THE CO₂ COMPENSATION POINT, HILL ACTIVITY AND PHOTORESPIRATION

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Summary

As a result of an examination of O_2 exchange in <u>Scenedesmus</u> and <u>Chlorella</u> using mass spectrometry, a scheme is outlined which depends on accepting CO_2 dependence for Hill activity in whole cells to explain changes in the CO_2 compensation point related to O_2 concentration. The scheme accommodates photorespiration and increased O_2 consumption in the light not related to CO_2 release.

Introduction

In recent years, a great deal of interest has centered around reports that many higher plants carry out photorespiration, a process in which losses of CO_2 increase during photosynthesis, especially at low concentrations of CO_2 and high levels of O_2 in saturating light (e.g., Forrester, Krotkov and Nelson, 1966). Zelitch (1966) and others have presented evidence implicating glycolate as the primary substrate. On the other hand, Brown and Weis (1959) found CO_2 production to be depressed in the light in the alga, Ankistrodesmus. Similar data were obtained by Ozbun, Volk and Jackson (1964) working with bean leaves. Hoch, Owens and Kok (1963) have suggested that increased O_2 consumption in the light observed to occur in certain algae may be a mechanism associated with ATP production. Criteria for photorespiration include a post-illumination burst of CO_2 and a finite CO_2 concentration at the CO_2 compensation point both of which increase as O_2 concentration is increased.

The study reported here was undertaken as part of an attempt to reconcile the above observations.

Experimental and Results

Data were obtained with the mass spectrometer technique of Hoch and Kok (1963) using isotopic 0_2 to allow separate measurement of rates of 0_2 production and consumption and monitoring for net CO2 exchange. Levels of dissolved CO_2 and O_2 in the sample vessel could be varied during sample preparation although precise control was not possible. The exact concentrations of each gas became apparent, of course, as soon as each run was initiated. Saturating light was used throughout with an optical system very similar to that described by Hoch et al. (1963). The algae Chlorella pyrenoidosa and Scenedesmus sp. were used as experimental material. Methods of culturing and harvesting have been described elsewhere (Bunt, MS). For making measurements, Scenedesmus was suspended in PO4 buffer at pH 7.0 and Chlorella in fresh medium of pH 4.2. Cell concentrations were adjusted to avoid undue changes in the concentrations of dissolved gases during the course of measurements in the closed sample vessel. In the experiments reported, Scenedesmus was exposed to 300 and Chlorella to $20^{\rm o}$. Rapid sequential monitoring of the two ${\rm o}_2$ isotopes and of CO2 gave an almost continuous record of gas exchange. Rates, however, were calculated only at as many points as was necessary to establish responses to declining CO2 concentration.

Figure 1 shows the pattern of O_2 exchange in <u>Chlorella</u> and <u>Scenedes-mus</u> at two levels of O_2 as the CO_2 concentration declined to the compensation point. Several features are noteworthy. At points where a net production or consumption of CO_2 ceased to be evident, i.e. at CO_2 compensation, there remained a finite exchange of O_2 with production somewhat exceeding consumption. In <u>Chlorella</u>, the magnitude of O_2 exchange at CO_2 compensation was not altered appreciably by a considerable increase in O_2 concentration whereas, in <u>Scenedesmus</u>, there was a markedly larger O_2 exchange at high than at low O_2 . With the exception of <u>Chlorella</u> exposed to low levels of O_2 , O_2 consumption declined markedly with approach

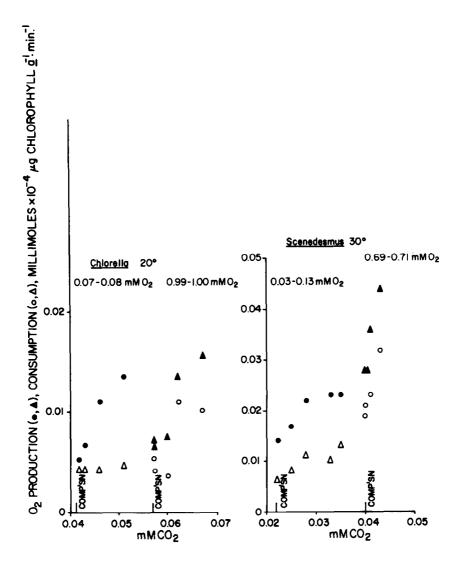


Figure 1. Rates of O_2 production and consumption in <u>Chlorella</u> and <u>Scenedesmus</u> approaching the CO_2 compensation point at two concentration ranges of O_2 .

to the CO_2 compensation point. Forrester <u>et al</u>. (1966) report that, in soybean, the CO_2 compensation point extrapolated to zero at zero O_2 concentration. This was not found to be the case in <u>Scenedesmus</u>. Based on a number of runs, extrapolation indicated that there should be quite pronounced O_2 exchange even at zero O_2 . Fewer data were obtained for <u>Chlorella</u> although they lead to a similar conclusion.

Discussion

Warburg and Krippahl (1958) appear to have been the first investigators to report that, in chloroplasts, Hill activity ceases in the absence of CO_2 . Their finding has since been confirmed by Izawa (1962), Good (1963) and others, all using chloroplasts but from a wide variety of plant sources. Mass spectrometry avoids the need to restrict measurements of Hill activity to chloroplasts and the data obtained in these experiments has been interpreted to indicate that dependence of photosynthetic O_2 evolution on CO_2 applies also to whole cells. Furthermore, these results suggest that some form of competition exists between O_2 and CO_2 so that the CO_2 requirement for Hill activity increases as the concentration of O_2 increases. If this assumption is correct, it offers a basis on which to explain dependence of the CO_2 compensation point on O_2 concentration which is independent of, but can accommodate, photorespiration. Figure 2 summarizes the hypothesis in general terms.

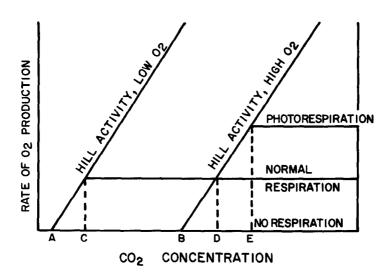


Figure 2. Postulated relation between Hill activity (limiting levels of CO_2) at several levels of O_2 , and the CO_2 compensation point without associated respiratory activity (A & B); with normal respiration (C & D) and with photorespiration (E).

Should conditions exist in which CO_2 production ceases in the light, it would be expected that, in a closed system, CO_2 should be depleted by photosynthetic activity, the cessation of its fixation being dependent on Hill activity which would cease at a CO_2 concentration related to O_2 concentration. These circumstances would be displayed as a set of CO_2 compensation points increasing with O_2 concentration.

In cells maintaining some level of normal respiratory activity, a function not affected by O_2 concentration over a wide range, CO_2 fixation would decline until its rate were controlled by the supply of CO_2 controlling Hill activity. The effect would be to shift the CO_2 compensation point to a higher level, the magnitude being independent of O_2 concentration. In cases where photorespiration were operating, one would expect the upward shift in the compensation point to become more and more pronounced up to levels of O_2 saturating photorespiratory activity.

As a possible extension of this hypothesis, increased O_2 consumption related to increased Hill activity and exceeding rates at the compensation point may be attributable to the process described by Hoch et al. (1963). Whether or not the latter may be identified with O_2 -dependent ATP formation (Forti and Jagendorf, 1961), as seems possible, has not been determined.

The scheme outlined appears compatible with a variety of apparently disparate observations. Its validity is currently being explored in detail.

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