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Effect of Thiourea and Substituted Thioureas on Dynein ATPase and on the Turbidity Response of Tetrahymena Cilia

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The effects of thiourea and of several substituted thioureas – phenylthiourea, α-naphtylthiourea, metiamide, and burimamide – on dynein ATPase have been studied. The substituted thioureas are over 30 times more potent than thiourea in causing enhancement of 30S dynein ATPase activity and inhibition of 14S dynein ATPase activity. The effects of thiourea and phenylthiourea can be prevented by very low concentrations of β-mercaptoethanol or dithiothreitol. Axonemal ATPase is also enhanced by the thioureas, but the reaction proceeds more slowly than for solubilized 30S dynein. Enhancement of 30S dynein ATPase by metiamide is prevented by low (∿ 1μM) concentrations of ATP and, less effectively, by AMP-PNP, but not by AMP-PCP even though the latter is a stronger inhibitor of 30S dynein ATPase than is AMP-PNP.

The thioureas inhibit the ATP-induced decrease in turbidity (measured as ΔA_{350}) of axonemal suspensions. Inhibition of the turbidity response is also prevented by low concentrations of β-mercaptoethanol, but, in contrast to the irreversible enhancement of ATPase activity, inhibition of the turbidity response is largely reversible. The ability of 30S dynein to rebind onto twice-extracted axonemes is not changed by treatment with phenylthiourea or metiamide.

These observations indicate that the thioureas react with at least two sets of SH or S–S groups on axonemes. Reaction with the group(s) on the 30S dynein causes an apparently irreversible enhancement of ATPase activity. Reaction with another group(s) causes a reversible inhibition of the turbidity response.

Key words: thiourea, cilia, dyneins, ATPase, sulfhydryl groups, turbidity response

The finding [1, 2] that ∿0.5 M thiourea inhibited the actomyosin system of mollusc muscle but not the "catch" mechanism led Brokaw and his colleagues to study the effects of thiourea on flagellar motility. Brokaw [3] found that ∿0.2 M thiourea rapidly reduced the beat frequency of Ciona and Lytechnius sperm with little change in other wave parameters. With increase of the time of exposure to thiourea, however, amplitude and fre-

Abbreviations or generic names used are: metiamide, N-methyl-N′(2 [(5-methylimidazol-4-yl) methylthiol] ethyl)thiourea; burimamide, N-(-2-[(imidazol-4-yl)methylthio] ethyl)-N′-methylthiourea; β-MSH, β-mercaptoethanol; DTT, dithiothreitol; AMP-PNP, adenylyl imidodiphosphate; AMP-PCP, β, γ-methylene-adenosine triphosphate; FNS, bis(4-fluoro-3-nitrophenyl) sulfone.

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quency decreased until the sperm became quiescent. The reduction in beat frequency was accompanied by about a 1.5-fold increase in ATPase activity [4]. It was confirmed that thiourea acts initially only to reduce frequency without changing the size and shape parameters of the waves for spermatozoa of Ciona in a study that also showed that the amount of movement-coupled oxidative metabolism per beat remained nearly constant as the beat frequently decreased [5]. The inhibition of beat frequency and increase of ATPase activity of isolated sea urchin sperm flagella caused by 0.3 M thiourea was accompanied by an increase in the apparent K_m for ATP from ∿0.02 mM to ∿0.10 mM [6], and it was shown that thiourea and urea did not affect the kinetics of solubilized dynein ATPase activity in the same way. Except for the reports that thiourea inhibited 14S dynein ATPase activity [7] and inhibited the pellet height response of ciliary axonemes [8], no further studies of the effect of thiourea on ciliary proteins have been performed. The report that urea and thiourea are equally potent as denaturants of a number of proteins [9] renders it highly unlikely that thiourea acts on the ciliary system as a nonspecific denaturant, and points up our lack of understanding of the mode of action of thiourea. In view of the report by Toennies in 1937 [10] of a reaction between cystine and the SH form of thiourea and the finding that cysteine protected rats against a lethal dose of α-naphthylthiourea [11], it seemed possible that thiourea was acting as an SH reagent. The latter studies had also shown that several substituted thioureas were much more potent than thioureas as inhibitors of tyrosinase. We therefore tested phenylthiourea and several other substituted thioureas for their effects on 30S and 14S dynein ATPase, on the ability of 30S dynein to rebind to axonemes, and on the ATP-induced change in turbidity of suspensions of axonemes.

