Cellular Opacity and the Activity of Chloroplast Pigments in Photosynthesis

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IN GREEN PLANTS, chloroplast pigments utilize the energy of sunlight for the production of oxygen and organic matter. The effectiveness of this unique photosynthetic reaction varies greatly in light of different wavelengths. This variation of photosynthetic activity with wavelength was observed first when plants plus motile, oxygen-sensitive microorganisms were placed in a visible spectrum (Engelmann) (*7*). It has been demonstrated repeatedly by numerous comparative measurements of photosynthesis in different spectral regions (*1, 4, 10–13, 16, 25*) and by careful physical measurements of photosynthetic efficiency (*3, 5, 8, 22–24*). Thus far, variation of photosynthetic efficiency with wavelength has been attributed primarily to variation in the amount of light absorbed by active pigments and to differences among the intrinsic reactivities of the individual pigments.

The diversity of the chloroplast pigments has presented serious obstacles to precise determinations of their photosynthetic effectiveness. Some five dozen or more of these pigments have been isolated from various autotrophic organisms. Several to a dozen or more occur in each plant. Fortunately, most of the chloroplast pigments are of two principal kinds, the chlorophylls and the carotenoids. In certain algae, red and blue proteinaceous phycobilins accompany the carotenoids and chlorophylls. Only one pigment, chlorophyll *a*, is a major component of the chloroplasts of virtually all autotrophic plants (*17–19*).

As shown by many investigations, photosynthetic efficiency is greatest in the spectral regions that are strongly absorbed by the active chloroplast pigments, and it is least in the spectral regions that are weakly absorbed by active pigments. The dependence of photosynthetic efficiency upon the absorption capacity of the pigments has been encountered when a single active pigment as a chlorophyll (*6*), a carotenoid (*3*), or a phycobilin (*5, 8, 9*) is the principal light-absorbing agent. This effect has also been observed when chlorophylls plus phycobilins (*4, 7, 9–14, 21*) or chlorophylls plus carotenoids (*3, 4, 6, 11–13, 22, 23*) are the active photosynthetic pigments, and it has been found in dense cell suspensions that absorb all the incident light (*2, 3, 5, 6, 9, 11, 22, 23*).

Dependence of the photosynthetic efficiency upon the absorption capacity of the pigments may now be correlated, in part, with the amount of light absorbed by active pigments relative to that absorbed by nonactive, opaque substances. Absorption by pigments is, in turn, a function of the concentration and the condition of the pigments in the chloroplasts. It is also a function of the volume of the chloroplasts relative to the volume and the opacity of the plant cells.

With high absorption capacity by active pigments, the incident light traverses little of the plant material before it is absorbed. Under these conditions only a small proportion of the incident energy is lost to inactive cellular constituents, such as cell walls, food-storage granules, nuclei, protoplasmic colloids, refractive interfaces, inorganic ions, and inactive cytoplasmic pigments. With low absorption capacity by active chloroplast pigments, the light traverses much cellular material before it is absorbed; hence a much larger proportion of the incident energy is lost to nonreactive, opaque materials, and the photosynthetic efficiency is reduced proportionately.

ABSORPTION CAPACITY OF INDIVIDUAL PIGMENTS AND PHOTOSYNTHETIC EFFICIENCY

Examples of the dependence of photosynthetic efficiency upon the absorption capacity of the active pigments have frequently been reported. In the green alga *Chlorella*, utilization of incident light by chlorophylls *a* and *b* is greatest in the spectral region of their absorption maxima but decreases at shorter and at longer wavelengths (*5*). In the diatom *Nitzschia* and in many brown algae, utilization of the light that is strongly absorbed by the carotenoid fucoxanthin is more efficient than utilization of light that is weakly absorbed by this carotenoid pigment (*3, 10–13, 16, 23, 24*). In many red algae, utilization of the yellow-green light that is most absorbed by phycoerythrin plus chlorophyll is more effective than utilization of the red light that is less absorbed by the chlorophyll alone (*1, 3, 7–11*). In the blue-green alga *Chroococcus,* photosynthetic efficiency is likewise greatest in the spectral regions that are strongly absorbed by chlorophyll *a* plus phycocyanin, and it is least in the regions that are weakly absorbed by chlorophyll alone or by phycocyanin plus carotenoids (*5*).

In the blue-green spectral regions, absorption by carotenoid pigments is usually greater than the low absorption by chlorophylls (*5, 6, 9, 11, 17, 22*); hence low photosynthetic efficiency in this region of weak absorption has commonly been attributed to low specific activity of the carotenoids (*5, 6, 22*). As this low photosynthetic activity may now be ascribed, in part, to a greater loss of the weakly absorbed light to nonreactive substances in the plant cells, the photosynthetic effectiveness of the carotenoids must be greater than was thought. Examination of this possibility depends upon knowledge of the concentration and the spectral absorption properties of the pigments, as well as upon knowledge of the opacity of the cellular material.

