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EFFECT OF CHEMICAL MODIFICATION OF AMINO GROUPS BY FLUORESCAMINE ON PARTIAL REACTIONS OF PHOTOSYNTHESIS

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Summary

4-Phenylspiro[furan-2(3H),1-phtalan]3,3'-dione (fluorescamine) was used to covalently modify amino groups of thylakoids. Subsequently its effect on parameters of energy transfer and phosphorylating activity was assessed. While electron transport, the extent of proton uptake, 515 nm change and 9-aminoacridine quench were relatively resistant to such treatment, the functions connected to coupling factor 1, namely ATP formation by acid/base transition, ATPase activity and photophosphorylation were affected much earlier. Photophosphorylation appears to be the most sensitive. The data are interpreted as indicating an involvement of free amino groups in energy transfer.

Introduction

The chemical modification of surface groups constitutes one of the approaches to the molecular architecture of the thylakoid system. Surface groups are covalently labeled and thus blocked by reaction with the chemical modifier. The altered system is then assessed for the remaining functional capabilities and conclusions are drawn about the involvement of the modified reactive groups in the photosynthetic process. In this way McCarty et al. [1,2] reacted sulfhydryl groups with *n*-ethylmaleimide and obtained information about their involvement in ATP formation by coupling factor 1 (CF_1). Similarly, certain aspects of the role of carboxy groups in grana stacking [3] and energy transfer between electron transport and CF_1 [4] were elucidated with the aid of complexing carbodiimides.

The present communication focusses on the role of free amino-groups on

Abbreviation: CF_1, coupling factor 1.

the thylakoid surface. Initial experiments were reported by De Kouchkovsky [5] using fluorescein-isothiocyanate as covalent coupling agent to monitor membrane pH changes. Kraayenhof and Slater [6] and Oliver and Jagendorf [7] showed a close connection between free aminogroups and the ability of CF_1 to undergo conformational change by labeling them covalently with fluorescamine and methyl acetimidate, respectively. The former label, developed by Udenfriend et al. [8] and used also in this study, combines with available H_2N-groups to form a fluorescent adduct with a half-time of 500 ms. The unreacted reagent is destroyed by hydrolysis within seconds, so that a quick, efficient modification of primary amino groups of the thylakoid surface can be achieved.

While the above mentioned studies emphasized the role of free amino-groups for the catalytic role of CF_1, this communication is concerned with their interaction in the coupling between electron transport and the ATP-synthesizing system. It complements results published before [9,10].

Materials and Methods

Two types of plastid membranes were used in the experiments. The main assay system consisted of chloroplasts, isolated and washed once in 0.3 M sucrose/50 mM NaCl/1 mM $MgCl_2$/10 mM Tricine, pH 7.8, and washed three times further in 50 mM NaCl/1 mM $MgCl_2$/2 mM Tricine, pH 7.8. Final suspension was in isolation medium. Electron-microscopic examination of such preparations showed them to consist of grana and membrane fragments. Only one type of surface protein, presumably CF_1, could be discerned (compare refs. 11 and 12].

Besides this very simplified system, class I type chloroplasts were used for comparative purposes. They were isolated, washed and resuspended in 0.3 M sucrose/50 mM NaCl/1 mM $MgCl_2$/10 mM Tricine, pH 7.8.

Fluorescamine was purchased from Serva. 20 μl acetone containing the label in various concentrations were added to chloroplasts, equal to 500 μg chlorophyll in a total volume of 2 ml isolation medium. The chloroplast suspension was kept in the dark for at least 30 min prior to addition of fluorescamine.

The various electron transport activities were measured in red, saturating light ($\lambda > 610$ nm) using FeCN as acceptor in the usual manner [10]. *P*-700 change and the 515 nm signal were determined with both steady-state relaxation spectrophotometry [13] and repetitive flash technique [14] using the assay conditions indicated in the literature. Both methods gave identical results. Proton uptake was determined by a Ingold LOT 405 glass electrode in combination with a Metrohm 505 pH-meter whose signal was fed through a differential amplifier to a Nicolet 1090 storage oscilloscope from whence it was recorded. The assay contained, in the cases indicated, phenazine methosulfate at a concentration of 30 μM slightly reduced by addition of ascorbate. Red light ($\lambda > 610$ nm) was used for illumination. The 9-amino-acridine quench was determined according to Harnischfeger [15]. The concentration of the dye was 10 μM. Photophosphorylating activity was determined by the ^{32}P method of Lindberg and Ernster [16]. The assay contained in 2 ml, 100 mM Tricine, pH 8.3/50 mM NaCl/5 mM $MgCl_2$/2 mM P_i including ^{32}P/

1 mM ADP/1 mM methylviologen/chloroplasts equal to 150 μg chlorophyll. ATP formation through acid base transition was measured according to Jagendorf [17]. ATPase activity of chloroplast membranes was determined after heat activation analogous to Lien and Racker [18].

Results

In salt-washed chloroplasts, used as a simpler system since they presumably contain only CF_1 as surface protein while still functioning in both electron transport and photophosphorylation, the following observations were made following fluorescamine modification of amino groups.

