Biochimica et Biophysica Acta, 1020 (1990) 278–284
Elsevier

BBABIO 43300

N-Ethylmaleimide inhibits the sulfite stimulation of spinach thylakoid ATPase

Ann L. Umbach, André T. Jagendorf and Bruce Howlett

Plant Biology Section, Cornell University, Ithaca, NY (U.S.A.)

(Received 17 May 1990)

Key words: CF_1; ATPase; Sulfite; *N*-Ethylmaleimide; Chloroplast; (Spinach)

Sulfite replaces light in inducing ATPase of pre-reduced thylakoids (Larson and Jagendorf (1989) Biochim. Biophys. Acta 973, 67–77). We find that *N*-ethylmaleimide treatment of these thylakoids inhibits a large part of the response to sulfite, even though primary activation due to reduction of the gamma disulfide group remains. Sulfite responsiveness, including insensitivity to excess uncouplers, is restored to reduced, NEM-treated thylakoids by further treatment with trypsin (post-illumination), by using methanol, or by solubilizing the CF_1. We suggest the NEM groups on the sulfhydryls may restrict flexibility of thylakoid-bound CF_1 needed for the sulfite effect; and a trypsin clip can restore sufficient flexibility to the complex, membrane-bound enzyme.

Introduction

The ATP synthase of thylakoid membranes (CF_1/CF_0) requires activation by light plus reductant in order to express ATPase activity. The role of light in this activation is indirect, through the ΔpH which is built up across the thylakoid membrane during photosynthetic electron transport. The ΔpH causes a conformational change in the enzyme which exposes a disulfide bond on the gamma subunit to reduction [1,2].

In addition to initial ATPase activation, ΔpH is necessary for subsequent activity maintenance. ATPase activity is lost if light-activated thylakoids are placed in the dark, and this has been correlated with loss of the protonmotive force and re-binding of an inhibitory ADP [1]. Also, superoptimal concentrations of uncouplers inhibit ATPase, presumably by making the enzyme inactive [3].

Recently, Larson and Jagendorf showed [4] that sulfite can substitute for the second ΔpH function, i.e., maintenance of activated, thylakoid-bound ATPase activity. Thus, if the ATPase has already been activated in the presence of light and reductant, very high rates of ATP hydrolysis are achieved by the enzyme in the dark, and even with saturating uncouplers (5 mM NH_4Cl plus 1 μM gramicidin) added. Under these conditions the rate of hydrolysis is essentially linear for the first 5 min, or longer (unpublished results; see also Fig. 1 in Ref. 27). A detailed comparison between sulfite and light (i.e., ΔpH) effects on ATPase characteristics is necessary to see how well sulfite may serve as a model chemical system for part of the normal energy transduction process.

For either sulfite or light to maintain activity of thylakoid ATPase, the gamma disulfide bond must stay reduced. High concentrations of DTT are used for this purpose [4]; otherwise, unpredictable, variable loss of the activated state occurs (Ref. 5; personal observation). An alternative way to preserve the activated state is to modify the reduced sulfhydryls by covalent binding of *N*-ethylmaleimide (NEM) [5], thereby preventing their reoxidation. This covalent binding does not interfere with the basic ATPase activity of the thylakoids. However, we have found that NEM-treated thylakoids have lost from 60 to 70% of their capacity to respond to sulfite. This intriguing difference between ΔpH and sulfite activation responses is explored further here.

Abbreviations: DL, thylakoids treated with DTT in the light; DLN, DL thylakoids exposed to NEM just after illumination; DLNT, DLN thylakoids treated with trypsin in the post-illumination dark; DLT, DL thylakoids treated with trypsin in the post-illumination dark; DTT, dithiothreitol; NEM, *N*-ethylmaleimide.

Correspondence: A.T. Jagendorf, Plant Biology Section, Plant Science Building, Cornell University, Ithaca, NY 14853, U.S.A.

Materials and Methods

Most chemicals except common salts were from Sigma.

Latent thylakoids were isolated from spinach leaves as described earlier [4]. Each experiment reported used thylakoids from a separate isolation of chloroplasts. Activation of these thylakoids by light in the presence of DTT (DL thylakoids) was also as described by Larson and Jagendorf [4]. Illumination was at 1100 $\mu E \cdot m^{-2} \cdot s^{-1}$ incandescent light at 20 °C for 5 min.