CONCENTRATION OF THE PIGMENTS AND THEIR ABSORPTION CAPACITY

The relative absorption capacity of the pigments at different wavelengths varies not only with their spectral absorption properties but also with their concentration (*13*). Chlorophyll *a,* the principal chloroplast pigment of most autotrophic plants, exhibits great variation in its absorption capacity for green light in comparison with red light (*19*). The higher the concentration of the chlorophyll, the greater the absorption capacity for green light relative to the absorption of red light. For a methanol solution with a light path of 5 microns and with a chlorophyll concentration of 2 percent, just over 80 percent of the incident blue and red light will be absorbed, but only 4 percent of the incident green light will be retained. Virtually all the incident red and blue light could, therefore, be absorbed by a single chloroplast containing this amount of chlorophyll, whereas most of the incident green light would be transmitted to penetrate other cellular materials (*18, 19*). Even with a chlorophyll concentration of 10 percent and with a light path of 10 microns, the absorption of green light by a single chloroplast would still be less than one-third that of red light. In the largest and greenest chloroplasts, without this much chlorophyll, the incident green light would penetrate many chloroplasts and much cellular material before it is absorbed.

CONDITION OF THE PIGMENTS AND PHOTOSYNTHETIC EFFICIENCY

Calculation shows that absorption of light by pigments relative to the absorption by nonphotosynthetic substances depends upon the volume of the chloroplasts relative to the volume and the opacity of the cells. For a given amount of chlorophyll, the smaller the volume of the chloroplasts relative to the cells, the lower the absorption by the active pigments and the greater the proportion of incident energy lost through absorption by nonreactive materials. In harmony with this conclusion, the highest photosynthetic efficiencies have been found in unicellular plants rich in pigments and with large chloroplasts filling most of the cells (*3, 5, 6, 20, 22*). The lowest photosynthetic efficiencies have been found in plants with a few small chloroplasts relative to the total amount of plant material (*2, 11*).

Absorption of light by active pigments relative to absorption by inactive substances also depends upon the condition or distribution of the pigments within the chloroplasts (*19*). The more diffuse the distribution, the greater the absorption by the pigment relative to the absorption by other less active pigments and by inactive substances. As indicated by the effect of heat and protein reagents upon the color of algae, the water-soluble proteinaceous phycoerythrin is diffusely distributed through the chloroplasts of red algae, and the similar phycocyanin is similarly distributed in the pigmented portions of blue-green algae (*19*). Qualitatively, there is a positive correlation between the condition of these phycobilin pigments in the cells and the photosynthetic efficiency (*8, 9, 11*).

CELLULAR OPACITY AND PHOTOSYNTHETIC EFFICIENCY

Absorption spectra of leaves, of bleached chlorophyllous tissue (*15, 17, 23*), and of pigment-free cells show that the absorption of light by the nonphotosynthetic components of plants varies rather uniformly with wavelength. Absorption is greatest in blue light and least in red. Qualitatively, therefore, the utilization of light by active pigments depends primarily upon their absorption capacity. Quantitatively, the utilization of light by active pigments depends upon their intrinsic photosynthetic activity and upon their absorption capacity relative to the total absorption capacity of the plant material.

Many qualitative observations and a few quantitative measurements indicate that great opacity of the plant cells is associated with low photosynthetic efficiency. Leaves that contain large amounts of opaque structural material utilize the incident light very inefficiently (*11, 15, 17*). In shallow-water brown algae of the family Fucaceae, great opacity is associated with low photosynthetic activity. In deep-water brown algae, by contrast, low opacity permits efficient utilization of the incident light (*11–14, 17*). As a rule, the highest photosynthetic efficiencies have been found in unicellular algae that exhibit great absorption capacity by active pigments and low absorption capacity by their cellular substance (*2, 5, 6, 11, 23*).

In leaves the quantitative relationship between absorption by nonreactive substances and absorption by active chloroplast pigments varies enormously with the leaf itself and with the wavelength of the incident light. In the green spectral regions, where absorption by active pigments is at a minimum, as much as 20–50 percent or more of the light may be lost to nonreactive, cellular substances (*11, 15, 17*) that occur outside the chloroplasts (*15*). But at wavelengths that are strongly absorbed by the chlorophylls, only 1–5 percent of the incident light may be lost (*15, 17*).

From these considerations, cellular opacity has a large effect upon the photosynthetic efficiency only in spectral regions of low absorption capacity by active pigments. Even in these regions photosynthetic efficiency is related to the concentration of the active pigments, to the condition of the pigments in the chloroplasts, and to the number, size, and orientation of the chloroplasts. Owing to the natural variation of all these conditions and to the complexity of the pigment mixtures, evaluation of the photosynthetic effectiveness of the individual pigments in different plants is subject to great uncertainty.

