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Regulation of the microtubule–lysosome interaction: activation by Mg^{2+} and inhibition by ATP

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We developed a sedimentation assay to characterize and quantify the association of purified lysosomes to reconstituted microtubules (Mithieux, G., Audebet, C. and Rousset. B. (1988) Biochim. Biophys. Acta 969, 121–130). In the present work, we have examined the potential regulatory role of ATP and Mg^{2+} on the microtubule–lysosome interaction. The formation of microtubule-lysosome complexes takes place in the absence of Mg^{2+}, but is activated by the addition of Mg^{2+}; both the rate of the interaction and the amount of complexes formed are increased. The maximal effect is observed between 1.5 and 3.5 mM free Mg^{2+}. Measured at the plateau of the interaction, the proportion of microtubules bound to lysosomes increases as a function of free Mg^{2+} concentration; at optimal concentration of free Mg^{2+}, 90% of the microtubules present in the incubation mixture are bound to lysosomes. ATP induces a concentration-dependent inhibition of the formation of microtubule-lysosome complexes. The half-maximal effect is obtained at an ATP concentration of 0.83 ± 0.11 mM ($n = 7$). The effect of ATP is not related to ATP hydrolysis, since ATP exerts its inhibitory action in the presence of EDTA. The ATP effect is mimicked by GTP, p[NH]ppA and tripolyphosphate, ADP and pyrophosphate, but not by AMP or phosphate. In the presence of 1 mM ATP, a Mg^{2+} concentration of 3 mM (corresponding to 2 mM free Mg^{2+}) is required to overcome the inhibition caused by ATP; above 3 mM, Mg^{2+} exerts its activating effect. Since the modulating effects of ATP and Mg^{2+} are obtained at concentrations closed to those occurring in intact cells, we conclude that the regulation of the microtubule–lysosome interaction reported in this paper could be of physiological significance.

Introduction

The intracellular distribution of subcellular organelles such as the Golgi apparatus [1–3], mitochondria [4,5] and lysosomes [6,7] has been shown to be strongly dependent on microtubule integrity, suggesting that, when not moving, organelles interact in a stable manner with microtubules. There is also increasing evidence, in various cell types, that interphase microtubules constitute the tracks supporting the intracellular movements of vesicles [8–15] among which are lysosomes [16–19].

Thyroid hormone secretion involves the intracellular motions of three types of vesicle which transport or process thyroglobulin, the prohormone: exocytotic vesicles, endocytotic vesicles and lysosomes. This secretory process is inhibited by anti-microtubular agents [20–22], suggesting

Abbreviations: Mes, morpholineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether) *N,N,N′,N″*-tetracetic acid; PPP_i, inorganic tripolyphosphate; p[NH]ppA, 5′-adenylyl imidodiphosphate.

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that vesicle motions could be microtubule-dependent. In a preceding paper, we developed a sedimentation method allowing us to monitor the association of lysosomes, purified from pig thyroid, with microtubules extracted from pig brain and repolymerized at steady state [23]. This method permits the separation of lysosomes and microtubule-lysosome complexes which sediment at 10000 $\times g$, from free microtubules and unpolymerized microtubule protein which remain in the soluble phase. We have shown that the association of microtubules to lysosomes is time- and temperature-dependent, is saturable and takes place without microtubule-associated proteins or cytosolic components. It was then decided to examine whether the adenosine 5′-triphosphate-magnesium (Mg-ATP) complex could modulate the association of microtubule to lysosomes. This was of particular interest in the light of numerous reports showing that movements of organelles along microtubules are energy-dependent processes coupled to ATP hydrolysis. The microtubule-translocating activity of kinesin, the better known cytosolic motor system for microtubule-based transport, has recently been correlated with ATPase activity [24]. In this work, using our sedimentation method, we have studied the effect of Mg-ATP, Mg-free ATP and free Mg^{2+} on the microtubule–lysosome interaction. We report that the formation of microtubule-lysosome complexes can be regulated in vitro by concentrations of ATP and Mg^{2+} in the range of those found in the in vivo situation.

Experimental procedures

Purification and labeling of microtubule protein. Microtubule protein was purified from pig brain using the temperature-dependent polymerization-depolymerization procedure of Shelanski et al. [25]. Twice-cycled microtubule protein was labeled by conjugation with the ^{125}I-Bolton-Hunter reagent. Polymerization competent-labeled microtubule protein was selected by an additional assembly-disassembly cycle, as previously described [23]. The specific radioactivity of the labeled proteins was 0.1–0.2 μCi/μg of microtubule protein.

