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ABSTRACT

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The photosynthetic assimilation of radioactive carbon dioxide for two-minute periods by Scenedesmus has been studied at temperatures ranging from 25° to 44° C. All labeled intermediates cease to be formed at about 45° C. With rising temperature, the radioactivity reaching the sugar phosphate reservoirs decreases regularly while there is a sharp maximum in sucrose at 37° C. and a less pronounced one in malic and aspartic acids above 40° C. A tentative interpretation of these effects is offered.

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(1) The work described in this paper was sponsored by the U. S. Atomic Energy Commission.

(2) Guggenheim Fellow 1949-50, on leave from Laval University, Quebec, Canada.

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INTRODUCTION

A change in temperature, especially near that at which photosynthesis ceases to take place, is likely to exert selective effects upon the rates of the various enzymatic reactions involved in carbon dioxide assimilation as revealed by the pattern of the labeled intermediates (1,2,3). In an attempt to bring out such effects, the photosynthetic fixation of radioactive carbon dioxide by Scenedesmus was studied at temperatures ranging from 25 to 44° C. Experiments of this kind suffer from one of two possible limitations: a) a disturbance of the steady state by the transition phenomena arising from a sudden temperature change or b) the long-range changes in enzyme constitution through which growing cells adapt themselves to a new temperature. Since the latter effect would more likely tend to restore a normal metabolic balance, brief thermal shocks and photosynthetic periods were used in this preliminary investigation.

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EXPERIMENTAL

For a series of experiments, a one-day growth from one liter of a continuous culture of Scenedesmus (4) was freed from the culture medium by centrifugation and resuspended in M/3000 phosphate buffer (pH 6.7) at a concentration of 1 gram wet packed cells in 100 cc. A stream of air was passed during one hour through this cell suspension kept at room temperature in subdued daylight. The reaction vessel was a 250 cc. Erlenmeyer flask in which a 20 cc. aliquot of cell suspension built a layer which was illuminated from underneath at 1200 foot candles from a bank of fluorescent lights. These lights were placed under a glass dish filled with water in which the reaction vessel was immersed. The temperature was read on a thermometer dipping into the layer of algae and held in position by a rubber stopper. In an experiment, the cells were first adapted to light of 1200 foot candles during a period of ten minutes. Hot water was then added to the water in the dish and the temperature in the cell layer rose in about two minutes to a final value where it could be kept constant within 0.5° C. At this point, 70λ of a radioactive sodium bicarbonate solution (11 microcuries, 0.001 millimoles) were added, the flask stoppered and the cells allowed to photosynthesize during two minutes, after which they were killed rapidly in boiling ethanol. During the whole adaptation and photosynthesis periods, the cell suspension was kept in motion by gentle horizontal shaking. The extract in 80% ethanol was analyzed by the radiochromatographic method (5).

RESULTS

Preliminary trials indicated a large increase in the percentage of labeled sucrose with rising temperatures for short experiments. The experiments described here were carried out at 25, 32, 37, 40, 42.5 and 44° C. within a period of about two hours and with samples from the same

growth of algae. Figure 1 shows the total radioactivity fixed and the part which is soluble in 80% ethanol. The radioactivities fixed during two minutes in the various intermediates were obtained by counting the corresponding spots on the paper chromatograms. Some of the data are shown in Figure 2. The main effects of raising the temperature are the gradual decrease in the labeled phosphates, the surge of labeled sucrose up to 37° C. and the remarkable convergence of all the curves toward the same limiting temperature of about 45° C. The sum of the radioactivities in glycine and serine (not shown on the diagram) is very nearly equal to the radioactivity in alanine and follows the same trend, i.e. remains constant almost up to the limiting temperature. On the other hand, the four-carbon compounds malic and aspartic acids show a significant increase near 43° C., just below the upper limit.

In a few experiments designed to test the reversibility of these effects, the algae were first subjected to the higher temperature for a period of two minutes and rapidly cooled down to room temperature for a two-minute photosynthetic fixation of $C^{14}O_2$. When the results of such two-minute shocks at 39 and 43° C. were compared with those of a blank experiment at 25° C., the only large difference was in the percentage of labeled sucrose, which was intermediate between that in the low and in the high temperature experiments. It thus seemed that no permanent change had taken place during the brief thermal shock.

