PROTON UPTAKE AND PHOSPHORYLATION IN DIGITONIN-TREATED CHLOROPLAST PARTICLES

F.A.McEVOY and W.S.LYNN

Department of Biochemistry, Duke University Medical Center Durham, North Carolina 27706, USA

Received 24 August 1970

1. Introduction

It has recently been shown [1] that chloroplast particles prepared by digitonin treatment were capable of cyclic phosphorylation although they were not capable of forming the reversible light-dependent pH gradient. It was concluded from this data that formation of a pH gradient was a process secondary to the formation of a postulated high-energy intermediate. However, previous work in this laboratory [2, 3] on subchloroplast particles prepared by sonication had shown that the extent of proton uptake from the external medium is determined by the buffering capacity within the chloroplast. When the internal buffers are removed by disruption of the chloroplasts, proton uptake from the external medium is diminished although the particles still form a pH differential of the same magnitude as shown with indicator dyes such as neutral red. This communication shows that subchloroplast particles prepared by digitonin treatment also display an internal pH differential comparable to that in the chloroplasts although proton uptake, as measured with electrodes, is almost entirely absent.

2. Methods

Proton uptake and phosphorylation were measured as before [2]. Neutral red absorbance changes at 570 nm were measured on an Aminco-Chance double-beam spectrophotofluorimeter using 595 nm as the reference wavelength [3].

Spinach leaf chloroplasts were prepared as before [2] and were swollen in 5 mM tris-HCl in the presence of ascorbic acid (10 mM) and bovine serum albumin (1 mg/ml). Subchloroplast particles were prepared by addition of solid digitonin at a chlorophyll concentration of 0.7 mg/ml according to the method of Nelson, Drechsler and Neumann [1].

3. Results and discussion

As shown in table 1, digitonin subchloroplast particles prepared from 0.25% digitonin had a very low rate and extent of proton uptake. The phosphorylation rate in the particles, however, was 40% of the control. The subchloroplast particles prepared from 0.5% digitonin had a low phosphorylation rate and low rate of proton uptake. The neutral red response was decreased proportionately to the rate of phosphorylation. The acidification of the lipophilic dye, neutral red, has in all cases been found to parallel the rate of ATP formation in chloroplasts [2, 3].

Imidazole, by serving as an internal buffer, was previously shown to increase the extent of proton uptake of intact chloroplasts without affecting the rate of ATP formation [3]. However subchloroplast particles prepared by sonication did not display a greater proton uptake in the presence of imidazole and these particles could not accumulate imidazole [3]. The low level of proton uptake in digitonin subchloroplast particles was also not stimulated by imidazole (table 1) suggesting that these particles had also lost their ability to accumulate imidazole for use as an internal buffer. Ammonia, unlike imidazole, diminishes the rate of proton uptake in chloroplasts [3] and in sonicated subchloroplast particles ammonia abolished the proton

Table 1
Phosphorylation, proton uptake and internal acidification in subchloroplast particles (SCP) compared with control chloroplasts.

	Proton uptake		Phosphorylation	Neutral red
	Rate (μmoles/mg/hr)	Extent (μmoles/mg)	(μmoles/mg/hr)	(ΔpH)
Chloroplasts	432	1.7	480	0.80
0.5% Digitonin SCP	⩽ 15	⩽0.12	60	⩽0.10
0.25% Digitonin SCP	⩽ 20	⩽0.15	180	0.38
+ 10 mM NH_4Cl	⩽ 15	⩽0.15	170	0.38
+ nigericin + KCl*	⩽ 15	⩽0.15	170	0.38
+ 0.2 mM imidazole	⩽ 15	⩽0.15	170	0.36

Proton uptake and phosphorylation are measured as units per mg chlorophyll. The internal acidification is expressed as a change in pH, calculated from the absorbance changes of neutral red at 570 nm [2].

* Nigericin and KCl concentrations were 2×10^{-7} M and 5×10^{-2} M, respectively.

gradient but did not diminish phosphorylation [4]. Neither ammonia for nigericin + KCl affected the level of phosphorylation or the change in neutral red absorbance in digitonin subchloroplast particles (table 1). This results would be expected if these uncoupling agents were to effect only the internal buffering capacity of the chloroplast. In intact chloroplasts the active increase in internal concentration of the cations NH_4^+ and K^+ is sufficient to exchange with membrane-bound protons. The resultant loss of membrane-bound proton then causes the decrease in phosphorylation.

The experiments shown here demonstrate that a pH differential, as shown by a neutral red absorbancy change, exists in phosphorylating subchloroplast particles even when the light-dependent proton uptake is not measurable. The deduction of Nelson et al. [1] that proton movements are processes secondary to the formation of a hypothetical high-energy intermediate which subsequently forms ATP would not seem to be strictly valid since it still appears that membrane-bound H^+ is required to drive the reaction,

$$H^+ + ADP^{3-} + Pi^{2-} \rightleftharpoons ATP^{4-} + H_2O.$$

Rather it appears that the increased concentration of H^+ within the membrane is, at least one of the proposed high energy intermediates.

Acknowledgement

This study has been supported by NIH Grant GM 14022–03.

References

[1] N.Nelson, Z.Drechsler and J.Newmann, J. Biol. Chem. 245 (1970) 143.
[2] W.S.Lynn, J. Biol. Chem. 243 (1968) 1060.
[3] W.S.Lynn, Biochemistry, 7 (1968) 3811.
[4] R.E.McCarty, Biochem. Biophys. Res. Commun. 32 (1968) 37.