DL thylakoids were treated with NEM (DLN thylakoids) as follows: after illumination the DL thylakoid suspension, at 0.75 mg chlorophyll/ml, was divided into two parts. A volume of 2 M NEM stock in ethanol or dimethylsulfoxide (made fresh every 2 to 3 days) was added to bring the NEM concentration to 45 mM. Since the DTT concentration during illumination was 20 mM, this provided a 5 mM concentration of free NEM. The same volume of ethanol (or dimethylsulfoxide) was added to the control sample. The thylakoids were incubated in the dark for 7 min at room temperature, then uncouplers were added along with excess DTT to quench the remaining NEM. The suspension was diluted from the original 3 ml, up to 15 ml with buffer containing 20 mM NaCl, 5 mM Tricine (pH 8), 20 mM DTT; centrifuged at $5000 \times g$ for 5 min, then resuspended in the same buffer. The thylakoids were kept on ice.

In some experiments, DL or DLN thylakoids were treated with trypsin (DLT or DLNT thylakoids) essentially as described earlier [4]. Trypsin was added to the thylakoid suspension at 1 μg per 10 μg chlorophyll. The mixture was incubated for 10 min on ice in the dark. Trypsin activity was terminated by adding soybean trypsin inhibitor at 10-times the weight of trypsin.

A small-scale version of the previously described method [6], consistent with the amounts of chlorophyll processed, was used to isolate CF_1 from DL and DLN thylakoids. The high salt eluate from the first DEAE column was used without further purification. NaBr particles (i.e., thylakoids stripped of CF_1) were prepared as described earlier [7] from either DL or DLN thylakoids. They were stored under liquid N_2 in a medium containing 50% glycerol, 5% dimethylsulfoxide, 20 mM dithiothreitol, 10 mM NaCl, 25 mM Hepes (pH 7.5) and 20 mg/ml bovine serum albumin. Before use they were diluted and washed in 0.4 M sorbitol, 10 mM Tricine (pH 8.0), 10 mM NaCl, 5 mM dithiothreitol. Recoupling of these thylakoids with CF_1 from either DL or DLN thylakoids was performed as described previously [8].

ATPase activity of thylakoids or of isolated CF_1 was assayed in 50 mM TAPS (pH 8.0 at 37 °C), 11 mM $MgCl_2$, 50 mM KCl, and 10 mM ATP. The uncouplers, 5 mM NH_4Cl and 1 μM gramicidin, were also included to insure complete dissipation of the proton gradient. For most experiments, reaction mixtures were made up in microtiter plate wells using 5 μl of thylakoids, 25 μl of a 2 × concentrated reaction buffer including everything but ATP and sulfite, and 10 μl of a sulfite solution at 5 × the desired final concentration. After equilibration at 37 °C for 2 min, the reaction was started by adding 10 μl of 50 mM ATP, and proceeded for 2 to 5 min. For assays performed in test tubes at 0.5 ml final volume, the reaction was initiated by adding thylakoids or CF_1 (about 5 to 10 μg chlorophyll or 2 μg protein). The 2 M stock sulfite solution had its pH adjusted to 8.0 and was stored under N_2 gas. Dilutions were made up in 10 mM DTT, to avoid auto-oxidation. For measuring organic solvent-stimulated ATPase activity, methanol was used at 33% (in preliminary experiments shown to be the optimum concentration). This is the same concentration as that optimal for latent thylakoid Mg^{2+}-ATPase [9].

The ATPase reaction was terminated by the addition of 20 μl of 5 M acetic acid, 50 mM $HgNO_3$. Centrifugation removed the Hg-DTT complex, avoiding interference with phosphate measurement by free DTT. Liberated inorganic phosphate was estimated by the method of Lebel et al. [10] as modified by Larson and Jagendorf [4], using a microtiter strip well reader. Kinetic constants for sulfite were derived from the original curves by non-linear regression, using the algorithm of Marquardt [11], in a computer program kindly supplied by Warren Zipfel. As noted previously [4], the dependence of ATPase rate on sulfite concentration follows a hyperbolic relation under these conditions; it is therefore possible to derive V_{max} values, and a concentration related to affinity of the system for sulfite. Since we do not want to imply that sulfite is a substrate, the affinity constant is designated as K_s, not K_m.

