A pH DEPENDENT ALTERATION OF PHOTOSYSTEM II ACTIVITY IN TRIS WASHED CHLOROPLASTS *

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Received 16 July 1970

1. Introduction

The inhibition of oxygen evolution in chloroplasts by washing with high concentrations of tris which was reported by Nakamoto et al. [1] has been used extensively to study the mechanism of oxygen evolution in chloroplasts. Yamashita and Horio [2] demonstrated that washing of chloroplasts with 0.8 M tris at pH 8.0 resulted in the complete inhibition of the Hill reaction. Yamashita and Butler [3, 4] have shown that the DCMU-sensitive † NADP photoreduction induced by such treatment can be restored by the addition of artificial electron donors such as *p*-phenylenediamine plus ascorbate and have concluded that the site of inhibition by tris washing is located prior to Photosystem II and more closely related to the oxidation of water. Similar results have been reported by Vernon and Shaw using diphenylcarbazide as an electron donor to Photosystem II [5].

This communication presents results of studies on the effect of pH during tris washing of chloroplasts on oxygen evolution and associated effects on photophosphorylation.

2. Materials and methods

Chloroplasts were prepared from market spinach by grinding 50 g of leaves in a Waring Blendor for 20 sec in 200 ml of 0.05 M tris-HCl buffer, pH 7.8, containing 0.4 M sucrose and 0.01 M NaCl (abbreviated as STN solution). The supernatant from centrifugation of the homogenate at 300 *g* for 90 sec was centrifuged again at 800 *g* for 10 min. The chloroplast pellets were resuspended in STN solution and filtered through glass wool to provide a uniform suspension of particles. The chloroplast concentration was adjusted to 0.5 mg of chlorophyl per ml with STN and the chloroplasts treated by washing with tris. Two ml portions of resuspended chloroplasts were diluted with 1.0 M tris at pH 7.2 (treated) to a concentration of 0.1 mg per ml. The chloroplasts were allowed to remain in the tris solution for 20 min at 0° and then recovered by centrifugation at 20,000 *g* for 10 min. Chloroplasts which had been washed with the STN solution were used as controls. Control and treated chloroplasts were resuspended in STN for use in the photochemical reactions reported. Concentrated tris and STN solutions were freshly prepared and the pH adjusted at 0° prior to each experiment.

Oxygen evolution was measured at 15° using a Clark type oxygen electrode. Photoreduction of ferricyanide was determined by measuring the de-

* This work was supported by grant number GB 7901 to E. Uribe from the National Science Foundation and by the Biomedical Sciences Support Grant number FR-07015 from the National Institutes of Health.

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† *Abbreviations*:
Tris: tris (hydroxymethyl) amino methane;
tricine: tris (hydroxymethyl) methylglycine;
DCMU: dichlorophenyl-1,1-dimethylurea.

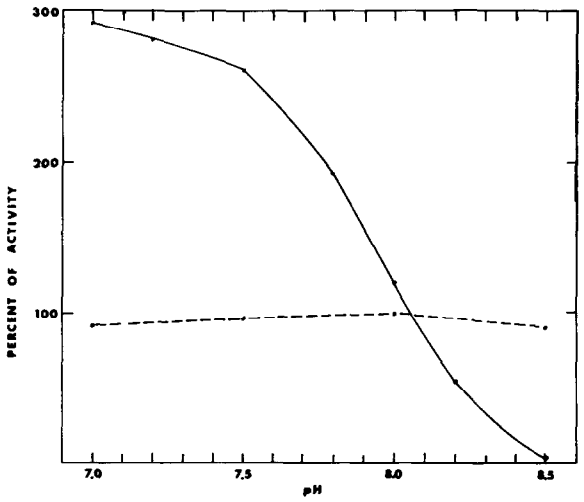

Fig. 1. Effect of pH of the washing buffer on oxygen evolution. Chloroplasts were washed with 0.8 M buffer at each pH indicated; tris: ——; tricine, – – – . The reaction conditions were the same as for table 1.

crease in absorbance at 420 nm and the photoreduction of NADP was followed by measuring the increase in absorbance at 340 nm. Reactions were illuminated at room temperature with heat filtered white light of intensity 5.8×10^5 ergs/cm^2/sec. The amount of ATP formed by the illuminated chloroplasts was determined by the method of Avron [6]. The spinach ferredoxin used in the NADP photoreduction reactions was purified by the method of Tagawa and Arnon [7]. Chlorophyll concentrations were measured by the method of Arnon [8].

3. Results and discussion

Preliminary experiments on the effect of tris washing on oxygen evolution indicated that a low pH during the treatment caused a DCMU sensitive stimulation of the oxygen evolution. Table 1 shows that the rate of oxygen evolution is enhanced some 2–3 fold by washing the chloroplasts with 0.8 M tris at pH 7.2 while washing with the same concentration of tris at pH 8.0 has little effect on the rate. The treatment with tris at low pH seems to sensitize the oxygen evolving system to tris inhibition as the resuspension of the treated chloroplasts in STN at pH 8.3 causes a reversal of the enhancement of oxygen evolution.

Table 1
Effect of tris washing of chloroplasts on oxygen evolution.