MATERIALS AND METHODS

Demembranated axonemes, 14S and 30S dyneins, and twice-extracted axonemes of Tetrahymena pyriformis, strain HSM, were prepared as described elsewhere [12] except that the final concentration of Triton X-100 used to demembranate the axonemes was reduced from 0.1% to 0.05% (v/v). Unless otherwise specified, all preincubations were at 0°C. The buffers used and the procedures for ATPase assay were as described [12].

Absorbance measurements (used as a measure of turbidity [13]) were made in 1-cm quartz cuvettes in a Gilford spectrophotometer with the chamber held at 25°. At the desired times, 0.95 ml of cilia were added to the cuvette, and the A_{350} level was measured. Then 0.05 ml of 1 mM ATP was added and the A_{350} measured several times during the next 2–3 min until a stable value was attained. Initial A_{350} readings were approximately 0.3, and were normalized to 0.300, so that all changes in turbidity refer to the same initial value. In these experiments, the change in A_{350} (corrected for the small dilution of the axonemes cause by ATP addition) was ∿0.07.

The rebinding of 30S dynein to twice-extracted axonemes (pellet II) was measured by preincubating the 30S dynein at 0° with the desired concentration of phenylthiourea or metiamide for 5 min. The volume was then brought to 1.0 ml by addition of 0.2 ml of pellet II suspension and the mixture was incubated at 0° for 10 min. The mixture was then centrifuged at 0° for 3 min at 12,000 g, and the supernatant was assayed for ATPase activity. Percentage binding was computed according to Equation 1 of Blum and Hayes [14].

Protein Determination

Protein concentrations were determined as described earlier [15].

Preparation of Reagents

Thiourea was dissolved in water. Substituted thioureas and FNS were dissolved in acetone or 50% acetone (v/v). When these reagents were used they were always used as 0.02 or 0.025 ml, giving a final acetone concentration of 1% or 1.25%. Control tubes received the same volumes of acetone. Unless otherwise specified, all solutions were made fresh daily and kept at 0°.

Reagents

AMP-PNP, phenylthiourea, and twice-recrystallized bovine liver catalase were purchased from Sigma, AMP-PCP from P-L Biochemicals, and α-naphthylthiourea from Aldrich; burimamide and metiamide were generious gifts of the Smith, Kline and French Laboratories. Sources of all other reagents were reported earlier [15]. The AMP-PNP appeared to be free of ATP when examined by ascending paper chromatography using solvent system A of Yount et al [16].

RESULTS

Table I shows the approximate concentrations of thiourea and several substituted thioureas required to cause half-maximal enhancement of 30S dynein or demembranated axonemal ATPase activities, and half-maximal inhibition of 14S dynein ATPase during a 17-h incubation at 0°. About 90 mM thiourea is required to cause half-maximal enhancement of axonemal ATPase, but an order of magnitude less is required for half-maximal enhancement of solubilized 30S dynein. Half-maximal inhibition of 14S dynein requires about half as much thiourea as do whole axonemes.

Phenylthiourea is 30–100 times more potent than thiourea, half-maximal enhancement of 30S dynein ATPase requiring only ∿0.3 mM. At this concentration, a nonspecific denaturant effect is extremely unlikely.

Burimamide and metiamide are substituted thioureas developed for use as blockers of histamine H_2-receptors [17]. They are polar hydrophilic molecules, with high dipole moments and low octanol-water partition coefficients, but are much less hydrophilic than thiourea. They are slightly less potent activators of 30S dynein ATPase and inhibitors of 14S dynein ATPase than is phenylthiourea (Table I). α-Naphthylthiourea is as potent as

TABLE I. Approximate Concentration (mM) for Half-Maximal Effects of Thiourea and Several Substituted Thioureas on ATPase Activity of Dyneins

	14S Dynein	30S Dynein	Axonemes
Thiourea	40	7	90
Burimamide	1	0.6	3
Metiamide	1	1	4
Phenylthiourea	0.4	0.3	1
α-Naphthylthiourea	2	0.2	-

Dyneins or demembranated axonemes were incubated at 0° and pH 7.5 for ∿17h with a range of concentrations of the indicated compounds, and the ATPase activity was then assayed as described in Methods. The approximate concentrations required to give half-maximal enhancement (30S dynein and axonemes) or half-maximal inhibition (14S dynein) of the ATPase activity are shown.

phenylthiourea as an activator of the latent ATPase of 30S dynein, but less potent than phenylthiourea as an inhibitor of 14S dynein. Unlike the other substituted thioureas in Table I, α-naphthylthiourea appears to cause some inhibition of 30S dynein and of axonemal ATPase at high concentrations (> 1 mM), but detailed studies have not been performed because of limited solubility.