The ability of light to activate thylakoid ATPase was tested in some experiments. Latent thylakoids or DLN thylakoids, in activation buffer with or without 20 mM DTT, were illuminated for 2 min at 20 °C with 1100 $\mu E \cdot m^{-2} \cdot s^{-1}$. Immediately after this illumination, ATPase activity was assayed in the dark in the presence or absence of sulfite.

Protein was assayed by dye binding [12], with a 1.6 correction factor for CF_1 [13] or by a modified Lowry procedure [14]. Chlorophyll was determined as described by Wintermans and De Mots [15].

Results

The ATPase activity of DL thylakoids showed a large stimulation by increasing concentrations of sulfite. This occurred even in the presence of saturating concentrations of uncouplers (Ref. 4; Fig. 1). If, however, DL thylakoids were exposed to NEM (DLN thylakoids), a large inhibition of ATPase was observed (Fig. 1). In the experiment of Fig. 1 the V_{max} with respect to sulfite was decreased from 635 μmol/mg chlorophyll per h in the DL thylakoids, to 199 in the DLN thylakoids; while the K_s value dropped slightly, from 68 mM in the

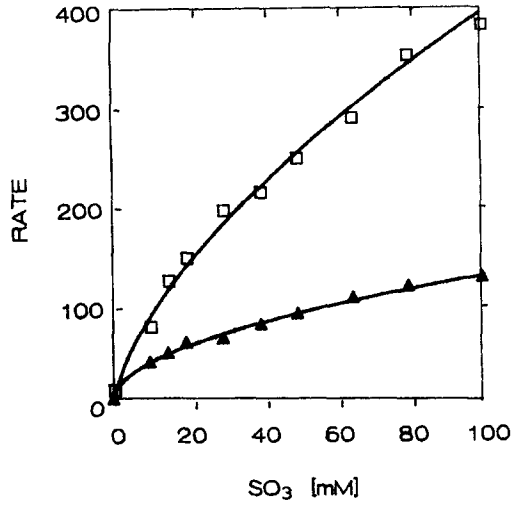

Fig. 1. Stimulation of thylakoid ATPase actvity by increasing sulfite concentration is inhibited by NEM. □, DL thylakoids; ▲, DLN thylakoids. Preparation of these, and assay conditions (including presence of uncouplers), are listed in Materials and Methods. Rates are in units of μmol P_i released/mg chlorophyll per h.

controls to 53 mM after NEM. A range of values for these parameters from several experiments is shown in Table I; in all of them, the V_{max} was decreased, usually to about 35% of DL thylakoid values; and the K_s values dropped, to variable extents. The degree of inhibition was similar if uncouplers were omitted from the assay (data not shown).

This inhibition was not expected, because the ordinary light (protonmotive force) + DTT activated ATPase, in the absence of uncouplers and sulfite, is not affected by the NEM treatment. Thus DLN thylakoids were just as active as DL thylakoids, in the ATPase elicited by light (Table II). This result confirms the earlier report of Ketcham et al. [5], that NEM causes no interference with ATPase activation by ΔpH.

TABLE II

NEM treatment of light + DTT-activated thylakoids preserves reduced disulfide bonds but diminishes HSO_3 stimulation

Thylakoids [a]	No uncouplers		Plus uncouplers	
	$-SO_3^{2-}$	$+SO_3^{2-}$	$-SO_3^{2-}$	$+SO_3^{2-}$
DTT present during illumination				
Latent	153 (13) [b]	155 (69)	14 (12)	159 (7)
DLN	169 (54)	163 (39)	0	51 (4)
DTT absent during illumination				
Latent	23 (12)	22 (9)	2 (2)	11 (4)
DLN	151 (47)	151 (28)	6 (6)	38 (12)

[a] Either latent or DLN thylakoids were illuminated in the presence or absence of 20 mM DTT for 2 min. Subsequently, ATPase activity in the dark was immediately assayed in the presence or absence of uncouplers (10 μM gramicidin and 5 mM NH_4Cl) and in the presence or absence of 60 mM SO_3^{2-}.

