# ACTION SPECTRUM OF PHOTOPHOSPHORYLATION IN VIVO BY ANKISTRODESMUS BRAUNII.

Wilhelm Simonis and Ernst Mechler
Botanical Institute, University Würzburg, Germany.

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In previous experiments with short time incorporation of labelled phosphate into living algal cells (Simonis 1960, Simonis and Urbach 1962,1963) it has been found that the $^{32}P$-incorporation and the fixation in certain phosphate-fractions is significantly higher in light. For a better understanding of the connection between this l i g h t d e p e n d e n t phosphorylation and p h o t o s y n t h e t i c phosphorylation in living cells it was desirable to measure the action spectrum of the former in v i v o.

The algae were cultured in weak light of about 185 footcandles (2000 Lux) in a 16:8 hours light and dark period and transferred into phosphate free medium (Kandler 1950) 3 days before experiments. The algae were treated for 5 minutes in the light or in the dark respectively with $^{32}P$-labelled phosphate of low concentration ($3,2 \cdot 10^{-8}$M) in a Tris-buffered solution (pH 8). The light was supplied by a slide projector (Leitz-Prado 750 Watt) and interference filters (Schott und Gen. Mainz, type FIL or IL) protected from infrared radiation by 1,5 cm water and BG 12 filter (Schott,Mainz). The cells were killed and extracted by 10% TCA solution. The radioactivity was determined in various phosphate-fractions according our previous methods.

Table I

Light dependent phosphorylation by Ankistrodesmus braunii in red light ($\lambda$ = 670 mμ), incident energy 720 ergs/cm$^2$ · sec. Time of $^{32}$P-incorporation 5 minutes. Counts/min · mg dry weight.

| phosphate-fraction | dark cpm | red light cpm | percent of dark |
|---|---|---|---|
| TCA-soluble organic (Po) | 8 250 | 16 500 | 200 |
| TCA-soluble ortho-phosphate (Pa) | 14 050 | 23 600 | 168 |
| TCA-insoluble (Pu) | 9 000 | 9 150 | 101 |
| total phosphate | 31 300 | 49 250 | 157 |

Experimental conditions:

10,0 ml suspension of algae in phosphate free medium; 1,65 mg dry weight; 1,5 ml Tris-buffer pH 8; 1,5 ml sodium chlorid 2 · $10^{-2}$M; 2,0 ml $^{32}$P-labelled phosphate solution (3,2 · $10^{-8}$M); 15,0 ml tot volume.

In red light of 670 mμ a distinct light dependent phosphorylation was observed even at a low incident energy of 720 ergs/cm$^2$ · sec. (Table I). Light saturation occured in this case at energies of about 6000 ergs/cm$^2$ · sec. The most significant differences between light and dark were obtained in the TCA-soluble organic phosphate fraction (Po). The measurement of the action spectrum was therefore confined to the incorporation into the Po-fraction and the light dependent phosphorylation was investigated in a series

of experiments at different spectral regions with constant incident energy (720 ergs/$cm^2$ · sec.). The values of $^{32}P$-incorporation at different wavelengths were related to the incorporation at 683 mμ as 100% and calculated on equal number of incident quanta (4,12 · $10^{-12}$ Einstein/$cm^2$ · sec.).

The action spectrum of the light dependent $^{32}P$-incorporation into the Po-fraction is given in figure I. It resembles essentially the absorption spectrum of living Ankistrodesmus cells. The peak in the red region is found at 670 mμ. The action spectrum of the total phosphate incorporation is similar to the spectrum in figure I.

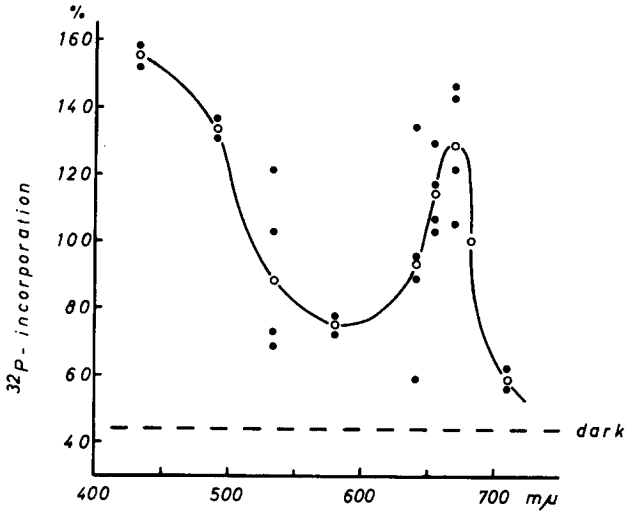


Figure I

Relative action spectrum of the $^{32}P$-incorporation into the TCA-soluble organic phosphate fraction (Po) by Ankistrodesmus braunii (Red light 683 mμ = 100%) calculated on the base of equal number of incident quanta (4,12 · $10^{-12}$ Einstein · $cm^{-2}$ · $sec^{-1}$). Dotted line = average dark incorporation.

Hence it follows that our spectrum of light dependent phosphorylation from living algal cells originates essentially from photosynthesis since it corresponds to the action spectrum of ATP-formation by isolated chloroplasts in the presence of phenazin methosulfate (Jagendorf 1958). There is also good agreement to the results of Black, Turner, Gibbs (1962) who measured the TPN-reduction and the simultaneous formation of ATP. The peak of the spectrum of the ATP-formation as measured by Arnon (1961) is very near to the spectral peak found by us. Finally there is a good agreement to the action spectrum of photosynthesis in Chlorella (Myers and French 1960, fig. 3).

In summary one can consider - the experimental conditions being as ours - the light dependent phosphorylation as an approximate measure of the photosynthetic phosphorylation occuring in l i v i n g algal cells.

A detailed description and descussion of our results will be published elsewhere. The experiments are being continued.

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