6 ml of a neutral solution of 0.02 M $MgSO_4$ and 0.034 M NaCl were shaken at 20° in the dark with different mixtures of CO_2 and air. When, after 30 minutes, the equilibrium concentrations of the "aerobic CO_2" had been reached, metaphosphate was determined as described in the text, Δ metaphosphate being x metaphosphate in CO_2 and air minus metaphosphate in air. In a parallel experiment the "aerobic CO_2" was determined with the fluoride method [1].

4. Parallelism between metaphosphate and aerobic CO_2

When the increments of the metaphosphate and the aerobic CO_2 were determined at different partial pressures of CO_2 in air, very similar curves were obtained, as shown in fig. 1. Thus the absolute increments of the metaphosphate and the aerobic CO_2 were equal at high CO_2 pressures, that is

$$\Delta \text{ metaphosphate} = \text{aerobic } CO_2 \ .$$

Both changes are completely reversible. If the CO_2 or the O_2 are removed, both increments disappear and the rates of disappearances are equal. It is not claimed that the aerobic CO_2 is a metaphosphate compound, but the findings reveal a close connection between metaphosphate and the "aerobic CO_2".

5. Light and metaphosphate

Light has no effect on the metaphosphate in Chlorella, if the CO_2 and O_2 pressures are kept constant in the dark and in the light. Only when light removes the CO_2 by photosynthesis, does the metaphosphate disappear together with the "aerobic CO_2".

References

[1] O.Warburg and G.Krippahl, Z. Naturforsch. 15b (1960) 788; Bioch. Z. 346 (1967) 418.
[2] Th.Rossel, Z. anal. Chem. 197 (1963) 333.
[3] J.M.Wiame, J. Biol. Chem. 178 (1949) 919.
[4] T.Mann, Biochem. J. 38 (1944) 345.