It should be noted that for each of the compounds tested, at least a three-fold higher concentration is needed to cause enhancement of axonemal ATPase than of solubilized 30S dynein.

Effect of pH and Temperature on Reaction of Phenylthiourea With Dynein

Previous studies [15] have shown that the rates of enhancement of 30S dynein ATPase by sulfhydryl reagents such as N-ethylmaleimide are rapid and increase markedly with temperature and with pH. Comparison of the earlier data with the data presented in Figure 1 shows that the rate of enhancement of 30S dynein by phenylthiourea is much slower than the rate of enhancement by N-ethylmaleimide and is relatively insensitive to change in pH from 7.5 to 8.6. All subsequent studies reported in this paper were performed at pH 7.5.

The reaction of 14S dynein with phenylthiourea appears to be very fast and may be largely completed during the 20-min assay interval at 25°. At 25°, most of this loss is due to the acetone used to dissolve the phenylthiourea (Fig. 1). At 0°, the acetone contributes very little to the loss of activity during a 30-min preincubation.

The maximum enhancement of 30S dynein or of axonemal ATPase we have observed with thiourea or any of the substituted thioureas is ∿ 2.8-fold. Because this is less than we

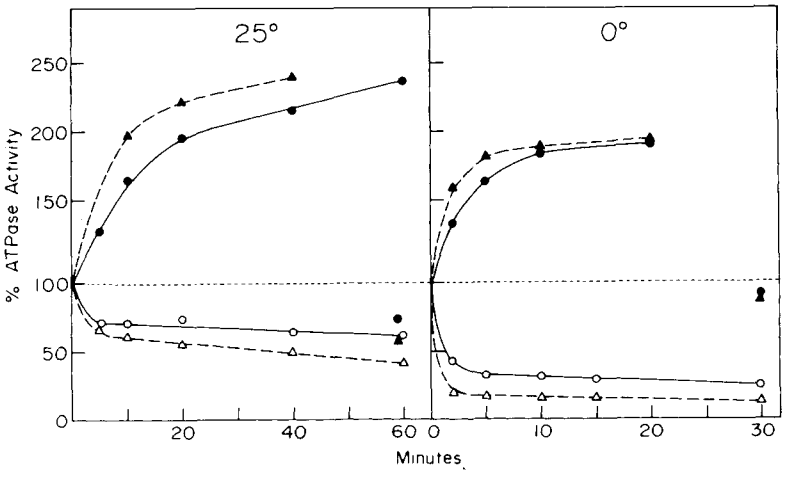

Fig. 1. Effect of phenylthiourea on 14S and 30S dynein ATPase. Dyneins were incubated for the times indicated on the abscissa with 0.44 mM (for experiments at 25°) or 1.11 mM phenylthiourea (for experiments at 0°) and the ATPase activity then assayed at 25° as described in Methods. Upper panels, 30S dynein; lower panels, 14S dynein; circles, pH 7.5; triangles, pH 8.6. 100% ATPase activity = 0.66 μmole/min·mg at pH 7.5 and 0.76 μmole/min·mg at pH 8.6 for 30S dynein; 1.14 μmole/min·mg at pH 7.5 and 1.33 μmole/min·mg at pH 8.6 for 14S dynein. The filled symbols in the lower panels show the effect of acetone alone on the ATPase activity at pH 7.5 (●) and 8.6 (▲).

consistently obtain with FNS, an experiment was performed in which 17 μM FNS and 4.4 mM phenylthiourea were incubated (separately) with 30S dynein for various times up to ∿18 h. The FNS caused a 4.5-fold enhancement of ATPase activity, whereas the peak enhancement cause by phenylthiourea was 2.6-fold.

Prevention of the Effect of Thiourea on Dynein ATPase by β-Mercaptoethanol

The upper panel of Figure 2 shows the results of an experiment in which phenylthiourea was preincubated with 30S dynein in the presence of varying concentrations of β-mercaptoethanol. In the absence of β-MSH, a 2.6-fold enhancement was observed. The presence of about 5 μM β-MSH was sufficient to give 50% protection against the effects of 4.4 mM phenylthiourea, and complete protection was observed with ∿90 μM β-mercaptoethanol. Similar results have been obtained with burimamide and thiourea. DTT appears to be as effective as β-mercaptoethanol; low concentrations of DTT afford complete protection against the inhibitory effect of 0.11 M thiourea and of 2 mM phenylthiourea on 14S dynein and the enhancing effects of thiourea on 30S dynein (data not shown). This behavior differs from that of FNS, where addition of 1 mM DTT increases the amount of enhancement of 30S dynein as compared to FNS alone.