Chloroplasts	O_2 evolved (μatoms/hr/mg/ chlorophyll)	Activity (%)
Control	92.2	100
Tris washed		
pH 8.0	119.8	130
pH 7.2	282.2	306
pH 7.2 (resuspend pH 8.3)*	115.2	125
pH 7.2 + DCMU	9.2	10

The reaction mixture contained the following components in μmoles: tris pH 7.8, 50; NaCl, 140; $KFe(CN)_6^{3-}$, 6 and chloroplasts containing 250 μg of chlorophyll in a volume of 6.0 ml. DCMU was added where indicated to a concentration of 1 μM. The reactions were run at 15° under illumination of 5,000 F.C. of white light.

* Chloroplasts treated with tris at pH 7.2 were resuspended in the STN solution at pH 8.3.

Table 2
Effect of tris washing at low pH on photophosphorylation.

Electron acceptor or cofactor	Photoreduction (μmoles/hr/mg/ chlorophyll)	ATP formed (μmoles/hr/ mg/chlor.)	P/2e
Ferricyanide			
control	498.6	195.5	0.78
washed	567.9	19.6	0.06
NADP			
control	86.1	58.9	0.68
washed	127.2	2.2	0.02
Pyocyanine			
control		439.3	
washed		138.9	

Washed chloroplasts were prepared as described in experimental. The reaction mixtures contained the following components in μmoles. For ferricyanide and pyocyanine supported phosphorylation; tris, pH 8.0, 50; $MgCl_2$, 10; $NaKHPO_4$, 10; ADP, 5; $KFe(CN)_6^{3-}$, 3 and chloroplasts containing 100 μg of chlorophyll in a volume of 2.0 ml. For NADP supported phosphorylation; tris, pH 8.0, 50; NaCl, 40; $MgCl_2$, 8; $NaKHPO_4$, 10; ADP, 5 and a saturating amount of ferredoxin and chloroplasts containing 50 μg of chlorophyll in a volume of 3 ml. All reactions contained $4–5 \times 10^5$ cmp ^{32}P.

The reversal is very similar to the inhibition of the oxygen evolution caused by the treatment of chloroplasts with high concentrations of tris at high pH

[3, 4]. The inhibition of oxygen evolution by resuspension in low concentrations of tris is also pH dependent and seems to be specific for tris as the inhibitory compound.

Fig. 1 reveals in detail the effect of the pH of the washing solution on oxygen evolution by spinach chloroplasts. The maximal enhancement of oxygen evolution is obtained at pH 7.0–7.5; at pH values lower than 7.0 the rate is decreased (unpublished experiments of N. Ikehara) as well as above 8.0 as noted by many workers. The pH curve resembles the pH dependence reported by Neumann and Jagendorf [9] for the uncoupling of photophosphorylation by treatment of chloroplasts with the detergent Triton X-100. Fig. 1 also shows that the enhancement of oxygen evolution is specific for tris as the identical treatment with tricine (a zwitterionic buffer) has no effect on oxygen evolution. Experiments on the time course of tris washing at low pH showed that the maximum rate of oxygen evolution was obtained after 20 min of treatment and that the rate was decreased when the time was lengthened to 60 min. These results suggest that the stimulation of oxygen evolution by tris washing may be due to enzymatic or structural changes induced by the tris acting as a membrane active agent.

Other studies have shown that enhanced electron flow (oxygen evolution) accompanies the uncoupling of phosphorylation from electron flow [9, 10]. It was important therefore to assess the effect of tris treatment at low pH on photophosphorylation. Table 2 shows that such treatment results in the uncoupling of both cyclic and non-cyclic photophosphorylation; and that the uncoupling is more complete for non-cyclic than for cyclic photophosphorylation. The reversal of the enhancement of oxygen evolution by 0.05 M tris at high pH as noted in table 1 is not accompanied by a restoration of the coupling of phosphorylation to electron flow.

The differential sensitivities of the cyclic and non-cyclic photophosphorylation to tris treatment and the DCMU sensitivity of the enhanced oxygen evolution are consistent with the evidence that tris treatment at high pH alters electron flow at the water side of Photosystem II and has little effect on Photosystem I [3, 4]. Although the uncoupling effect of tris at high pH has been demonstrated [10] there is no evidence that tris can cause significant uncoupling at low pH. This communication shows that high concentrations of tris at low pH can be included with treatments capable of uncoupling phosphorylation from electron flow. An interesting fact which emerges from this study is that high concentrations of tris can induce totally opposite effects dependent on the pH of the buffer during the treatment of the chloroplasts. It is attractive to consider that both effects are due to the alteration of the same site in Photosystem II.

The uncoupling of photophosphorylation by tris treatment may be due to the alteration of membrane properties or components essential to the energy transfer process such as coupling factor [11] or the selective permeability of the grana membranes [12]. Detailed studies on the cause of tris uncoupling are presently being carried out in this laboratory.

Acknowledgements

The authors acknowledge with thanks the expert technical assistance of Miss Betty Li. We thank R. DiNello, R.Drivdahl, P.Melcarek, J.Novak and A.Di-Pasquale, students of the Biochemical Methods course in Yale College, for the purified Spinach Ferredoxin.

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