Bioenergetics (1970) **1**, 367–377.

K^+-Independent Effects of Valinomycin in Photosynthetic Systems*

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Abstract

With chromatophores of *Rhodospirillum rubrum*, valinomycin inhibited electron transport in the presence or absence of K^+. NH_4Cl had no effect on photophosphorylation but uncoupled with valinomycin present. ATPase activity was stimulated by NH_4Cl plus valinomycin but not by either alone. K^+ partially reversed the inhibition of phosphorylation and the stimulation of ATPase by valinomycin plus NH_4Cl.

With chloroplasts, valinomycin inhibited coupled but not basal electron transport. The inhibition was only partially reversed by uncouplers. Valinomycin stimulated the light-activated Mg^{2+}-dependent ATPase similar to several uncouplers such as quinacrine, methylamine, and S-13. In addition, valinomycin inhibited delayed light emission and stimulated the H^+/e^- ratio. These contrasting activities in chloroplasts are not easily explained.

Introduction

The antibiotic valinomycin is being used widely in studies of ion transport and energy-linked reactions in mitochondria, chromatophores, chloroplasts, and artificial membranes, and we have used this antibiotic, alone and in conjunction with other ion-transport-inducing antibiotics, in chromatophores from the photosynthetic bacterium *Rhodospirillum rubrum*. We observed that valinomycin inhibited photophosphorylation at concentrations greater than those normally used to stimulate ion transport[1] and that this inhibition was apparently independent of K^+ or other alkali metal cations.

Due to the wide use of this antibiotic in studying characteristics of membrane systems, we have studied the K^+-independent effects of valinomycin in chromatophores and chloroplasts. In a preliminary report of this work[2] we concluded that in the absence of K^+, valinomycin was an energy-transfer inhibitor in chloroplasts. Further studies reported in this paper have not totally supported this conclusion.

Methods and Materials

R. rubrum, S1, was grown and chromatophores prepared as previously described.[3] Photophosphorylation was determined as previously described.[4] The reaction mixture for chromatophore ATPase contained TrisCl, pH 8, 50 mM; 3·3 mM $MgCl_2$; 0·67 mM

* Contribution number 389 of the Charles F. Kettering Research Laboratory.

ATP; and bchl* in 3 ml. The reaction products were fixed with 0·3 ml of 50% TCA and analyzed for P_i by the Fiske-Subba Row method.

Chloroplasts were prepared from market spinach or lettuce, var. Romaine. The depetiolated leaves were ground with sand in an ice-cold mortar in buffer containing 50 mM HEPES, pH 7·8, 0·4 M sucrose, and 0·01 M NaCl. The homogenate was filtered through cheesecloth and centrifuged for 2 min at 200 × *g*. The chloroplasts were sedimented from the supernatant by centrifuging at 1000 × *g* for 8 min and resuspended in the same buffer. Chloroplast ATPase activity was determined as follows: 1·5 ml of reaction mixture containing 50 mM HEPES, pH 7·8, 6·7 mM $MgCl_2$, 50 mM cysteine, 50 μM PMS and not more than 75 μg chl was illuminated for 5 min with 2×10^6 ergs cm^{-2} sec^{-1} of red light at 20° C to activate the ATPase. This mixture was added to 1·5 ml containing 50 mM HEPES, 6·7 mM $MgCl_2$, 1·33 mM ATP, and the compounds to be tested. The reaction was incubated for 5 min in the dark, fixed with TCA to 5%, and analyzed for P_i as above. The rate of the ATPase was proportional to the chl concentration and was linear with time for at least 5 min under these conditions, plus or minus uncouplers.

Valinomycin was a gift from Dr. J. M. McGuire of the Lilly Research Laboratories, and S-13 was a gift from Dr. P. C. Hamm of the Monsanto Company.

Results

Effect of Valinomycin on Photophosphorylation in Chromatophores

The effect of valinomycin on photophosphorylation in *R. rubrum* chromatophores in the presence of sucrose, K^+, Na^+, or NH_4^+ is illustrated in Fig. 1. The inhibition is essentially linear with the log of the antibiotic concentration up to at least 15 μM and is not dependent on the presence of K^+. In fact, the inhibition observed with 50 mM K^+ or Na^+ present is somewhat less than with sucrose. The concentration of valinomycin required for 50% inhibition of ATP formation is somewhat greater than that required to stimulate the light-induced pH change[5,6] or to inhibit phosphorylation in the presence of nigericin and K^+.[1,6,7] Therefore, this inhibition may or may not be related to effects of this antibiotic on K^+ or H^+ transport.