RELATIVE EFFECTIVENESS OF INDIVIDUAL CHLOROPLAST PIGMENTS

As indicated already, the photosynthetic effectiveness of the individual pigments in each organism has been estimated from relative or comparative measurements of photosynthesis at different wavelengths (*11, 22*). For these comparisons the intrinsic activity of each pigment has been assumed to remain constant, irrespective of the absorption capacity. When corrected for the light lost to opaque substances, the differences among the activities of the several pigments are reduced. An example cited above is the greater effectiveness of the weakly absorptive carotenoids in higher plants, in green algae, and in blue-green algae. Similarly, the effectiveness of the abundant phycoerythrin in red algae (*8, 9, 11*) is reduced relative to the effectiveness of the chlorophyll.

In *Chlorella* photosynthetic efficiency of chlorophylls *a* and *b* in weakly absorbed red light (700 mμ) is very low relative to the efficiency in strongly absorbed red light (680 mμ) (*6*). This effect may now be ascribed to a proportionately greater loss of the weakly absorbed light to nonreactive materials, rather than to variation of the photosynthetic efficiency of the green pigments (*6*).

In diatoms and in deep-water brown algae, the photosynthetic efficiency in the spectral range of 500–600 mμ approaches that in red light (*3, 10–14, 16, 23, 24*). As chlorophyll *c* absorbs much of the incident light in this spectral region (*3, 20*), the pigment must be effective in the photosynthetic process. This conclusion and the activity of diatom carotenoids support the view that deep-water plants deriving their energy primarily from light of low intensity cannot afford high concentrations of noneffective pigments that absorb strongly in this spectral region (*19*).

PIGMENTS AND THE MECHANISM OF PHOTOSYNTHESIS

The photosynthetic effectiveness of the chloroplast pigments is more intimately related to their spectral absorption capacity than to any other established physical or chemical property. Aside from their great spectral absorption capacity, the diverse pigments of the chloroplasts do not have many chemical properties in common. Thus far, the use of the most sensitive chromatographic adsorption methods has not revealed colored alteration products of the chloroplast pigments in photosynthesizing plants (*18*). Yet, if one molecule of chlorophyll were involved in the transfer of two hydrogen atoms or of one oxygen atom per molecule of assimilated carbon dioxide, some 30 grams of chlorophyll would undergo change per gram of carbohydrate produced. In view of these facts, the transformation of radiant energy to chemical energy during photosynthesis must depend upon instantaneously reversible reactions of the pigments, or upon reactions confined to colorless components of the pigment-chloroplast system.

In the utilization of sunlight by chloroplast pigments, chlorophyll *a* plays a predominant role. It is the most abundant and, in many plants such as the yellow-green algae, it is the only green chloroplast pigment. Chlorophyll *a* has great spectral absorption capacity; it is effective in spectral regions where carotenoids do not absorb light; and it exhibits great photosynthetic efficiency. As a consequence, elucidation of the mechanism of energy transfer in autotrophic plants hinges upon knowledge of the activity of this chlorophyll.

The efficiency of photosynthesis in monochromatic light varies with the spectral absorption capacity of the pigments and with the opacity of the plant cells. With high pigment concentration, with great spectral absorption capacity, with large chloroplasts, and with transparent plant cells, little of the incident energy is lost to nonreactive opaque substances, and the efficiency of photosynthesis is high. With low spectral absorption capacity, with a small volume of chloroplast material relative to the volume of the cells, and with opaque plant material, a large proportion of the incident energy is lost to the inactive opaque substances, and the photosynthetic efficiency is low.

When the opacity of the plant material is considered as a factor affecting the efficiency of photosynthesis, most chloroplast pigments must be regarded as effective, though not necessarily equally effective, in the process. The available evidence suggests that energy absorbed by pigments is utilized without concomitant chemical change of the pigments themselves.

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Concerning the Measurement of pH, Ion Activities, and Membrane Potentials in Colloidal Systems

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IN THE FIELDS of biology and agriculture and in many industrial laboratories, pH measurement of colloidal systems, such as gels and thick suspensions, plays a prominent role in research and testing activities.

Measurement of pH with H_2-, quinhydrone-, and glass-electrodes, and of cation activity with various charged membranes, always involves a liquid junction, usually at a KCl bridge. In measurements made in true solutions the liquid junction potential probably is negligible, but this is not necessarily true for systems containing a great number of electrically charged particles of extremely high valencies, such as those represented by large clay and resin particles.

We are proposing a theory, supported by experimental data, showing that in certain colloidal systems liquid junction potentials may assume considerable magnitudes.

Consider microscopic particles of cation exchange material (e.g., resin) bathed in a dilute solution of KCl. Let ionic equilibrium be established. When a salt bridge containing saturated KCl is inserted into such a system, a junction between solutions having two different KCl concentrations within the matrix of the negatively charged particles results. If the negatively charged particles affect the relative diffusion rates of K^+ and Cl^-, a potential will arise at the interface of bridge and assemblage of particles, which we shall describe in terms of the activities of KCl in the liquid between the particles and in the bridge and the transference numbers of K^+ and Cl^- in the interface.

Transference numbers of K^+ and Cl^- in systems comprising KCl solution and cation exchanger material were determined by a modification of the Hittorf method. An 8-mm layer of the cation exchange material was interposed between the anode and cathode compartments of a Bradfield cell so that ions trans-

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