All reactions indicative of intermediate energy provision for ATP formation react similarly to amino-group modification by fluorescamine. Fig. 1 shows that the size of proton-uptake is affected in the same way as 515 nm change and 9-amino-acridine quench. They start to decline exponentially at a fluorescamine/chlorophyll ratio of 0.3 and still possess 65% of their former value at a fluorescamine/chlorophyll ratio of 1.

Photophosphorylation, on the other hand, is more susceptible to modification of free amino groups. Fig. 2 shows that its rate starts declining at a fluorescamine/chlorophyll ratio of 0.1 and no activity is found when the ratio reaches 1. Acid base-induced ATP formation and ATPase activity, although beginning to be influenced at the same fluorescamine/chlorophyll value, still possess 40—50% of their original activity at a fluorescamine/chlorophyll ratio of 1.

It is worth noting that the initial rate of proton uptake is also much more sensitive to amino-group modification than either its final size (compare Fig. 1) or the rate of dissipation (Fig. 3).

Electron transport is only gradually affected by fluorescamine treatment. Both uncoupled activity (Fig. 4) and *P*-700 change (Fig. 5) show this very clearly. Under phosphorylating conditions the same effect is noticed, although

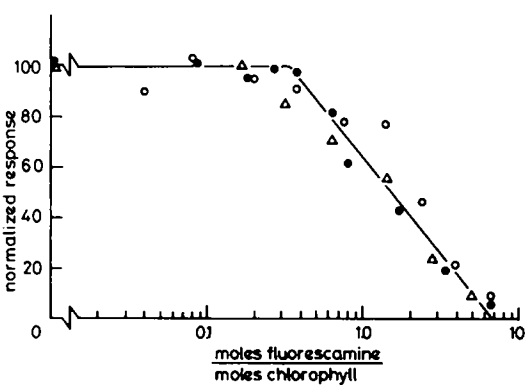

Fig. 1. Influence of fluorescamine concentration in the labeling assay on proton uptake, 515 nm absorption change and 9-aminoacridine quench in salt-washed chloroplasts. ●———●, proton uptake; ○———○, 515 nm change; △———△, 9-aminoacridine quench. The data are normalized to the average control values which were: 135 nmol $H^+ \cdot mg^{-1}$ chlorophyll, $4 \cdot 10^{-3}$ ΔI 515/I 515 $\cdot mg^{-1}$ chlorophyll and 35%, respectively.

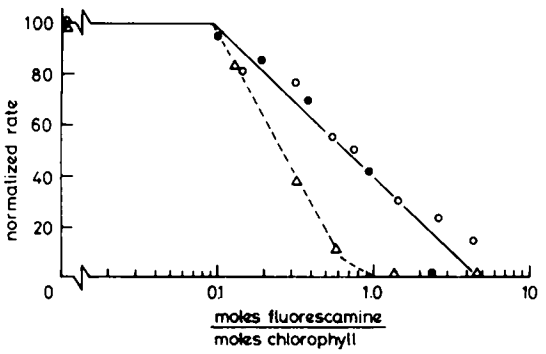

Fig. 2. Influence of fluorescamine concentration in the labeling assay on various CF_1-connected activities in salt-washed chloroplasts. ●———●, heat activated ATPase; ○———○, acid/base transition-induced ATP formation; △———△, photophosphorylation. The data are normalized to the average control values which were: 64 μmol · mg^{-1} chlorophyll · h^{-1} for heat-activated ATPase, 190 nmol · mg^{-1} chlorophyll for acid/base transition-induced ATP and 30 μmol · mg^{-1} chlorophyll · h^{-1} (methylviologen acceptor) for photophosphorylation.

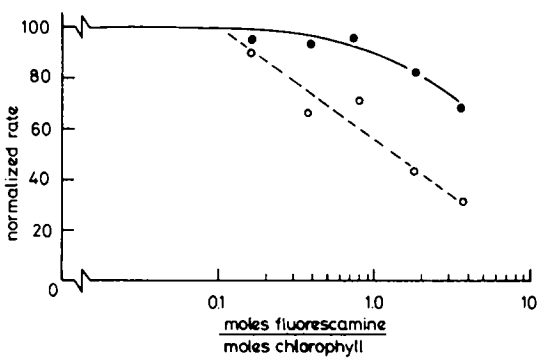

Fig. 3. Influence of fluorescamine concentration in the labeling assay on the measured rate of proton uptake and proton extrusion in salt-washed chloroplasts. Open circles denote the rate of proton uptake, closed circles that of proton extrusion. The rates were determined through the tangent to the initial trace of the electrode signal after switching light on or off. They represent only the lower limit of the actual value. Control values: inflow was 45 nmol · mg^{-1} chlorophyll · s^{-1}, extrusion 11 nmol · mg^{-1} chlorophyll · s^{-1}, both measured in the presence of phenazine methosulfate; without addition of PMS the rates were 12 and 6 nmol · mg^{-1} chlorophyll · s^{-1}, respectively.