In further studying this inhibition we observed that low concentrations of NH_4Cl enhanced the valinomycin inhibition. NH_4Cl alone has no effect on photophosphorylation in *R. rubrum* chromatophores, in contrast to the uncoupling effect in chloroplasts. In previous papers, we found that nigericin plus K^+ inhibited the light-induced pH change in *R. rubrum* but had no effect on photophosphorylation.[8] The combination of valinomycin, nigericin (or monensin A or dianemycin) and K^+ was strongly inhibitory.[1,6,7] We attributed this inhibition of ATP formation to an energy-linked cyclic ion transport which utilized a high-energy intermediate of phosphorylation. Like nigericin plus K^+, NH_4^+ inhibited the light-induced pH change in *R. rubrum*† as it does in chloroplasts.[9] Therefore, we suspected that NH_4Cl plus valinomycin was emulating the K^+ plus valinomycin plus nigericin effect described above. To differentiate between inhibition of phosphorylation due to uncoupling and inhibition due to blocking of electron trans-

* Abbreviations used are: bchl, bacteriochlorophyll; chl, chlorophyll; S-13, 5-chloro-3-t-butyl-2′-nitrosalicylanilide; TMPD, N,N,N′,N′-tetramethyl-p-phenylenediamine; PMS, N-methyl phenazinium methyl sulfate; DCCD, N,N′-dicyclohexylcarbodiimide; TTFB, 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole; CMU, *p*-chlorophenyl-1,1-dimethylurea.

† Unpublished observation.

port, we measured the effect of valinomycin, plus and minus NH_4Cl, in the presence of a dye, TMPD, which allows electrons to by-pass the site of inhibition of known inhibitors of electron transport such as antimycin *a* and 2-heptyl-4-hydroxyquinoline N-oxide.[10] The results, presented in Table I, illustrate that the inhibition of phosphorylation produced by valinomycin alone was reversed by TMPD and, as shown by Baltscheffsky and Arwidsson,[11] by PMS. Thus, the valinomycin effect can be attributed to an inhibition of electron transport at a site which is by-passed by TMPD.

The extra inhibition induced by NH_4Cl, however, was not affected by TMPD, and thus can be attributed to uncoupling. This is substantiated by the studies presented in the next section.

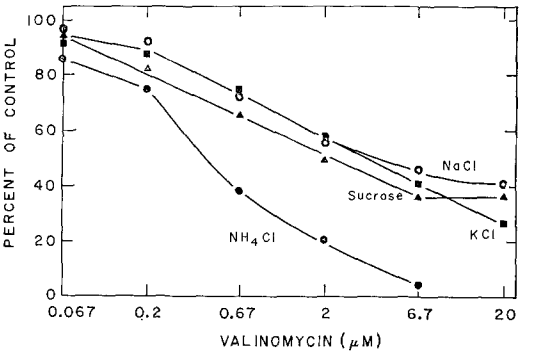

Figure 1. The effect of valinomycin on photophosphorylation in *R. rubrum* chromatophores. The reaction mixture contained 50 mM TrisCl, pH 8·0, 1 mM $MgCl_2$, 1·67 mM sodium succinate, 3·3 mM sodium $^{32}P_i$, 0·83 mM ADP, and 50 mM sucrose, KCl or NaCl. NH_4Cl was 2 mM. The reaction was illuminated for 3 min with a light intensity of $2{\cdot}6 \times 10^5$ ergs cm^{-2} sec^{-1}. The reaction products were fixed with TCA to 5% and analyzed for organic-^{32}P as previously described.[38]

Baltscheffsky and Arwidsson[11] reported that low concentrations of valinomycin inhibited photophosphorylation by 50% maximally in *R. rubrum*, and they interpreted this observation to indicate that there were two sites of phosphorylation, only one of which was inhibited by valinomycin. Sato *et al.*[12] also found that valinomycin inhibited phosphorylation by 50%, but that almost 100 times more valinomycin was required than reported by Baltscheffsky and Arwidsson. This discrepancy was not discussed. Our results do not confirm these previous observations. Although, as illustrated in Fig. 1, the inhibitory effect seems to level off somewhat at the highest valinomycin concentration that we used, the amount of the inhibition was always 60% or greater and in some experiments no break in the curves was found and the inhibition approached 80% at 20 μM.