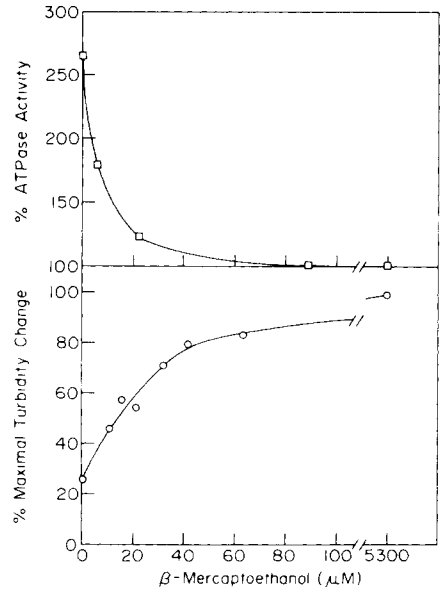

Fig. 2. Prevention of enhancement of 30S dynein ATPase and loss of turbidity change of axonemes by β-mercaptoethanol. Lower panel: Demebranated axonemes (71 μg) were preincubated with 4.2 mM phenylthiourea and the concentration of β-mercaptoethanol indicated on the abscissa for 15 min at 0° in a total volume of 0.95 ml. At the end of the preincubation the A_{350} was measured and 0.05 ml of mM ATP was added and the A_{350} measured again. The maximum turbidity change (no phenylthiourea) was 0.072, computed as described in Methods. Upper panel: 30S dynein was preincubated with 4.4 mM phenylthiourea for 30 min at 0° in the presence of the indicated concentrations of β-mercaptoethanol, and the ATPase activity was then assayed at 25° as described in Methods. 100% ATPase activity = 0.60 μmole/min·mg.

Effect of Thioureas on the ATP-induced Turbidity Change of Axonemes

Because thiourea was known to inhibit flagellar motility and the pellet height response of Tetrahymena axonemes, it was expected that the turbidity response of demembranated axonemes, due in large part to the sliding apart of the doublets [18], would also be inhibited by the thioureas. Panel A of Figure 3 shows the results of experiments in which axonemes were preincubated for 5 min at 25° with various concentrations of thioureas and then assayed for $\triangle A_{350}$ and for ATPase activity. Both thiourea and phenylthiourea inhibited the turbidity response, phenylthiourea being much more potent. Metiamide, at concentrations up to 5 mM, however, scarcely inhibited the turbidity response. Whereas thiourea caused a 2.5-fold increase in ATPase activity, phenylthiourea caused only a 1.4-fold and metiamide a 1.3-fold enhancement. Thus there was no evident correlation between the ability of these reagents to enhance ATPase activity and to inhibit the $\triangle A_{350}$ response. This point is made more forcefully by the data shown in panel B of Figure 3, where a very low concentration of thiourea (11 mM) caused a 1.4-fold enhancement of axonemal ATPase activity at 25° without causing any inhibition of $\triangle A_{350}$. The disparity was even greater with metiamide, where a 1.8-fold enhancement of ATPase activity was achieved without any loss of the turbidity response. Because the metiamide, and phenylthiourea, were dissolved in acetone, which was known to enhance dynein ATPase [12], it was of interest to examine the effects of acetone on the $\triangle A_{350}$. It can be seen that a