[b] μmol P_i/(mg chlorophyll per h). All values are averages of two experiments. Half the range is indicated in parentheses, for each value.

As expected, latent thylakoids required the presence of reductant during illumination to express ATPase activity (compare 'latent' values, rows 1 and 3, Table II). DLN thylakoids, on the other hand, required no reductant during the second illumination in order to activate their ATPase. NEM modification had prevented the newly exposed Cys residues from oxidizing to a disulfide bond again (see 'DLN' values, rows 2 and 4, in Table II). Both kinds of thylakoids showed a marked response to sulfite if activity was assayed in the presence of uncouplers, again illustrating the ability of sulfite to replace ΔpH in maintaining ATPase activity. Sulfite was effective only if DTT had been present during the illumination, reducing the the gamma disulfide bond. The data of Table II also illustrate again the reduced response to sulfite by the NEM-treated

TABLE I

Kinetic constants for the sulfite effect with DL and DLN thylakoids

Expt.	Trypsin [a]	V_{max} (μmol ATP hydrolysed/mg chlorophyll per h)			K_s (mM)	
		DL [b]	DLN	Inhib. (%)	DL	DLN
1	–	747	153	79	129	55
2	–	654	241	63	69	40
3	–	865	306	65	68	21
4	–	618	243	61	68	41
5	–	635	199	69	68	53
6	–	668	383	43	85	65
	+	1898	1870	2	5	7
7 [c]	–	505	187	63	63	36
	+	792	766	3	15	9

[a] When present, used just after activation in the light.

[b] DL refers to thylakoids with ATPase by light + DTT; DLN to those treated with NEM just after light activation.

[c] Experiment performed with previously frozen thylakoids.

TABLE III

Thylakoid treatment with NEM before reduction has no effect on response to sulfite

SO_3^{2-} (mM)	DL [a]	DLN	NEM before reduction [b]
0	0 [c]	0	0
20	53	17	47
60	117	40	126

[a] DL refers to thylakoids with ATPase activated by light + DTT; DLN to those treated with NEM just after light activation.
[b] Thylakoids in the last column were exposed to NEM in the dark, prior to activation by reduction with DTT in the light.
[c] Rates are shown as μmol/mg chlorophyll per h.

ATPase (compare 'latent' and "DLN" values in 'uncouplers' columns).

The conditions of the experiments insured that the reduced disulfide on gamma was exposed to NEM [16], and the results shown so far are consistent with NEM interaction with these two sulfhydryls. However, additional sulfhydryls are present in CF_1 [17,18], and we needed to assess their possible participation in the inhibition of the sulfite response.

To test for involvement of the dark-exposed sulfhydryls of gamma and epsilon subunits in the NEM inhibition, we exposed latent thylakoids to 2 mM NEM in the dark. After subsequent light + DTT activation, these thylakoids showed no inhibition of response to sulfite; while thylakoids from the same isolation, but treated with NEM after activation, were inhibited (Table III).

A second sulfhydryl group of gamma is exposed to the medium and sulfhydryl reagents only during light activation of the ATPase [17]. Since thylakoids in our study were treated with NEM only after light activation, never during, it is unlikely that NEM could have reacted with this sulfhydryl. In addition, modification of this sulfhydryl by NEM causes severe inhibition of either light-activated [17] or sulfite-stimulated [19] ATPase. However, under our conditions there was no inhibition of light-activated ATPase by NEM (Table II), or of ATPase further stimulated by other treatments (see below).

Although NEM inhibition of sulfite-dependent activity was substantial, the inhibition could not be found under any other reaction condition. The first of these was the light-activated ATPase, noted above. Secondly, trypsin digestion, which induces remarkably high rates in DL thylakoids assayed with sulfite [4], removed the inhibition by NEM. Following trypsin treatment, the rates of ATPase were identical for DLT (control) and DLTN (NEM-treated) thylakoids (Fig. 2). Under the conditions employed here, trypsin treatment produces a small peptide from the gamma subunit, which contains one of the NEM-complexed sulfhydryls [18].