It has recently been demonstrated that ubiquinone is rapidly photochemically reduced by the reaction center (P865) at the temperature of liquid nitrogen and with

TABLE I. Restoration of valinomycin-inhibited phosphorylation by TMPD

Additions	Percent inhibition	
	Minus TMPD	Plus TMPD
NH_4Cl, 2 mM	0	—
Valinomycin, 0·67 μM	26	0
Valinomycin, 6·7 μM	46	0
NH_4Cl plus valinomycin, 0·67 μM	61	30
NH_4Cl plus valinomycin, 6·7 μM	92	51
KCl, 50 mM plus valinomycin, 6·7 μM	41	11

The reaction mixture was the same as described in Fig. 1, except that it also contained 20 mM glucose and ~ 1 unit hexokinase dialyzed free of $(NH_4)_2SO_4$. TMPD was 1·5 mM and bchl was 12·3 μg/ml. The control rate of phosphorylation was 165 μmoles/mg/hr and was stimulated to 309 with TMPD.

high quantum efficiency.[13,14] These conditions apparently exclude a phosphorylation site preceding ubiquinone, and the potential span between ubiquinone (~ 0·05 V) and cytochrome c_2 (+ 0·32 V) apparently exclude more than one site of ATP formation on the photosynthetic electron transport chain.

Stimulation of ATPase by NH_4Cl plus Valinomycin

Figure 2(A) illustrates that valinomycin (6·7 μM) plus NH_4Cl (1 mM) stimulated ATP hydrolysis by three-fold, whereas valinomycin alone had little effect. Nonactin

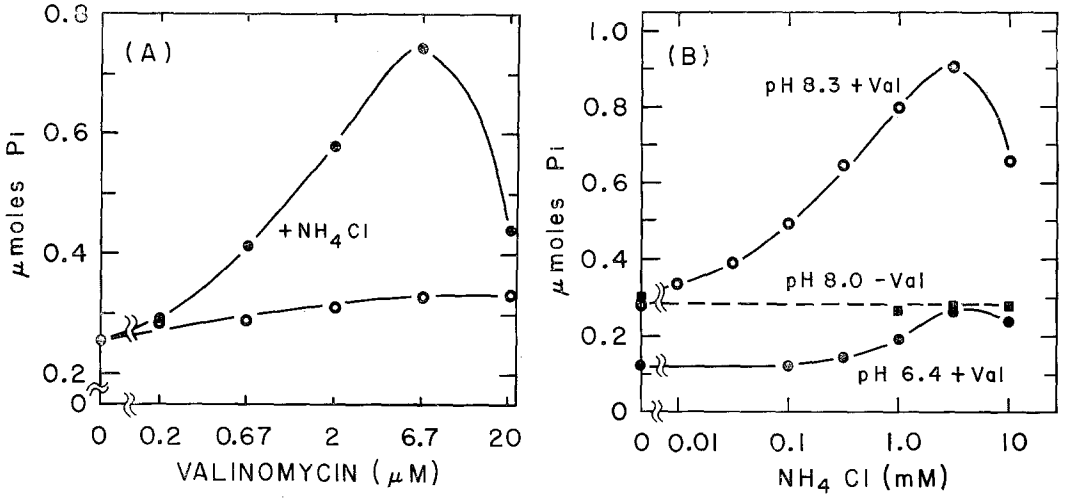

Figure 2. The effect of valinomycin and NH_4Cl on ATPase in chromatophores. The reaction mixture (A) contained 50 mM TrisCl, pH 8·0, and 3 mM NH_4Cl where indicated. (B) The pH 8·0 and 8·3 reactions were run in TrisCl and the pH 6·4 in HEPES. Valinomycin was 2 μM and the rate at pH 8 was 52 μmoles/mg bchl/hr.

had essentially the same effect as valinomycin. Figure 2(B) illustrates that NH_4Cl alone had no effect even at 100 mM (not illustrated), whereas with valinomycin present, the optimal stimulation was observed at 3 mM at both pH 6·4 and 8·3. This indicates that the rate-limiting step is not the diffusion of NH_3 into the chromatophore. A strong inhibition of ATPase activity was observed at concentrations of valinomycin above 6·7 μM.

