Short Communications and Preliminary Notes

THE ELECTRON SCATTERING POWER OF PROTEIN STRUCTURE IN THE SPINACH CHLOROPLAST

by

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In a preceding paper (THOMAS, BUSTRAAN, AND PARIS[1]) the occurrence of protein fibrils in the stroma of spinach chloroplasts has been mentioned. These fibrils consist of "globules" linked together by "threads", in a way resembling the structure of chromosomes. They were considered to be identical with "chromidia" and "interchromidia"—*cf.* MONNÉ[2] and RONDONI[3]. In contradistinction to interchromidia, the chromidia are assumed to contain phospholipids, ribonucleic acid, and metals. If the above were true, we may expect that the chromidia show a higher electron scattering power per unit of volume than the interchromidia do.

A preliminary experiment, mentioned in our preceding paper[1], seemed to confirm this hypothesis. However, it has been emphasized that a more accurate procedure is required in order to state things with certainty. In the meantime such experiments have been carried out. A brief account of the results follows here.

Preparations of spinach chloroplasts, deprived of lipoids by means of either acetone or lipase, were studied under a Philips electron microscope. The above-mentioned protein fibrils were photographed. Then, they were shadowed and photographed again. In this way we obtained pictures of the same fibril before and after shadow-casting. Consequently, the ratio of the thickness of a chromidium and an interchromidium could be computed in a twofold way: by measuring of the shadow lengths as well as by determining the electron scattering power of both structures, provided this property is the same per unit of volume of both. If this were true the values of both ratios must coincide. If, on the contrary, the electron scattering power per unit of volume of the chromidia differs from that of the interchromidia these values must diverge.

The estimation of the thickness from the electron scattering power was done according to a device of MARTON AND SCHIFF[4]. To this purpose the ratio between the incident electron current density and that transmitted by the object and recorded on the photographic film must be known. This ratio was determined by microphotometrical evaluation of the density of the non-shadowed electron micrographs. The validity of this procedure was proved to be valid for WO_3 crystals as well as for silica films too.

The results obtained pointed out that the ratio of thicknesses of the chromidia and the interchromidia, when computed from the electron scattering power, surpasses that obtained by measuring the shadow lengths. So we conclude that the electron scattering constant of the chromidia indeed exceeds that of the interchromidia. In fact, the mean ratio of these constants is 1.34 for acetone-treated preparations whilst it numbers 1.56 for lipase-treated ones. This clearly indicates a difference in constitution. Whether this is due either to the presence of heavier elements or to a higher density of the chromidia as compared to the interchromidia cannot be decided by the applied technique.

A full account of this investigation will be published later in this journal.

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REFERENCES

[1] J. B. THOMAS, M. BUSTRAAN, AND C. H. PARIS, *Biochim. Biophys. Acta*, 8 (1952) 90.
[2] E. MONNÉ, *Advances in Enzymol.*, 8 (1948) 1.
[3] P. RONDONI, *Ergebn. Enzymforsch.*, 10 (1949) 264.
[4] L. MARTON AND L. I. SCHIFF, *J. Appl. Phys.*, 12 (1941) 759.

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SUCROSE SYNTHESIS IN HIGHER PLANTS AND HIGH ENERGY PHOSPHATE

by

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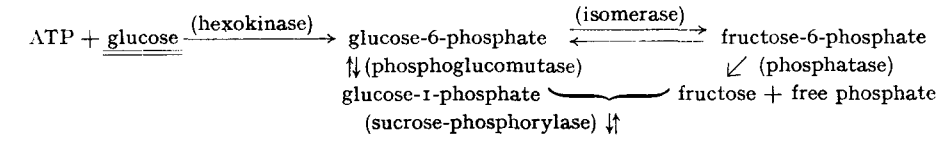
Literature data[1,2,5,6] as well as experiments carried out on germinating rice by the present authors, clearly show that aerobic respiration is necessary for the synthesis of sucrose in higher plants. The following experiments offer an explanation for this phenomenon. Potatoes (variety Doré) were kept in the dark at 30° C for a couple of weeks and slices were cut from the small tubers ("submarines") formed by them. These slices were exposed to the action of 20% sugar solutions at pH 7 for 1 hr; after this, they were superficially dried and kept in a moist desiccator for 18–20 hr (compare 6). Both glucose and fructose gave a great increase in sucrose, *viz.*, up to 177% in addition to the amount already present. 20% mannitol gave a 72% decrease, presumably owing to respiration; this means that the plasmolysis caused by the hexose solutions probably is not, or not solely, responsible for their effect. Inorganic phosphate strongly inhibited sucrose formation, which *may* mean that, in the final step of sucrose synthesis, a liberation of inorganic phosphate takes place. Vacuum infiltration of the slices with the solutions used[4] or replacement of air by nitrogen prevented sucrose synthesis, *unless ATP was added beforehand.* It is a well-established fact that much more of this substance is produced in aerobic than in anaerobic respiration. AMP or ADP could not replace ATP, so that the effect of the latter substance cannot be due to the presence of the adenosine ring system alone. Magnesium ions had an activating effect upon sucrose synthesis, certain concentrations of fluoride an inhibiting one. Glyceraldehyde and its biological precursor, L-sorbose-1-phosphate (both known to be hexokinase poisons!) inhibited synthesis. As the present authors have been able to obtain a fairly active hexokinase concentrate from potato press-juice, and as the enzyme has since then also been reported in the same material by KOTELNIKOVA[3], it is reasonable to assume that the function of ATP in sucrose synthesis is to make possible the action of hexokinase. Experiments with glucose-6-phosphate have shown that this must be the only function of ATP here, for glucose-6-phosphate under anaerobic circumstances gives only an insignificant decrease in sucrose, and sometimes even a small increase. Other enzymes demonstrated in potato press-juice by the present authors are: phosphoglucomutase, phosphohexoisomerase and, surprisingly, a phosphatase which, at pH 7, exerts its action mainly on fructose-6-phosphate. It is the presence of this latter enzyme with its power to produce free phosphate which may account for the lack of success encountered by the present authors when they tried sucrose synthesis from fructose-6-phosphate or from Robison-ester. It is clear that, if only sucrose phosphorylase could be demonstrated in potatoes, sucrose synthesis there might be pictured as follows:



The thin evidence for the presence of sucrose phosphorylase that could be obtained is mainly based on experiments in which potato press-juice was allowed to act, at pH 7, on a mixture of sucrose and inorganic phosphate, in the presence of a small amount of notatin added to eliminate selectively the interfering substance glucose. In most cases, a decrease in inorganic phosphate and a roughly corresponding increase in 7-min-phosphate was observed. This 7-min-phosphate could not be identified with certainty yet, but at least part of it seems to be glucose-1-phosphate. Sucrose could not be replaced by other sugars, with the exception of maltose, a substance known to be phosphorylated