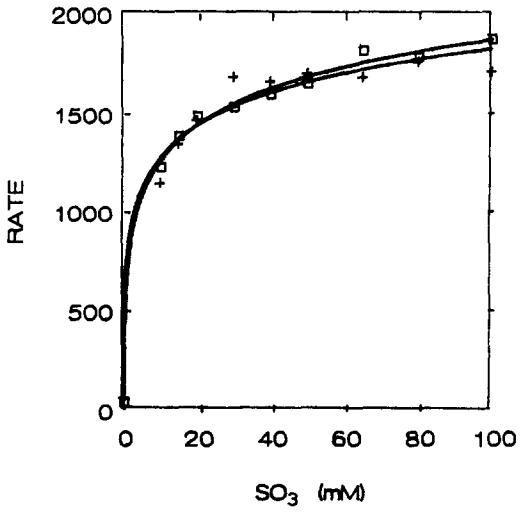

Fig. 2. Recovery of sulfite stimulation by trypsin treatment after activation. □, DLT thylakoids (trypsin treated after activation by light and DTT); +, DLNT thylakoids (treated with NEM after activation by light and DTT, then treated with trypsin). Preparation of these, and assay conditions, are listed in Materials and Methods. Rates are in units of μmol P_i released/mg chlorophyll per h.

Addition of methanol to DLN thylakoid ATPase assays also reversed NEM inhibition. In methanol, the Mg^{2+}-ATPase is inhibited by small amounts of free Mg^{2+} and this inhibition is reversed by sulfite [9]. When assayed in the presence of 33% methanol, the response of DLN thylakoid ATPase to sulfite was the same as that of the DL controls; and as usual, the same DLN thylakoids were inhibited if assayed without methanol (Table IV).

A third treatment which reversed NEM inhibition was solubilization of CF_1. DL and DLN thylakoids, showing the usual inhibition by NEM treatment, were used as the source for isolation of CF_1. The CF_1 from DLN thylakoids responded to sulfite just as well as that from DL thylakoids (Table V).

The failure of isolated CF_1 to show the NEM inhibition raised the possibility that the effect was caused by

TABLE IV

Methanol activation restores sulfite stimulation

Methanol % (v/v)	SO_3^{2-} (mM)	DL [a]	DLN
0	0	0 [b]	0
	20	101 (30)	30 (6)
	60	336 (50)	103 (9)
33	0	477 (17)	457 (27)
	20	640 (4)	623 (30)
	60	825 (22)	843 (25)

[a] DL refers to thylakoids with ATPase activated by light + DTT; DLN to those treated with NEM just after light activation.
[b] Rates shown are the average of two experiments. Half the range is indicated in parentheses. The units are μmol P_i/mg chlorophyll per h.

TABLE V

Mg-ATPase of soluble CF_1 responds to sulfite ± NEM

The units are μmol P_i/mg chlorophyll per h for thylakoids, μmol P_i/mg protein per min for soluble CF_1.

State of CF_1	SO_3^{2-} (mM)	DL [a]	DLN
Thylakoids	0	0 [b]	0
	20	56 (16)	27 (2)
	60	238 (48)	80 (3)
Soluble	0	0.5 (0.1)	0.8 (0.3)
	20	1.7 (0.4)	2.2 (0.5)
	60	3.7 (0.5)	4.3 (1.5)

[a] DL refers to thylakoids with ATPase activated by light + DTT; DLN to those treated with NEM just after light activation.

[b] Rates shown are the average of two experiments. Half the range is indicated in parentheses.

binding of NEM to thylakoid component(s), rather than to CF_1. To assess this possibility, CF_1 was stripped from DL and DLN thylakoids using 2 M NaBr [7], and these NaBr particles were cross-reconstituted with CF_1 from either DL or DLN thylakoids. When the CF_1 derived from two kinds of thylakoids was reconstituted with control NaBr particles, the response to sulfite was inhibited strongly (Fig. 3). However, reconstitution of control CF_1 with the two different types of NaBr particle showed the same response to sulfite, with no inhibition apparent (Fig. 4).Thus NEM modification of the CF_1, not of the thylakoids, is responsible for decreased response to sulfite.