The specificity of this uncoupling effect of valinomycin plus NH_4^+ is illustrated in Fig. 3, in which it is shown that cations which are known to be complexed by valinomycin (K^+, Rb^+) actually reverse the NH_4Cl-induced stimulation of ATPase activity. Na^+ had much less effect. This

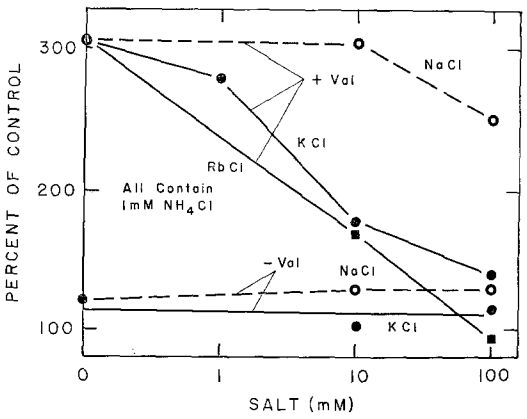

Figure 3. Effect of cations on the valinomycin plus NH_4Cl stimulation of ATPase in chromatophores. The reaction was run in 50 mM TrisCl at pH 8·0 and contained 80 μg bchl and 2 μM valinomycin where indicated. The control rate was 50 μmoles ATP hydrolyzed per milligram bchl per hour.

TABLE II. Partial reversal of valinomycin-inhibited photophosphorylation by KCl

	Percent inhibition of valinomycin (μM)		
	0·67	2	6·7
Sucrose, 50 mM	29	40	62
KCl, 50 mM	17	28	48
NH_4Cl, 2 mM	54	66	99
NH_4Cl plus KCl	30	48	96

The control rates of phosphorylation (μmoles/mg bchl/hr) were: sucrose, 316; KCl, 297; NH_4Cl, 299; NH_4Cl plus KCl, 273.

reversal of the valinomycin plus NH_4Cl effect by K^+ was also observed on photophosphorylation, as is illustrated in Table II. The most apparent explanation of the reversal of the NH_4^+ effect by K^+ is that when the valinomycin molecule is complexed with K^+, it is unavailable to complex and transport NH_4^+.

Effect of Valinomycin in Chloroplasts

The inhibitory effect of valinomycin plus NH_4Cl in chromatophores can be attributed to an enhancement of the permeability of the membrane for NH_4^+ by valinomycin, thus inducing a rapid turnover of H^+ and a dissipation of the H^+ gradient via a $NH_3 \rightleftharpoons NH_4^+$ cycle. In green plant chloroplasts, NH_4Cl alone is an uncoupling agent, and the most plausible explanation of the difference in the two systems is that the lamellar membrane of the chloroplast is more permeable to NH_4^+ than is the chromatophore membrane. If NH_4^+ permeability is the limiting factor in uncoupling, then valinomycin should enhance the uncoupling due to NH_4Cl. McCarty[15] has recently demonstrated that this is true; the inhibition of phosphorylation by valinomycin plus NH_4^+ was considerably more than the additive inhibition due to each compound separately, using both chloroplasts and subchloroplast particles.

As was the case with chromatophores, valinomycin alone inhibited photophosphorylation to a significant degree. Although not discussed, this inhibition is also apparent in the results of Plengvidhya and Burris,[16] Karlish and Avron,[17] and McCarty.[15] To determine if the inhibition of phosphorylation was due to an uncoupling effect or an inhibition of electron transport, as was the case in chromatophores, we studied the effect of the antibiotic on ferricyanide reduction. The results, illustrated in Fig. 4, show that valinomycin had little effect on the rate of electron transport in the absence of ADP and P_i (basal rate) but inhibited ferricyanide reduction in the presence of the ADP plus P_i. In separate experiments, we confirmed that ATP formation was inhibited concomitantly with electron transport. These results are typical of those observed with energy-transfer inhibitors such as phlorizin,[18] DIO-9,[19] and Synthalin.[20]

The uncoupler, S-13,[21] reversed the inhibition of electron transport by low concentrations of valinomycin but was less effective at higher concentrations. Essentially, the same results were previously reported with methylamine.[2] Quinacrine was much less effective than either S-13 or methylamine.

The effect of valinomycin in inhibiting ATP formation is enhanced slightly by the presence of K^+, as contrasted with Li^+ (Table III). However, we do not regard the difference as significant, since less difference was observed in some other experiments.