Purification of thyroid lysosomes. Pig thyroid lysosomes were purified by subfractionation of thyroid vesicle fractions (800–26000 $\times g$) by isopycnic centrifugation on 30% Percoll gradients [26] prepared in hyper-osmotic medium (0.5 M sucrose/10 mM Tris-HCl, pH 7.4). This purification procedure yielded 20 mg of lysosomal protein per 100 g of fresh tissue with a 50 to 60-fold enrichment in lysosomal marker enzymes [23].

Quantitative assay of lysosome-microtubule complexes. Microtubule protein was mixed with ^{125}I-labeled microtubule protein in buffer A (100 mM Mes/0.5 mM $MgCl_2$/1 mM EGTA/0.1 mM GTP, pH 6.4) supplemented with 0.5 M sucrose and maintained for 30 min at 4° C. The mixture was then centrifuged at 50000 $\times g$ for 20 min to remove undepolymerized material. After addition of 1 mM GTP to the soluble microtubule protein, ^{125}I-labeled microtubules were assembled during 20 min of incubation at 37° C. Microtubules at steady state were sheared by five passages through a 25 gauge needle of a syringe and kept for 5 min at 25° C. The lysosome suspension was equilibrated at 25° C for 5 min and mixed with microtubules. Substances to be tested were added to the lysosome suspension just before mixing with microtubules. Interaction experiments were carried out at 25° C for various periods of time in a final volume of 200 μl of buffer A containing 0.5 M sucrose. At the end of the incubation period, the mixture was layered on 100 μl of buffer A containing 0.75 M sucrose in a 1 ml conical tube and centrifuged at 10000 $\times g$ for 5 min. Under these conditions, lysosomes and microtubule-lysosome complexes were found in the pellet, whereas free microtubules were recovered in the supernatant [23]. The supernatant and the cushion were removed and the pellet was counted for radioactivity in a Packard scintillation gamma counter. The amount of microtubules recovered in the pellet was calculated from radioactivity measurements and the specific radioactivity of the microtubule protein solution.

Measurement of assembled microtubule protein. To determine the amount of assembled microtubule protein under the different experimental conditions, incubation mixtures were centrifuged at 150000 $\times g$ for 60 min at 25° C. The supernatants (free tubulin) and the pellet (microtubules) were assayed for radioactivity and protein.

Other methods. Protein was assayed according to Lowry et al. [27] using bovine serum albumin as

a standard. GTP was assayed using the 3-phosphoglycerate kinase method [28].

Calculation of free Mg^{2+} concentration. Free Mg^{2+} concentrations were calculated using a computer-aided program taking into account the Mg^{2+} complexing species: EGTA, EDTA, ATP/GTP and ADP/GDP. Calculations were made using the following apparent association constants which were corrected for temperature (25°C), ionic strength (0.07 M) and pH (6.4): EGTA-Mg complex, $K_a \approx 9\ M^{-1}$; for EDTA-Mg complex, $K_a \approx 4.3 \cdot 10^4\ M^{-1}$; for ATP/GTP-Mg complex, $K_a \approx 5 \cdot 10^3\ M^{-1}$; for ADP/GDP-Mg complex, $K_a \approx 3.6 \cdot 10^2\ M^{-1}$.

The free Mg^{2+} concentration was calculated at the beginning of the incubation period assuming that GTP which has been hydrolyzed during the microtubule assembly process is in the form of GDP.

Results

In a first attempt to determine whether the association of microtubules with lysosomes could be modulated by the presence of Mg-ATP, we performed interaction experiments by adding 1 mM ATP and 2 mM $MgSO_4$ to buffer A (conditions used to activate microtubule-dependent translocation of organelles). Control experiments carried out in the presence of Mg-free ATP, or Mg^{2+} alone or EDTA were carried out in parallel. As reported previously [23], the kinetics of the microtubule–lysosome interaction presented three phases: a lag-period, a phase of association, during which the amount of complexes increased with time and a plateau, during which the amount of microtubules bound to lysosomes remained constant. We observed that the addition of Mg-ATP led to the inhibition of the interaction. The addition of ATP alone yielded a comparable effect; contrarily, Mg^{2+} alone increased both the rate of association and the amount of microtubules bound to lysosomes at the plateau (Fig. 1). In the presence of 5 mM EDTA, the amount of microtubules bound to lysosomes at the plateau slightly decreased. This first set of data led us to consider that Mg^{2+} and ATP could have distinct and opposite effects on the microtubule–lysosome interaction. The effects of ATP and Mg^{2+} alone or in various combinations have been further studied.