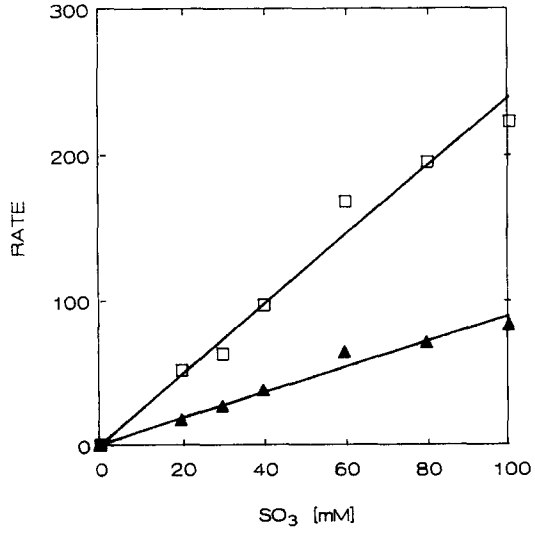

Fig. 3. ATPase activity by reconstituted NaBr particles. □, CF_1 taken from DL thylakoids; ▲, CF_1 taken from DLN thylakoids. These were reconstituted with NaBr treated latent thylakoids, lacking endogenous CF_1. Preparation of these, and assay conditions, are listed in Materials and Methods. Rates are in units of μmol P_i released/mg chlorophyll per h.

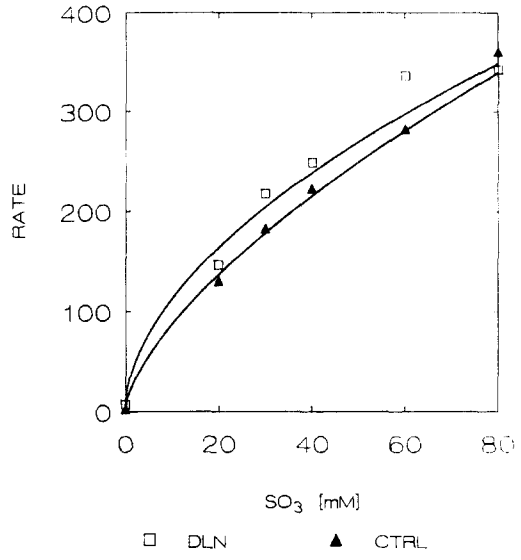

Fig. 4. ATPase by reconstituted NaBr particles. CF_1 was taken from latent thylakoids, and reconstituted with NaBr particles made from DL thylakoids (□) or DLN thylakoids (▲). Preparation of these, and assay conditions, are listed in Materials and Methods. Rates are in units of μmol P_i released/mg chlorophyll per h.

Discussion

Treatment of pre-illuminated thylakoids with NEM was shown previously not to inhibit the ATPase elicited by light + DTT [5,16], and this result was confirmed here (Table II). However, the NEM treatment did inhibit the sulfite-dependent ATPase found in the presence of uncouplers (Fig. 1), where sulfite replaces the protonmotive force in maintaining activity [4]. Thylakoid ATPase is expressed under a variety of activating conditions; since the sulfite-dependent activity was the only one inhibited by NEM, we can conclude that the sulfite activation is affected, rather than ATPase in general.

There are 4 Cys residues on the gamma subunit of CF_1 [17,20]. One of these (Cys-322), and an additional Cys residue on the epsilon subunit, are exposed to maleimide in the medium, on thylakoids in the dark as well as in the light. Since use of NEM prior to illumination did not result in the inhibition of the sulfite response (Table III), we can rule out their participation in this phenomenon.

Application of NEM in our experiments followed the protocol used previously [5]: illumination of thylakoids with DTT to reduce the disulfide bond on the gamma subunit (between Cys-199 and Cys-205), then destruction of the protonmotive force by added uncouplers in the dark, prior to adding the NEM. Under these conditions, about 2/3 of the CF_1 molecules in thylakoids are susceptible to DTT reduction and subsequent reaction with NEM [5]. It is probably significant that the extent of inhibition which we found was usually on the order of 60 to 65% (Table I). This leaves open the possibility

that NEM addition might inhibit sulfite-stimulated ATPase completely, for those molecules which have been modified. However the variable decrease in K_s value (representing apparent increase in affinity for sulfite) caused by NEM treatment argues against this.