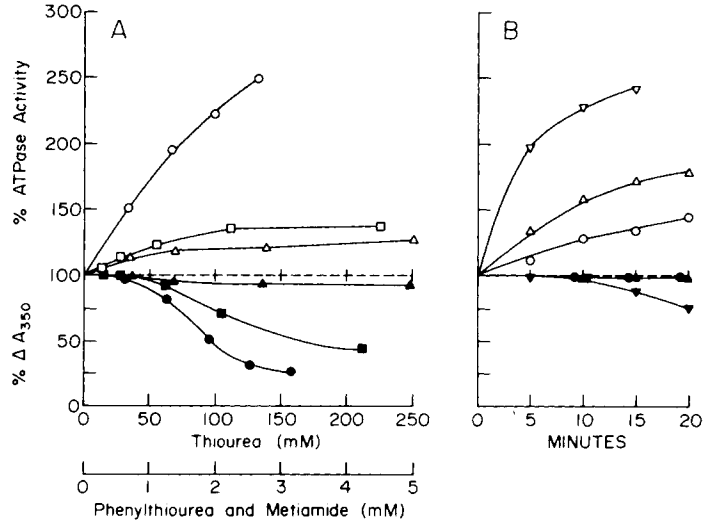

Fig. 3. Effect of some substituted thioureas on axonemal ATPase activity and on turbidity response. Panel A: Demembranated axonemes (∿0.10 mg) were preincubated with the indicated concentrations of reagent for 5 min at 25°. At the end of the preincubation, 0.1 ml 10 mM ATP was added and the ATPase activity was assayed for 20 min at 25° as described in Methods, or 0.05 ml of 1 mM ATP was added and the $\triangle A_{350}$ was determined as described in Methods. 100% ATPase activity corresponds to ∿100 nmoles/min·mg, and 100% turbidity response corresponds to a $\triangle A_{350}$ of 0.067. Panel B: Axonemes (∿0.07 mg) were preincubated at 25°with 11 mM thiourea, 5.6 mM metiamide, or 4.2 vol % acetone for the times indicated on the abscissa and the ATPase activity and $\triangle A_{350}$ was measured as described for A. Symbols used: thiourea, ○, ●; phenylthiourea, □, ■; metiamide, △, ▲; acetone, ▽, ▼.

15-min preincubation with 4.2% (v/v) acetone caused a 2.4-fold enhancement of ATPase activity but little loss of the turbidity response.

Because low concentrations of β-mercaptoethanol prevented the enhancement of ATPase by phenylthiourea (Fig. 2), it was of interest to establish whether inhibition of the turbidity response would also be protected. The lower panel of Figure 2 shows that preincubation of axonemes with 2.4 mM phenylthiourea for 15 min caused about a 75% loss of the turbidity response. If ∼25 μM β-MSH was present during preincubation, 50% protection against the loss of $\triangle A_{350}$ was obtained. Similar results have been obtained with thiourea. Thus low concentrations of β-MSH (and DTT) can prevent the effects of high concentrations of thiourea and phenylthiourea on the ATPase activity of 14S and 30S dyneins and on the turbidity response of axonemes.

The data in Figure 1 show that enhancement of 30S dynein by 1.1 mM phenylthiourea at 0° takes about 10 min. Even with 4.4 mM phenylthiourea it takes about 2 h to achieve nearly full (two-fold) enhancement of axonemal ATPase, and very little enhancement occurs during the first 15–30 min of incubation (data not shown). Thus not only is a larger concentration of phenylthiourea required to yield half-maximal enhancement of axonemal ATPase compared with 30S dynein in a 17-h incubation at 0° (Table I), but the rate of enhancement of ATPase in situ is much slower than the rate of enhancement of solubilized 30S dynein. Similar results have been obtained with thiourea and with metiamide.

Effect of Phenylthiourea and of Metiamide on Rebinding of 30S Dynein to EDTA-Extracted Axonemes

Because phenylthiourea caused a loss of the turbidity response of demembranated axonemes to ATP (Fig. 2), it was of interest to ascertain whether preincubation with phenylthiourea would interfere with rebinding of 30S dynein to axonemes that had been twice extracted with Tris-EDTA. The data in Table II show that neither phenylthiourea nor metiamide caused an appreciable reduction in the ability of 30S dynein to rebind to the axonemes. Since in these experiments the axonemes were also exposed to these reagents for 10 min, it is evident that the competency of the axonemes to rebind 30S dynein was not adversely affected by treatment with 4 mM phenylthiourea or 5 mM metiamide.

TABLE II. Effect of Phenylthiourea and Metiamide on the Rebinding of 30S Dynein to Extracted Axonemes

30S Dyenin	Pellet II		ATPase activity in supernatant (μmole/min·mg)	% Rebinding	% Dynein ATPase
+	–		0.680	–	100
–	+		0.040	–	–
+	+		0.155	79	–
+	–	(+ Metiamide)	1.37	–	200
+	+	(+ Metiamide)	0.287	80	–
+	–	(+ Phenylthiourea)	1.70	–	250
+	+	(+ Phenylthiourea)	0.459	72	–

30S dynein (20 μg) was preincubated with 5.0 mM phenylthiourea or 6.3 mM metiamide for 5 min at 0°, pH 7.5, as indicated, in a total volume of 0.8 ml, and then 0.2 ml of twice-extracted axonemes (pellet II, 0.56 mg) were added and the mixture was allowed to incubated for 10 min more. The tubes were then centrifuged at 0° and the supernatants were assayed for ATPase as described in Methods.