These results would seem to indicate that valinomycin is an energy-transfer inhibitor in chloroplasts, although the inhibitory effects of valinomycin on coupled electron transport are not completely reversed by uncouplers. McCarty and Racker[22] could only partially reverse the inhibitory effects of the energy-transport inhibitor, DCCD, with ammonium chloride, and thus valinomycin resembles this inhibitor in this respect. The results presented in the following sections, however, do not support the concept that valinomycin is an energy-transfer inhibitor.

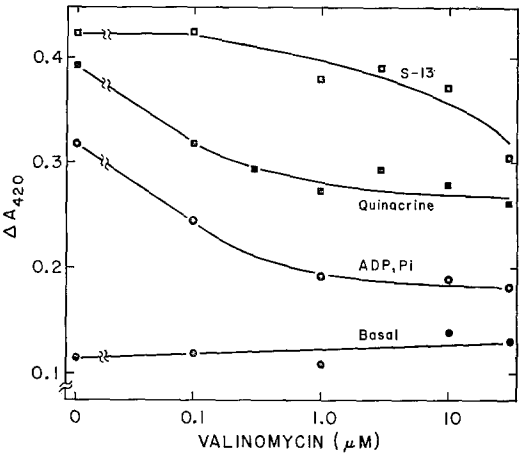

Figure 4. The effect of valinomycin on ferricyanide reduction in chloroplasts. The reaction mixture contained 50 mM HEPES, pH 7·8, 3·3 mM $MgCl_2$, 0·67 mM ferricyanide, and 36 μg chl in 3 ml. Where indicated, ADP was 0·83 mM; P_i, 13·3 mM; S-13, 0·3 μM; quinacrine, 20 μM. The chloroplasts were prepared from lettuce and were illuminated for 4 min. Qualitatively, the same results were obtained with spinach chloroplasts.

Effect of Valinomycin on Delayed Light Emission

In further studying the properties of valinomycin, we studied its effect on delayed light emission. Mayne[23] has shown that the energy-transfer inhibitors phlorizin and DIO-9 reverse the inhibition of delayed light emission caused by ADP, P_i,

TABLE III. Effect of valinomycin on photophosphorylation in chloroplasts in Li^+ or K^+

Valinomycin μM	ATP formation: Ferricyanide Li^+	Ferricyanide K^+	PMS Li	PMS K^+
	Percent inhibition			
0·1	14	27	0	13
1·0	50	57	14	38
10	80	90	40	54
30	91	96	57	69

The reaction mixture contained 0·05 M HEPES, pH 7·8, 3·3 mM $MgCl_2$, 0·8 mM ADP, 6·7 mM $^{32}P_i$ as the K^+ or Li^+ salt, 0·6 mM ferricyanide or 0·02 mM PMS, and 26 μg chlorophyll. Illumination was for 4 min with 3×10^5 ergs cm^{-2} sec^{-1} red light.

TABLE IV. Effect of valinomycin on delayed light emission in chloroplasts

Additions	Delayed light (relative units)	
	Minus ADP, P_i	Plus ADP, P_i
None	3·8	3·0
Phlorizin, 1 mM	3·7	3·7
DCCD, 0·5 μM		3·1
1·5 μM	3·9	3·5
5 μM		2·9
Valinomycin, 1μM	3·4	2·6
3μM	2·8	2·4
33μM		1·9

Delayed light emission was measured as described by Mayne,[23] except the instrument was altered to measure 0·5 msec delayed light and the emission was detected on an oscilloscope. The reaction mixture contained HEPES, 0·05 M, pH 7·8, 3 mM $MgCl_2$, 0·5 mM ferricyanide, and 16 μg chlorophyll/ml; 0·75 mM ADP and 4 mM lithium phosphate were added where indicated.

and Mg^{2+}. The results presented in Table IV, which were done in collaboration with Dr. Berger C. Mayne, illustrate that phlorizin completely reversed the inhibition due to ADP and P_i; DCCD partially reversed the inhibition, but valinomycin did not restore the delayed light emission, and in fact caused a further inhibition. Thus, these results are not consistent with valinomycin being an energy-transfer inhibitor.

