LIGHT-INDUCED POTASSIUM EFFLUX FROM SPINACH CHLOROPLASTS*

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Received September 28, 1964

Several laboratories have shown that chloroplasts exhibit light-dependent increases in light-scattering (Packer, 1963; Itoh, et al., 1963; Jagendorf and Hind, 1963; Dilley and Vernon, 1964a). Itoh, et al., (1963) demonstrated slow scattering changes related to chloroplast shrinkage. Using a Coulter counter, Dilley and Vernon (1964c) measured a rapid shrinkage having the same kinetics as the light-scattering changes. Such shrinkage could result from: (a) contraction of a contractile protein or (b) loss of ions and water from within the chloroplast structure. We have observed a light-dependent K^+ efflux from chloroplasts which appears related to the observed shrinkage phenomena in its kinetics and response to selected inhibitors.

METHODS

Chloroplasts were prepared (Dilley and Vernon, 1964a) in sucrose - 0.1M Tris-acetate pH 7.5. Potassium ion was measured

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†Post-doctoral trainee under sponsorship of the Public Health Service.

potentiometrically with a Beckman "cation" electrode using a Leeds and Northrup Model 7401 pH meter with a Ag:AgCl reference electrode. The meter output was recorded on a Brown recorder, using a variable resistance (100 to 300 Ω) across the meter output to give adequate sensitivity. Potassium was also qualitatively and quantitatively measured with a Perkin-Elmer 303 Atomic Absorption Spectrophotometer. These measurements were graciously performed by Dr. H. K. Anders, Chemistry Department, Bowling Green State University, Bowling Green, Ohio. For these measurements the potassium content of the suspending medium was determined for illuminated and non-illuminated systems after removing the chloroplasts by filtration through a Millipore filter having an average diameter of 0.65 μ.

Illumination was provided by a Unitron Research Illuminator Model LKR. A Corning 2304 red filter and a Corning infrared absorbing (No. CS1-69) heat filter were placed in the light beam. The light intensity incident to the suspensions was 1.5×10^5 erg cm^{-2} sec^{-1}.

RESULTS

Figure 1 shows a typical signal obtained from the "cation" electrode upon illuminating a chloroplast suspension in the presence of an electron transfer agent ($TMQH_2$[††] in this case). The reaction is apparent first order with a $t_{1/2}$ of approximately 5 sec, depending on the chloroplast preparation, degree of aging, etc. A true first

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[††] Abbreviations used: $TMQH_2$, reduced trimethyl-1, 4-benzoquinone; Cl-CCP, meta-chlorocarbonyl cyanide phenylhydrazone; DCMU, 3-(3, 4-dichlorophenyl)-1, 1-dimethyl urea.

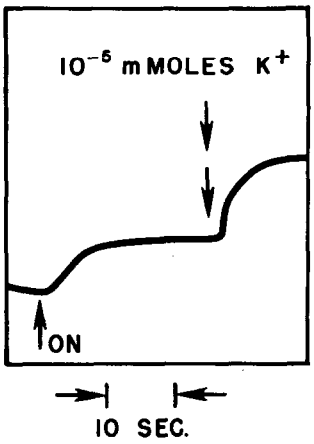

Fig. 1. Potassium efflux from chloroplasts. The reaction mixture contained 0.1 M Tris-acetate pH 7.0, 0.132 μm/ml $TMQH_2$, and chloroplasts equivalent to 314 μg chlorophyll in a total volume of 15 ml. The light was turned on at the arrow indicated by "on".

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order reaction is indicated by two facts: (1) the $t_{1/2}$ values for the reaction are the same for chloroplast concentrations ranging from 11 to 22 μg in the reaction system, and (2) a log plot of time versus $K^+_{t_\infty - t}$ ($[K^+]$ at the steady state minus $[K^+]$ at time t) gives a linear plot.

The signal obtained from the "cation" electrode was identified with K^+ through the use of atomic absorption spectrophotometry. Table I shows that the suspending medium from light-treated chloroplasts contained significantly more K^+ than a non-illuminated control. The K^+ efflux calculated from these data is 0.26 mole K^+ per mole chlorophyll. Similar values for K^+ efflux were obtained with the Beckman K^+ electrode (see Table II).

The K^+ efflux reaction is inhibited much the same as the chloroplast shrinkage reaction (Dilley and Vernon, 1964a). In Table II it is seen that DCMU inhibited the K^+ efflux almost completely when an electron acceptor (TMQ) is added. However, if the reduced form

Table I

K^+ Efflux from illuminated spinach chloroplasts determined by measuring K^+ concentrations in chloroplast suspension media.