On the gamma subunit, Cys-89 is hidden from the medium in the dark, and only exposed while a protonmotive force is present across the thylakoid membrane [21]. If Cys-89 is modified by maleimides, ATPase activity is strongly inhibited. Had this Cys been the target for NEM inhibition of the sulfite response, we would have seen inhibition of both soluble CF_1 and thylakoid ATPase activity under all circumstances. However the sulfite effect on solubilized CF_1, representing a reversal of inhibition by free Mg^{2+} [22], was not diminished by NEM (Table V). On the thylakoids, neither the light-stimulated (Table II), the methanol-dependent (Table IV) nor the trypsin (after light) activated thylakoid ATPase (Fig. 2) were affected. The last activity, in particular, shows very high rates, so that any NEM induced rate limitation should have easily appeared, but none did.

If, during exposure to NEM, the gamma subunit sulfhydryls re-oxidized, some inhibition would have been expected. However, the NEM inhibition of response to sulfite was not reversed by DTT added during a second illumination (Table II), so this possibility can be ruled out.

Finally, results of the cross-reconstitution experiments (Figs. 3 and 4) rule out the NEM effect being due to modification of some as yet undefined component of the thylakoids. We feel the combined evidence strongly supports the idea that NEM covalent binding to the reduced disulfide bond components, Cys-199 and Cys-205, is responsible for inhibition of the response to sulfite. Another necessary component is attachment of CF_1 to the thylakoids, since soluble NEM-treated CF_1 is not affected (Table V) until it is re-bound to thylakoids (Fig. 3).

The simplest mechanism for preventing a response to sulfite would be if the anion itself interacts directly with free Cys-199 and -205, and so could not interact fruitfully with the NEM modified groups. But this interaction seems unlikely, since using methanol, or solubilizing the CF_1, permitted a full response to sulfite even though NEM remained bonded to the sulfhydryls.

It seems more likely that sulfite acts at a site on CF_1 distinct from the disulfide sulfhydryls. We suggest that the presence of two NEM groups may sterically hinder sulfite-induced conformational changes responsible for the stimulation of enzyme activity. The release of sulfite-stimulated enzyme activity from NEM inhibition by treatments able to increase the flexibility of the enzyme (trypsin, methanol or solubilization) is consistent with this idea. Methanol, for instance, may weaken the interactions of the CF_1 subunits [23]. It is significant that 20% methanol causes release of the epsilon subunit from CF_1 bound to a DEAE column [24]. Such increased flexibility could allow the enzyme to assume a more active conformation despite the presence of NEM on gamma.

The inhibition of bicarbonate-stimulated mitochondrial F_1 ATPase by sulfhydryl-binding reagents may have a similar basis. Pedersen [25] observed that F_1 ATPase, in the presence of bicarbonate, was inhibited about 50% by 1 mM *p*-chloromercuribenzoate or *p*-chloromercuribenzoylsulfonate, although not by NEM. Inasmuch as the bicarbonate stimulation of F_1 may be similar in kind to the sulfite stimulation of CF_1, it is interesting that both activities are only partly inhibited by sulfhydryl-binding reagents.

Given a steric mechanism for NEM inhibition of the sulfite effect, the place on CF_1 where sulfite acts may be at a site distant from gamma or at least Cys-109 and -205. The binding of sulfite to this site may produce 'global' conformational changes in the enzyme which, in turn, affect gamma. A related situation has recently been described for the cytoplasmic domain of red blood cell band 3 protein. Binding of hemoglobin at the cytoplasmic end of the protein dimer causes a change in structure such that sulfhydryl groups at the other membrane-associated end are no longer accessible to sulfhydryl reagents. In this way, cytoplasmic hemoglobin may affect the membrane transport properties of the protein [27]. Transduction of activating, conformational changes throughout a large enzyme such as CF_1 seems a prerequisite for the action of any regulatory molecules.

The actual function of sulfite in activation of CF_1 seems more clear, thanks to the discovery of Du and Boyer [28] that it is not needed for activation of the reduced enzyme, providing the tightly bound ADP was released by dilution after illumination. Thus the major function for sulfite, and of light, is likely to be in bypassing the inhibitory effects of or in causing early release of a tightly bound adenine nucleotide.