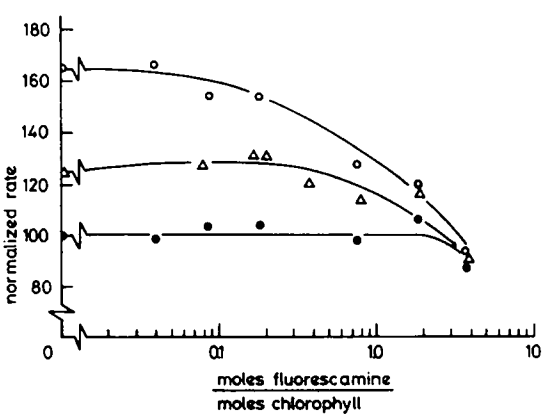

Fig. 4. Influence of fluorescamine concentration in the labeling assay on electron transport activity of salt-washed chloroplasts. ●———●, basal rate (100% equals 73 μmol · mg^{-1} chlorophyll · h^{-1}); △———△, rate in the presence of ADP and phosphate (100% equals 95 μmol · mg^{-1} chlorophyll · h^{-1}); ○———○, methylamine uncoupled electron transport (100% equals 135 μmol · mg^{-1} chlorophyll · h^{-1}).

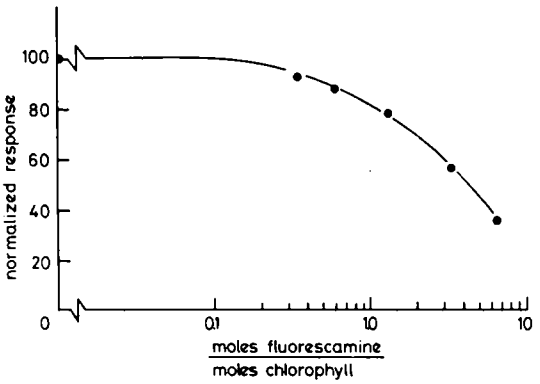

Fig. 5. Influence of fluorescamine concentration in the labeling assay on the *P*-700 signal in salt-washed chloroplasts. The control value was $22.5 \cdot 10^{-3}$ $\Delta I/I \cdot mg^{-1}$ chlorophyll.

to a lesser degree, while basal rates start to become influenced only when they are indistinguishable from the uncoupled activity (fluorescamine/chlorophyll of approx. 2).

The fluorescamine/chlorophyll ratios at which a 50% reduction of the indicated activities takes place are summarized in Table I. For comparative purpose data obtained with class I chloroplasts are incorporated which show that essentially the same phenomena can be observed. Due to the presence of additional surface proteins in this case, the fluorescamine/chlorophyll ratios are shifted to higher values. The behaviour of the 9-aminoacridine quench, however, is a notable exception. This parameter can only, under well defined

TABLE I

RATIO OF FLUORESCAMINE TO CHLOROPHYLL LEADING TO A 50% REDUCTION OF THE INDICATED ENERGETIC PARAMETERS

Plastid type	Parameter	Ratio
Chloroplast fragments	Electron transport	
	Basis	>3
	+ ADP, + P_i	>3
	uncoupled	>3
	P-700 absorption change	>3
	Proton gradient	
	Size	1.5
	Influx rate	1.5
	Efflux rate	>3
	515 nm change	1.5
	9-Aminoacridine quench	1.5
	Photophosphorylation	0.25
	Acid/base induced ATP formation	0.7
	ATPase activity	0.7
Class I chloroplasts	Proton gradient	
	Size	2
	Photophosphorylation	0.6
	9-Aminoacridine quench	0.21

circumstances, serve as indicator of ΔpH since it derives essentially from a concentration quench due to absorption to negative membrane-bound charges [15].

Discussion

The data show, that the most sensitive reaction responding to modification of amino groups by fluorescamine is photophosphorylation. This sensitivity cannot reside in a loss of catalytic activity of CF_1 due to blockage of lysine groups since ATPase activity and ATP formation through acid-base transition are much less affected.

This leaves two possible interpretations for the observed data. The first alternative is a differential decrease of photophosphorylation due to diminished electron transport in the phosphorylating system. Evidence comes from the rate of proton uptake, measured through the slope of the tracings in the glass electrode experiments. Fig. 3 shows that this is influenced similarly to electron transport. Although the time resolution of the glass electrode is limited, it shows clearly the decrease in the rate of uptake. One can, thus, interpret the rate of proton pumping as the rate limiting step for photophosphorylation, although in the absence of ADP and P_i the energetic pool can still be filled.

The second alternative is less specific but cannot be excluded by the data. This interpretation assumes that labeling first affects amino groups critical in the transfer of intermediate energy between the electron transport chain and CF_1. It is conceivable that an electrostatic interaction occurs between protonated amino groups of CF_1 and light-generated carboxyl groups of the thylakoid surface. Such interactions might possibly lead to conformational changes in CF_1, necessary for efficient phosphorylation. Labeling with fluorescamine removes these necessary amino groups of CF_1 and results in a damage of its link to energy-providing reactions.

Another uncertainty is whether the covalent blockage of the amino group or the simultaneous creation of a carboxyl group in the reaction between fluorescamine and amine is the determining factor. This cannot be resolved by the present data.

Acknowledgements

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