DISCUSSION

The simultaneous decrease in rate of formation of all the labeled intermediates at about 45° C. suggests that a key step is blocked at that temperature, possibly by the heat denaturation of an enzyme or by the alteration of some structure in the cell. The point of break-down must be located either at the earliest stage of carbon dioxide fixation or at an anterior stage

connected with the photolysis of water by the chloroplasts, which are known to become rapidly inactivated in that temperature region (6,7). If the latter were the case, one could expect enhanced dark fixation to occur above 45° C. after preillumination at room temperature.

A tentative explanation of the effects shown in Figure 2 can be offered on the basis of a reaction scheme of the type of that which has been proposed earlier (8). In a regenerative cycle comprising two-, three- and four-carbon intermediates, a reversed glycolytic sequence branches off from a three-carbon intermediate and leads to sucrose, while another branch leads from a four-carbon intermediate to malic and aspartic acids (9). As the temperature rises from 25 to 37° C., the passage from the hexose phosphates to free sucrose is greatly accelerated, so that more of the assimilated carbon reaches sucrose and less accumulates in the phosphates. Above 37° C., the access to the glycolytic branch becomes more difficult, presumably as the result of heat deactivation of an enzyme, so that the radioactivity decreases simultaneously both in the phosphates and in sucrose. However, this decrease benefits the competing malic-aspartic reservoir, into which C^{14} now accumulates more rapidly, until a temperature is reached at which C^{14} ceases to be incorporated into the regenerative cycle. The fact that the rate of fixation into alanine seems independent of temperature between 25 and 40° C. suggests that the rate of an important step is controlled by diffusion.

An attempt to apply the Arrhenius equation to the individual variations led to the results shown in Figure 3, which permit a rough evaluation of the temperature increments. The slopes for the phosphates and for sucrose above 40° C. correspond to energies of -100 to -150 kcal./mole, which are of the right order of magnitude for the activation energies of enzyme inactivation. The sucrose curve between 25 and 37° C. yields 50 kcal./mole, possibly the

resultant activation energy of a few consecutive anabolic steps. In spite of the complexity of the curves for malic and aspartic acids, the ratio aspartic/malic between 32 and 44° C. shows a constant slope corresponding to an energy of 28 kcal./mole. Assuming that these two acids accumulate outside the regenerative cycle (9) and are in equilibrium with each other, one might consider this temperature effect as an equilibrium shift and the above energy as the free energy of the corresponding reaction. Although these interpretations are only speculative, the results of these preliminary experiments indicate that some of the intermediate steps in photosynthesis are accessible to thermal analysis.

It should be made clear that the rate of appearance of radioactivity in any intermediate depends not only on the rates of formation and utilization of the intermediate but upon the concentration of previous intermediates as well. Consequently the experimentally observed results are dependent upon the effects of temperature upon the numerous rate constants of this rather complex system.

SUMMARY

The photosynthetic assimilation of radioactive carbon dioxide for two-minute periods by Scenedesmus has been studied at temperatures ranging from 25 to 44° C. All labeled intermediates cease to be formed at about 45° C. With rising temperature, the radioactivity reaching the sugar phosphate reservoirs decreases regularly while there is a sharp maximum in sucrose at 37° C. and a less pronounced one in malic and aspartic acids above 40° C. A tentative interpretation of these effects is offered.

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CAPTIONS TO FIGURES

Fig. 1. Temperature dependence of the rate of fixation of carbon dioxide by Scenedesmus in two-minute photosynthesis at 1200 foot candles. Radioactivity in millions of counts per minute per gram cells: Total ○, soluble in 80% ethanol (◑).

Fig. 2. Radioactivity in some soluble intermediates, in counts per minute per 50 mg. cells, on paper chromatograms. Lower section on enlarged scale.

Fig. 3. 1/T vs logarithm of A) the radioactivities in the phosphates and in sucrose; B) the ratio of the radioactivities in aspartic and malic acids.