Reversibility of Effect of Thiourea and Phenylthiourea on the Turbidity Response

A number of experiments were performed in which thiourea was incubated with axonemes at 0° and at 25° for various times and 0.1 ml of the incubated mixture was transferred to a cuvette containing 0.85 ml buffer, the A_{350} was measured, and the $\triangle A_{350}$ measured upon addition of 0.05 ml of 1 mM ATP, as described in Methods. These experiments always showed much less inhibition of the $\triangle A_{350}$ response than identical experiments in which thiourea was also present in the buffer at the same concentration as during the preincubation. These experiments strongly suggested that the effect of thiourea on $\triangle A_{350}$ was reversible by dilution. Proof that this is so was obtained in experiments in which axonemes were incubated for 20 min at 25° with thiourea (115 and 230 mM) or phenylthiourea (3.1 and 6.2 mM) and 0.1 ml aliquots were taken for ATPase assay (ie, at one-tenth the concentration of drug). The remainder of each sample was washed by centrifugation at 0° and resuspended in fresh buffer. A sample of the washed axonemes was tested for ATPase and found to have the same enhanced activity (up to 1.7-fold for thiourea, 1.3-fold for phenylthiourea) as the unwashed axonemes. The same results were obtained if the washed axonemes were incubated with 1 mM DTT for 15 min at 25° before assay of ATPase activity. These axonemes were also tested for their turbidity response ($\triangle A_{350}$) to 0.1 mM ATP. The $\triangle A_{350}$ of untreated cilia was not changed by the washing procedure. Treatment of axonemes with thiourea or phenylthiourea for 4 min caused an appreciable inhibition of $\triangle A_{350}$, while pretreatment for 20 min caused an almost total inhibition of the turbidity response. If, however, the axonemes were washed free of the drugs and then assayed for $\triangle A_{350}$, there was a partial recovery of the $\triangle A_{350}$, and this was further increased if the washed cilia were incubated with DTT before the turbidity assay was performed. Thus the enhancement of axonemal ATPase activity by thiourea or phenylthiourea appears to be irreversible, whereas the inhibition of turbidity response is partially or almost completely reversible, depending on the concentration of drug used and whether the washed axonemes are incubated wtih DTT.

Protection by ATP and AMP-PNP Against the Enhancing Effects of Thiourea and Metiamide on 30S Dynein

The enhancement of ATPase activity that occurs when 30S dynein is preincubated with 6.3 mM metiamide for 30 min was completely prevented by 5 μM ATP and about 50% protection was obtained at 0.4 μM ATP (Fig. 4). Although we have have not studied the protection by ATP against all the other substituted thioureas in detail, we have shown that complete protection against the enhancing effects of burimamide and phenylthiourea can be obtained at 11 μM and 110 μM, respectively. With thiourea, very low concentrations of ATP give a partial protection against enhancement of ATPase activity, but even 90 μM ATP gave only slightly greater than 50% protection in the experiment shown in Figure 4, although somewhat higher protection has been obtained with other preparations of 30S dynein. Similar results were obtained with AMP-PNP, but at a 10 times higher concentration range. In a similar experiment (data not shown), 11 μM ATP gave almost complete protection against the enhancing effects of 56 mM thiourea during a 2.5 h preincubation, while 280 μM AMP-PNP gave almost 50% protection. Thus although the presence of a high-affinity binding site for ATP is evident for both thiourea and several thioureas, full protection against the enhancing effects of these substances is best observed with the substituted thioureas, perhaps because at the lower concentrations required there are fewer "nonspecific" effects.

The data in Figure 4 also show that neither ATP nor AMP-PNP gave any protection against the inhibitory effects of thiourea on 14S dynein ATPase. Similar studies (data not presented) showed that neither ATP nor AMP-PNP gave any protection against the inhibitory effect of phenylthiourea on 14S dynein. AMP-PCP did not protect against the enhancing (30S) or inhibiting (14S) effects of thiourea, nor did the potency of AMP-PCP as an ATPase inhibitor change if added after the enhancement of inhibition of ATPase activity by the thiourea.