Conditions	Absorbance* at 770 mμ	K^+ efflux in light moles/mole Chl
Dark	153 ± 4	
Light	165 ± 4	0.26

Reaction mixtures contained chloroplasts equivalent to 7.3 μg chlorophyll, 0.1 M Tris buffer pH 7.0 and 0.132 mM $TMQH_2$ in 3 ml total volume. Ten samples each were incubated in darkness and in the light (one minute at 1.5×10^5 erg cm^{-2} sec^{-1} red light). Atomic absorption spectrophotometry was used for the K^+ assay, making use of 770 mμ emission line of potassium. Concentrations of K^+ were obtained from a standard curve prepared by adding known amounts of KCl to samples under similar conditions.

*$\times 10^{-3}$

of the acceptor is added ($TMQH_2$), which is capable of catalyzing cyclic electron flow and phosphorylation, the K^+ efflux was inhibited only 33% by DCMU at 3.2×10^{-5} M. Quinacrine, an uncoupler of photophosphorylation, reduced the extent of K^+ efflux by about 40%, but it did not measurably affect the $t_{1/2}$ value. Cl-CCP, also an uncoupler, inhibited both the initial rate and the extent of the K^+ efflux at a very low concentration (6×10^{-7} M).

Ouabain, an inhibitor of active cation transport in mammalian membrane systems (Schatzmann, 1953), did not affect the K^+ efflux at 5×10^{-5} M.

If one assumes that chlorophyll is about 0.2 M in chloroplasts (Rabinowitch, 1945) the data in Table II may be used to calculate a minimum K^+ concentration in these chloroplasts (assuming all K^+ is effused)

Table II

Effect of inhibitors and uncouplers upon K^+ efflux from illuminated spinach chloroplasts.

Conditions	μmoles K^+ effused / μmole chl.	Per Cent Inhibition
1 Control (TMQ)	0.21	-
plus 1.6×10^{-5} M DCMU(TMQ)	0.025	90
plus 3.2×10^{-5} M DCMU($TMQH_2$)	0.14	33
2 Control	0.20	-
plus 6×10^{-7} Cl-CCP	0	100
3 Control	0.26	-
plus 4×10^{-5} M Quinacrine	0.14	44
plus 8×10^{-5} M Quinacrine	0.14	44
plus 5×10^{-5} M Ouabain	0.26	0

Exp. one: 0.1 M Tris-acetate pH 7.0, 314 μg chlorophyll and 0.11 mM TMQ (or 0.13 mM $TMQH_2$ as indicated). Exp. two: 0.1 M Tris-acetate pH 7.0, 0.132 mM $TMQH_2$ and 180 μg chlorophyll. Exp. three: Same as exp. two except 340 μg chlorophyll were used. The final volume in each case was 15 ml. The illumination period was one minute, and K^+ concentration was determined with a Beckman "cation" electrode.

of 0.04 M K^+. This is consistent with published values for K^+ concentration in chloroplasts of higher plants (Saltman, et al., 1962).

DISCUSSION

The data presented above show that chloroplast fragments effuse K^+ ions through a mechanism which depends on light-induced electron flow. The reaction is first order and has a $t_{1/2}$ value similar to that of the shrinkage reaction. It appears that both the K^+ efflux and chloroplast shrinkage are coupled to photosynthetic electron transfer reactions, since DCMU inhibits both reactions and a

differential effect is observed for systems containing TMQ or $TMQH_2$. The reduced form of the quinone overcomes DCMU inhibition of light-induced electron transfer, K^+ efflux, and chloroplast shrinkage (Dilley and Vernon, 1964a).

A further analogy between the shrinkage reaction and the K^+ efflux is found in the effect of the uncouplers Cl-CCP and quinacrine. The former completely inhibits the rate and extent of both K^+ efflux and chloroplast shrinkage. Quinacrine has no appreciable effect on the initial rate of K^+ efflux but it does decrease the extent of K^+ efflux by about 40% at 8×10^{-5} M. Quinacrine does not alter the initial shrinkage rate, but allows the initial rate to continue until shrinkage is complete (Dilley and Vernon, 1964b).

The above evidence indicates that the K^+ efflux is related to the chloroplast shrinkage reaction which in turn appears to be related to the energy conservation mechanism of chloroplasts. Experiments are now in progress to further clarify the relationship of the K^+ efflux to other energy-related chloroplast phenomena.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the encouragement and counsel of Dr. L. P. Vernon and the competent technical assistance rendered by Mrs. Inger Stoughton.

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