The Effect of Valinomycin on Chloroplast ATPase

Many conflicting reports appear in the literature regarding the effect of uncouplers on the light- and sulfhydryl-activated Mg^{2+}-dependent ATPase of chloroplasts. In surveying the literature we found that in many studies the uncouplers were present during the light activation phase of the reaction.[24, 25, 26, 27] Under these conditions we have found that almost any type of inhibitor of phosphorylation partially inhibits the activation of the ATPase, and the results cannot be related to their effect on the ATPase in the dark following light activation. Several papers report the inhibition of the ATPase by uncouplers when added in the dark following light activation,[28, 29] no effect,[26] or stimulation.[30, 31, 32]

We have found that, depending on the particular preparation of chloroplasts (either spinach or lettuce), we find varying effects of an uncoupler ranging from marked stimulation (four-fold) to marked inhibition (80%) and that the effects are apparently not related to the control rate of the ATPase or the age or source of the spinach or lettuce that we used.* Since it is apparent that uncouplers do stimulate the ATPase under the proper conditions, only when stimulation by these compounds is observed

* Less stimulation was observed when Tris buffer was used than with HEPES. Indeed, in some experiments, uncouplers such as quinacrine and methylamine stimulated when assayed in HEPES but inhibited when Tris was used. Therefore, Tris should be avoided in ATPase experiments.

TABLE V. Effect of various compounds on the light-activated ATPase of chloroplasts

	Additions	μmoles P_i	Percent of control
Exp. 1.	None	0·80	
	Quinacrine, 30 μM	1·71	214
	Methylamine, 5 mM	1·90	238
	S-13, 0·1 μM	2·66	332
	Nigericin plus K^+, 0·1 μg, 50 mM	1·79	224
	Valinomycin, 10 μM	1·70	213
Exp. 2.	None	0·50	
	Methylamine, 5 mM	1·01	203
	TTFB, 3 μM	1·07	215
	Synthalin, 0·2 mM	0·31	62
	Phlorizin, 1 mM	0·17	34
	DCCD, 3 μM	0·24	48
	CMU, 10 μM	0·54	109

The rate of the control reaction in experiments 1 and 2 was 135 and 92 μmoles ATP hydrolyzed per milligram chl per hour.

can the effect of unknown compounds be assessed. Table V illustrates the effects of several uncouplers and inhibitors in experiments in which we obtained marked stimulation of the ATPase by the uncouplers quinacrine and methylamine. S-13 is the most potent uncoupler of the ATPase that we found, which is in agreement with its potent uncoupling activity in mitochondria[21] and chromatophores.* The most surprising results were the discovery that valinomycin and TTFB stimulated the ATPase, and therefore appear to be uncouplers. The results in the previous section on the inhibition of coupled electron transport by valinomycin, and similar results with TTFB,[33] indicated that the inhibition produced by these compounds was similar to that of the energy-transport inhibitors such as phlorizin, DCCD, DIO-9, and Synthalin. Thus, these compounds (valinomycin and TTFB) constitute a separate group of inhibitors characterized by inhibition of coupled electron transport, limited reversal of the inhibited electron transport by uncouplers, and stimulation of ATPase activity.

Typical electron-transport inhibitors such as 2-heptyl-4-hydroxyquinoline N-oxide (HOQNO), as expected, had no effect on ATPase activity; however, another well-known inhibitor of electron transport, m-butyl-3,5-diiodo-4-hydroxybenzoate, inhibited ATPase activity at the same concentrations as those which inhibited electron transport. Thus, its mechanism also is hard to explain.

Effect of Valinomycin on Proton Uptake

Dilley[34] has recently reported that energy-transfer inhibitors such as DIO-9 and Synthalin inhibit electron transport in chloroplasts but have little effect on the rate of proton uptake. This leads to a large increase in the H^+/e^- ratio. The effect of polylysine was even more dramatic, since this compound stimulated the proton uptake markedly.

* Unpublished experiments.

In his experiments, valinomycin inhibited both electron transport and proton uptake, but the inhibition of electron transport was greater than the inhibition of proton uptake, thus leading to an increase in the H^+/e^- ratio similar to that found with Synthalin. These results would be consistent with an inhibition of energy transfer.

Discussion

In a recent publication, Gomez-Puyou *et al.*[35] found that valinomycin-inhibited 2,4-dinitrophenol stimulated ATPase activity and respiration in mitochondria and that these effects were not dependent on K^+ and apparently not related to ion transport. Three additional effects of valinomycin have been described in this paper: (1) an inhibition of photophosphorylation in *R. rubrum* chromatophores which, apparently, is due to an inhibition of electron transport; (2) an inhibition of coupled electron transport in chloroplasts which resembles an inhibition of energy transfer; and (3) a stimulation of ATPase activity in chloroplasts. These effects are apparently independent of K^+.