DISCUSSION

The finding that relatively low (millimolar) concentrations of several substituted thioureas cause the same enhancement of 30S dynein ATPase and inhibition of 14S dynein ATPase as does a much higher ($\sim$ 100 mM) concentration of thiourea makes it virtually certain that these compounds act as specific reagents rather than as unspecific denaturants. A similar conclusion could be reached from data showing that inhibition of tyrosinase required about 5×10^{-3} M thiourea, 8×10^{-6} M α-naphthylthiourea, or 2.5×10^{-7} M phenylthiourea [11]. The latter data are compatible with the view that there is a strong hydrophobic region of tyrosinase which binds the substituted thioureas in the indicated order or that the various substituents on the thiourea increase the reactivity of the C=S bond in the indicated order. With dynein, however, the increase in potency was about 30-fold to 90-fold, ie relatively independent of the substituent on the thiourea. This suggests that the increased potency of the substituted thioureas for dynein (as compared to thiourea) may be due to a fairly nonspecific hydrophobic region at the site where the thioureas react. A similar situation may obtain for rat lung acetylcholinesterase, where

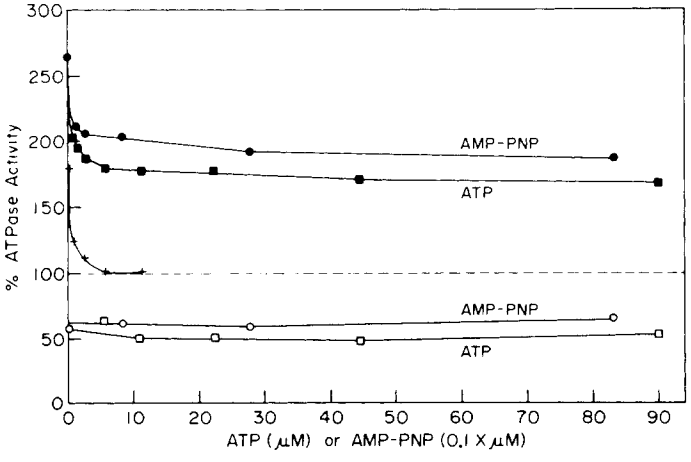

Fig. 4. Protection by ATP and by AMP-PNP against the enhancing effects of thiourea and of metiamide on 30S dynein ATPase. For the curves with filled symbols (●, ■) 30S dynein was preincubated with 0.11 M thiourea for 15 min at 0° in the presence of ATP or AMP-PNP at the concentrations indicated on the abscissa, and the ATPase activity was then assayed at 25° as described in Methods. For the curve with + symbols the 30S dynein was preincubated with 6.3 mM metiamide for 30 min in the presence of the indicated concentration of ATP. The curves with open symbols (○, □) are for 14S dynein preincubated with 0.11 M thioruea for 15 min.

α-naphthylthiourea and phenylthiourea ($K_I \sim 10^{-4}$ M) are about 10-fold to 100-fold more potent inhibitors than thiourea [19].

Although cysteine protects rats against lethal doses of α-naphthylthiourea, it neither prevents nor reverses the inhibition of tyrosinase by 10^{-6} M α-naphthylthiourea [11]. The effects of thiourea and of several substituted thioureas on 14S and 30S dyneins, however, were prevented by remarkably low concentrations of β-MSH or DTT, as was the inhibitory effect of the thioureas on the turbidity response of axonemes to ATP. The finding that β-MSH and DTT can prevent the effects of the thioureas strongly suggests that the thioureas are reacting with an SH (or –S–S) group(s) on the 30S dynein. Toennies [10] showed that dithioformamidine, the disulfide corresponding to thiourea, reacts with two molecules of cysteine to form cystine. Dithioformamidine can be formed by reaction of thiourea with H_2O_2. Although there are no known H_2O_2-generating systems present in axonemes or in solubilized dyneins, an experiment was performed in which the enhancement of axonemal ATPase by 4.4 mM phenylthiourea was measured in the presence of 10 μg catalase. The presence of the catalase did not alter the rate of enhancement of ATPase activity (data not presented). Experiments were also performed in which air was bubbled through a solution of thiourea (1 M) for 4 h and its effect on enhancing ATPase activity and inhibiting the turbidity response of axonemes was compared to a portion of untreated thiourea. There was a small increase (∼ 10–30%) in ability to cause enhancement of the ATPase activity, but no change in the ability to inhibit $\triangle A_{350}$. Furthermore, if a solution of thiourea (1 M) was left at room temperature for several days there was a reduction in potency as an inhibitor of $\triangle A_{350}$ but no change in potency as an enhancer of axonemal ATPase or of 30S dynein. The potency of phenylthiourea as an activator of axonemal ATPase was also not changed by storage at room temperature for 2 days. If oxidized glutathione (1.7 mM) was preincubated with axonemes for 15 min, it did not cause enhancement of ATPase activity, nor did it increase the inhibition of $\triangle A_{350}$ by thiourea that had been treated by bubbling air, although the oxidized glutathione by itself does cause a small inhibition of turbidity response. It therefore seems unlikely that the effective reagent was the disulfide form of the reagent. Toennies [10] showed that a reversible equilibrium occurs between cystine and thiourea on the one hand and cysteine and S-(guanylthio-) cysteine on the other. It is possible, therefore, that the thioureas are reacting with a S–S bond on the dynein, but further work is needed to establish the identity of the group(s) on dynein that react with the thioureas.