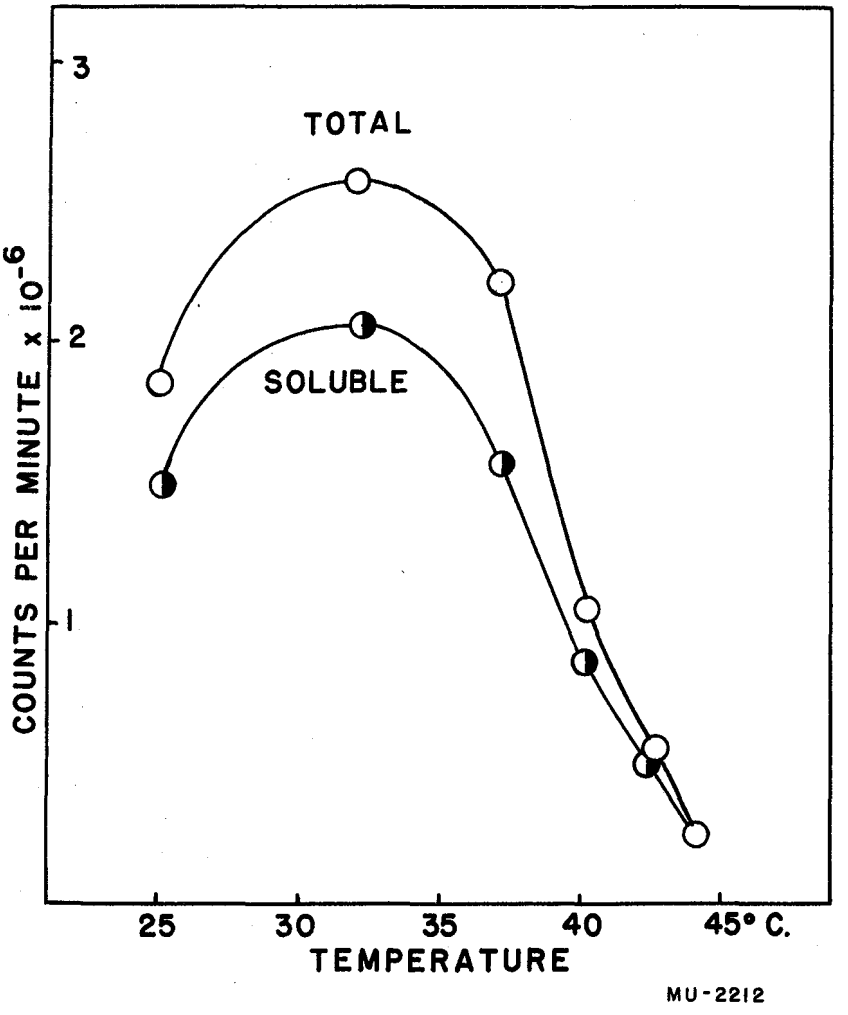
3
2
1
TOTAL
SOLUBLE
COUNTS PER MINUTE x 10-6
25
30
35
40
45° C.
TEMPERATURE
MU-2212

Fig. 1

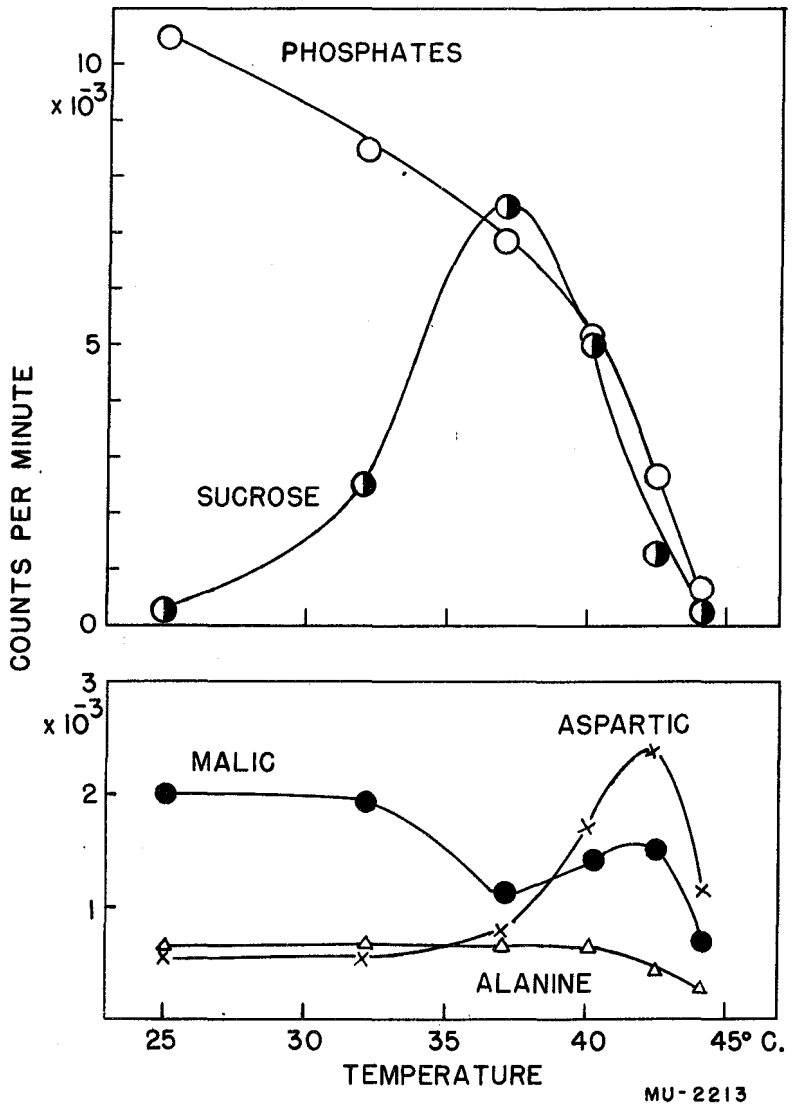
PHOSPHATES
SUCROSE
MALIC
ASPARTIC
ALANINE
COUNTS PER MINUTE
x 10⁻³
10
5
0
3
2
1
25
30
35
40
45° C.
TEMPERATURE
MU-2213