In sum, our results confirm that sulfite does support and stimulate activated thylakoid ATPase activity, and in this way resembles the ΔpH in its effect. They also indicate that sulfite does not act in a manner identical to ΔpH, since the sulfite effect is inhibited by NEM and that of ΔpH is not. The basis for the NEM inhibition may be steric since several treatments which increase the flexibility of CF_1 also release it from the NEM inhibition of the response to sulfite.

Acknowledgement

This work was supported by grant DCB 88-17527 from the National Science Foundation, and by Hatch Grant 185-417.

References

1 Strotmann, H. and Bickel-Sandkötter, S. (1984) Annu. Rev. Plant Physiol. 35, 97–120.
2 Nalin, C.M. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7275–7280.
3 Jagendorf, A.T. (1977) in Encyclopedia of Plant Physiology, New Series (Trebst, A. and Avron, M., eds.), Vol. 5, Photosynthesis I, pp. 307–337, Springer, Berlin.
4 Larson, E.M. and Jagendorf, A.T. (1989) Biochim. Biophys. Acta 973, 67–77.
5 Ketcham, S.R., Davenport, J.W., Warncke, K. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7286–7293.
6 Binder, A., Jagendorf, A.T. and Ngo, E. (1978) J. Biol. Chem. 253, 3094–3100.
7 Nelson, N. and Eytan, E. (1979) in Cation Flux Across Biomembranes (Mukohata, Y. and Packer, L., eds.), pp. 409–415, Academic Press, New York.
8 Telfer, A., Barber, J. and Jagendorf, A.T. (1980) Biochim. Biophys. Acta 591: 331–345.
9 Anthon, G.E. and Jagendorf, A.T. (1983) Biochim. Biophys. Acta 723, 358–365.
10 Lebel, D., Poirier, G.G. and Beaudoin, A.R. (1978) Anal. Biochem. 85, 86–89.
11 Press, W.H., Teukolsky, S., Flannery, B. and Vetterling, W. (eds.) (1986) "Numerical Recipes, the Art of Scientific Computing" pp. 533–535, Cambridge University Press, Cambridge.
12 Bradford, M. (1976) Anal. Biochem. 72, 248–254.
13 Viale, A., Vallejos, R. and Jagendorf, A.T. (1981) Biochim. Biophys. Acta, 496–503.
14 Larson, E., Howlett, B. and Jagendorf, A.T. (1986) Anal. Biochem. 155, 243–248.
15 Wintermans, J.F.G.M. and De Mots, A. (1965) Biochim. Biophys. Acta 109, 448–453.
16 Moroney, J.V., Fullmer, C.S. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7281–7285.
17 Nalin, C.M., Béliveau, R. and McCarty, R.E. (1983) J. Biol. Chem. 258, 3376–3381.
18 Schumann, J., Richter, M.L. and McCarty, R.E. (1985) J. Biol. Chem. 260, 11817–11823.
19 Cohen, W.S. (1989) Plant Physiol. 91, 1107–1111.
20 Miki, J., Maeda, M., Mukohata, Y. and Futai, M. (1988). FEBS Lett. 232, 221–226.
21 McCarty, R.E., Pittman, P.R. and Tsuchiya, Y. (1972) J. Biol. Chem. 247, 3048–3051.
22 Anthon, G.E. and Jagendorf, A.T. (1986) Biochim. Biophys. Acta 848, 92–98.
23 Anthon, G.E. and Jagendorf, A.T. (1984) Biochim. Biophys. Acta 766, 354–362.
24 Richter, M.L., Patrie, W.J. and McCarty, R.E. (1984) J. Biol. Chem. 259, 7371–7373.
25 Pedersen, P.L. (1976) Biochem. Biophys. Res. Commun. 71, 1182–1188.
26 Salhany, J.M. and Cassoly, R. (1989) J. Biol. Chem. 264, 1399–1404.
27 Larson, E.M., Umbach, A. and Jagendorf, A.T. (1989) Biochim. Biophys. Acta 973, 75–85.
28 Du, Z. and Boyer, P.D. (1990) Biochemistry 29, 402–407.