Inhibition of 14S dynein ATPase by the thioureas requires higher concentrations of these reagents than is required for enhancement of 30S dynein ATPase, and it occurs rapidly. In these respects the thioureas differ from the behavior of, eg, N-ethylmaleimide or p-phenylendimaleimide [15]. If the 14S dynein behaves in situ as it does when solubilized, this would imply that the slow enhancement of axonemal ATPase activity caused by the thioureas reflects a slow reaction of 30S dynein in situ.

A 1/V vs 1/[ATP] plot of axonemal ATPase activity yields straight lines, indicating the presence of high-affinity ($K_m \sim 1$ μM), and a low affinity, ($K_m \sim 12\mu$ M) ATP-binding sites [20]. The presence of a high-affinity binding site on solubilized 30S dynein was demonstrated by the ability of low concentrations of ATP to protect against the enhancing effects of FNS [21]. Low concentrations of ATP also protected against the enhancing effect of phenylthiourea on 30S dynein ATPase. For both FNS and the thioureas AMP-PNP is an effective protector (though less potent than ATP), but AMP-PCP, despite the

fact that it is a stronger inhibitor of ATPase activity than is AMP-PNP [21], does not confer any protection. This suggests that AMP-PCP binds differently than ATP to 30S dynein, possibly at the low-affinity ATP-binding site. This is consistent with the report [22] that AMP-PNP allows relaxation of axonemes in rigor whereas AMP-PCP does not. There is no evidence of the presence of a similar high-affinity binding site on 14S dynein, since ATP does not protect against the inhibitory effect of the thioureas on 14S dynein ATPase. The presence of a high-affinity binding site on 30S dynein was also evident when thiourea was the enhancing reagent. In this case, however, less than complete protection was afforded by ATP. Perhaps at the high concentrations of thiourea required for enhancement some other effect of the thiourea, not preventable by ATP, occurs.

The present experiments show that there are at least two effects of the thioureas on axonemes. One is an apparently irreversible enhancement of the ATPase activity of the 30S dynein, presumably by reaction at or near the high-affinity ATP-binding site which is localized at the distal end of the arms, facing the B-subfiber [23]. The other is a largely reversible inhibition of the turbidity response. Takahashi and Tonomura [20] have shown that the decrease in turbidity caused by ATP correlates with a detachment of the dynein arms from attachment sites on the B-subfibers, but a large part of the turbidity decrease may be due to sliding apart of the doublets, which occurs without trypsinization in Tetrahymena axonemes [18]. Inhibition of the turbidity response by the thioureas could indicate the presence of a thiourea-reactive site(s) at or near the B-subfiber attachment sites on the dynein arms. If the thiourea-reactive group(s) responsible for inhibition of $\triangle A_{350}$ are on the B-subfiber attachment sites, then the failure of phenylthiourea or metiamide to prevent rebinding of the dynein arms could merely imply that the arms are being rebound via the (thiourea-insensitive) attachment sites on the A-subfibers. If the thiourea-reactive sites are on the dynein arms, it seems unlikely that they are identical to the groups responsible for enhancement of the ATPase activity, not only because of the large differences in degree of reversibility of the ATPase-enhancement and the $\triangle A_{350}$ inhibition, but also because of the lack of correlation between degree of ATPase enhancement and degree of inhibition of $\triangle A_{350}$. Further work is necessary to establish the nature – ie, SH or S–S – and localization of thiourea-reactive groups in the axonemes.

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