Fig. 2

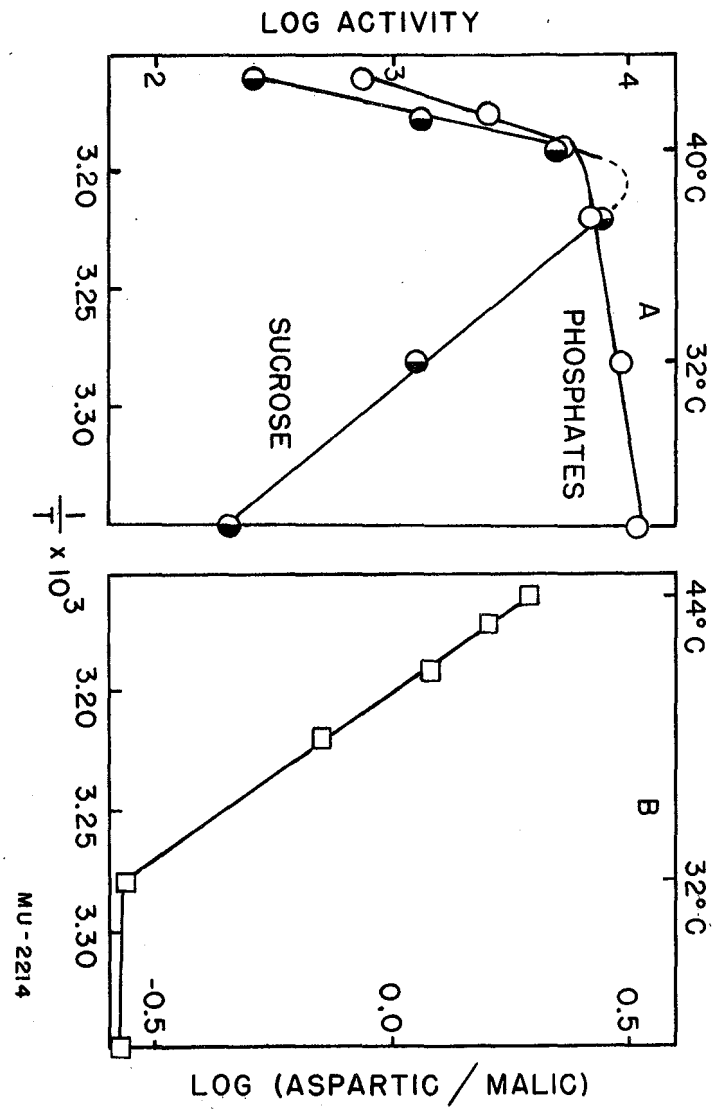
LOG ACTIVITY
2
3
4
40°C
32°C
A
PHOSPHATES
SUCROSE
3.20
3.25
3.30
1/T x 10^3
44°C
32°C
B
-0.5
0.0
0.5
LOG (ASPARTIC / MALIC)
MU-2214

Fig. 3