NH_4^+ and amines are well-known uncoupling agents in chloroplasts but have no effect on phosphorylation in chromatophores. The present observation, that NH_4Cl becomes an uncoupler in chromatophores with valinomycin present, allows us to postulate a common mechanism of NH_4Cl uncoupling in chloroplasts and chromatophores. NH_4Cl is a well-known uncoupler of photophosphorylation in chloroplasts, and Crofts[36] has postulated the following mechanism: Chloroplast membranes are freely permeable to NH_3 and this compound equilibrates across the membrane; upon illumination, chloroplasts accumulate H^+ which then equilibrates with the internal NH_3 to form NH_4^+. Since the NH_4^+ concentration becomes higher inside the chloroplast than out, the NH_4^+ diffuses outward, and this leads to a cyclic uptake and dissipation of H^+. The potentiation of the NH_4Cl effect by valinomycin[15] indicates that the diffusion of NH_4^+ across the chloroplast membranes is the rate-limiting process. Whether the inhibition of phosphorylation is due to a loss of the pH gradient or whether it is due to competition between energy-linked H^+-uptake and ATP formation for a common high-energy intermediate is a matter for debate at the present time.

In chromatophores, NH_4^+ has no effect on photophosphorylation and we ascribe this to a relative impermeability of the chromatophore membrane to NH_4^+. In contrast, the membrane is freely permeable to NH_3, as is illustrated by the inhibition of the formation of a pH gradient by NH_4Cl. In the presence of valinomycin, the enhanced permeability of the membrane to NH_4^+ leads to increased cyclic transport of H^+ via the $NH_3 \rightleftharpoons NH_4^+$ cycle and, therefore, an inhibition of phosphorylation. A similar uncoupling action of valinomycin and NH_4Cl in submitochondrial[37] and subchloroplast particles[15] has been described.

The K^+-independent effects of valinomycin in chloroplasts are more complex and defy explanation, assuming only one mechanism of action. As shown in Fig. 4, valinomycin inhibited coupled electron transport but had essentially no effect on the basal electron flow. This is a property associated with energy-transfer inhibitors, but has also been observed with antimycin *a*[18] and TTFB.[33] Whereas the inhibition of coupled electron transport by the energy-transfer inhibitors is reversed by uncouplers, the inhibition by antimycin *a* and TTFB is not reversed by these compounds. The inhibition of coupled electron transport by valinomycin was only slightly reversed by uncouplers,

but this is also characteristic of the energy-transfer inhibitor, DCCD.[22] Thus, it is impossible to characterize the activity of valinomycin on the basis of its effect on electron transport.

The effect of valinomycin on the light-activated ATPase increases the confusion. We have shown that valinomycin stimulated ATPase activity similar to compounds which are uncouplers and, therefore, is unlike electron-transport inhibitors such as CMU, HOQNO, and antimycin *a*. It is also unlike energy-transfer inhibitors such as DCCD, phlorizin, and Synthalin, which inhibited ATPase activity. TTFB stimulated the ATPase, and thus is similar to valinomycin in its activity. Unfortunately, the relation of the light-activated ATPase and other energy-linked activities in chloroplasts is not well elucidated and, therefore, it is impossible to decide if the activity of these compounds in stimulating ATPase activity is correlated with the inhibition of coupled electron transport.

The inhibitory effect of valinomycin on delayed light emission correlates with the stimulation of ATPase activity, and not with an energy-transfer effect which would increase delayed light emission. The effect of valinomycin in increasing the H^+/e^- ratio[34] is similar to the effect of energy-transfer inhibitors on the H^+/e^- ratio, and, therefore, not correlated with the effect found on delayed light emission or on the ATPase.

Apparently, the only way to resolve these observations, at least at our present state of knowledge, is to postulate several independent effects of valinomycin.

Acknowledgements

We wish to thank Dr. Berger Mayne for collaboration with the delayed light experiments and Dr. Richard Dilley for many helpful ideas and discussions.

References

1. A. Thore, D. L. Keister, N. Shavit, and A. San Pietro, *Biochemistry*, **7** (1968) 3499.
2. D. L. Keister and N. J. Minton, in: *Electron Transport and Energy Conservation*, J. Tager, S. Papa, E.Quagliariello, and E. Slater (eds.), Bari, Italy, Adriatica Editrice, in press.
3. A. Thore, D. L. Keister, and A. San Pietro, *Arch. Mikrobiol.*, **67** (1969) 378.
4. D. L. Keister and N. J. Yike, *Biochemistry*, **6** (1967) 3847.
5. L.-V. von Stedingk and H. Baltscheffsky, *Arch. Biochem. Biophys.*, **117** (1966) 400.
6. A. Thore, D. L. Keister, N. Shavit, and A. San Pietro, in: *Progress in Photosynthesis Research*, H. Metzner (ed.), International Union Biological Science, Tubingen, 1969, p. 1402.
7. J. B. Jackson, A. R. Crofts, and L.-V. von Stedingk, *European J. Biochem.*, **6** (1968) 41.
8. N. Shavit, A. Thore, D. L. Keister, and A. San Pietro, *Proc. Natl. Acad. Sci., U.S.A.*, **59** (1968) 917.
9. J. Neumann and A. T. Jagendorf, *Arch. Biochem. Biophys.*, **107** (1964) 109.
10. A. Trebst, E. Pistorius, and H. Baltscheffsky, *Biochim. Biophys. Acta*, **143** (1967) 257.
11. H. Baltscheffsky and B. Arwidsson, *Biochim. Biophys. Acta*, **65** (1962) 425.
12. H. Sato, K. Takahashi, and G. Kikuchi, *Biochim. Biophys. Acta*, **112** (1966) 8.
13. T. Beugeling, *Biochim. Biophys. Acta*, **153** (1968) 143.
14. B. Ke, *Biochim. Biophys. Acta*, **172** (1969) 583.
15. R. E. McCarty, *J. Biol. Chem.*, **244** (1969) 4292.
16. P. Plengvidhya and R. H. Burris, *Plant Physiol.*, **40** (1965) 997.
17. S. J. D. Karlish and M. Avron, *FEBS Letters*, **1** (1968) 21.
18. S. Izawa, T. N. Connolly, G. D. Winget, and N. E. Good, *Brookhaven Symp. Biol.*, **19** (1967) 169.
19. R. E. McCarty, R. J. Guillory, and E. Racker, *J. Biol. Chem.*, **240** (1965) 4822.
20. E. Gross, N. Shavit, and A. San Pietro, *Arch. Biochem. Biophys.*, **127** (1968) 224.
21. R. L. Williamson and R. L. Metcalf, *Science*, **158** (1967) 1694.
22. R. E. McCarty and E. Racker, *J. Biol. Chem.*, **242** (1967) 3435.
23. B. C. Mayne, *Photochem. Photobiol.*, **6** (1967) 189.
24. M. Avron, *J. Biol. Chem.*, **237** (1962) 2011.
25. A. Bennun and M. Avron, *Biochim. Biophys. Acta.*, **109** (1965) 117.

26. B. Petrack, A. Craston, F. Sheppy, and F. Farron, *J. Biol. Chem.*, **240** (1965) 906.
27. N. Shavit and A. San Pietro, *Biochem. Biophys. Res. Commun.*, **28** (1967) 277.
28. C. Carmeli and M. Avron, *Biochem. Biophys. Res. Commun.*, **24** (1966) 923.
29. K. G. Rienits, *Biochim. Biophys. Acta.*, **143** (1967) 595.
30. G. Hoch and I. Martin, *Biochem. Biophys. Res. Commun.*, **12** (1963) 223.
31. C. Carmeli and Y. Lifschitz, *FEBS Letters*, **5** (1969) 227.
32. C. Carmeli, *Biochim. Biophys. Acta*, **189** (1969) 256.
33. Z. Gromet-Elhanan, *Arch. Biochem. Biophys.*, **123** (1968) 447.
34. R. A. Dilley, *Arch. Biochem. Biophys.*, (1970) in press.
35. A. Gomez-Puyou, F. Sandoval, M. Tuena, E. Chavez, and A. Peña, *Arch. Biochem. Biophys.*, **129** (1969) 329.
36. A. R. Crofts, *Biochem. Biophys. Res. Commun.*, **24** (1966) 127.
37. R. S. Cockrell and E. Racker, *Biochem. Biophys. Res. Commun.*, **35** (1969) 414.
38. D. L. Keitser, *J. Biol. Chem.*, **240** (1965) 2673.