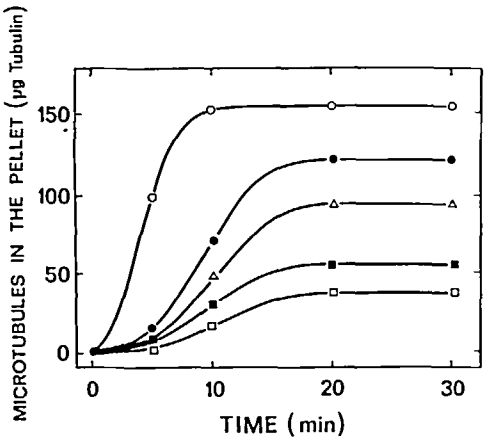

Fig. 1. Time course of the microtubule–lysosome interaction. ^{125}I-labeled microtubules (340 µg, 110 cpm/µg of tubulin) were incubated at 25°C in the presence of 0.6 mg/ml of lysosomes in a total volume of 200 µl. At the end of the incubation period, the amount of microtubules bound to lysosomes was determined as described under Experimental procedures. Each point is the mean of duplicate incubations of a representative experiment. Experimental conditions were as follows: buffer A (●); buffer A plus 5 mM EDTA (△), buffer A plus 1.5 mM $MgSO_4$ (○), buffer A plus 1 mM ATP (□), buffer A plus 1.5 mM $MgSO_4$ plus 1 mM ATP (■).

Effect of magnesium on the microtubule–lysosome interaction

The effects of increasing concentrations of Mg^{2+} are reported in Fig. 2. Results plotted at 0 Mg^{2+} were obtained by adding 5 mM EDTA to buffer A which contains 0.5 mM Mg^{2+}; under these conditions, the calculated free Mg^{2+} concentration was about 2 µM. Mg^{2+} increased both the rate of the reaction and the amount of microtubules bound to lysosomes at the plateau (Fig. 2A). The lag-phase decreased when the Mg^{2+} concentration increased. At high Mg^{2+} concentrations, the lag-phase disappeared provided the lysosome concentration was high (conditions of Fig. 2). At low lysosome concentrations, the lag-phase extended over 5–10 min and Mg^{2+} was unable to suppress it completely. The proportion of microtubules bound at the maximum of interaction, i.e., after 25 min of incubation (Fig. 2), increased from about 50% at 0 free Mg^{2+} concentration to 70% in the presence of 0.5 mM

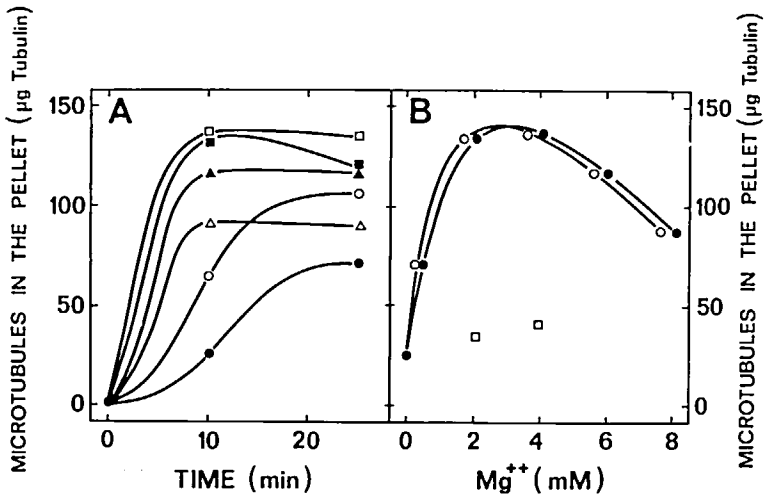

Fig. 2. Effect of Mg^{2+} on the formation of microtubule-lysosome complexes. ^{125}I-labeled microtubules (295 µg, 95 cpm/µg of tubulin), were incubated with lysosomes (0.7 mg/ml). Panel A: kinetics of the interaction performed in buffer A (○) or in buffer A supplemented with 5 mM EDTA (●) 1.5 mM $MgSO_4$ (■) 3.5 mM $MgSO_4$ (□) 5.5 mM $MgSO_4$ (▲) or 7.5 mM $MgSO_4$ (△). For clarity, the curves are interrupted at 25 min, but, whatever the experimental conditions, the amount of complexes did not augment after 25 min of incubation. All determinations were performed in duplicate. Panel B: plot of the amount of microtubules bound to lysosomes after 10 min of incubation as a function of total Mg^{2+} concentration (●) or free Mg^{2+} concentration (○). Zero Mg^{2+} values correspond to those obtained in the presence of 5 mM EDTA. The symbols (□) refer to the values obtained in the presence of 10 mM EDTA and the indicated total Mg^{2+} concentrations.

Mg^{2+} (conditions of buffer A corresponding to 0.32 mM free Mg^{2+}) and to 85–90% in the presence of 4 mM Mg^{2+} (i.e., 3.5 mM free Mg^{2+}). The plot of the amount of microtubules bound to lysosomes after 10 min of incubation as a function of the Mg^{2+} concentration shows that the Mg^{2+}

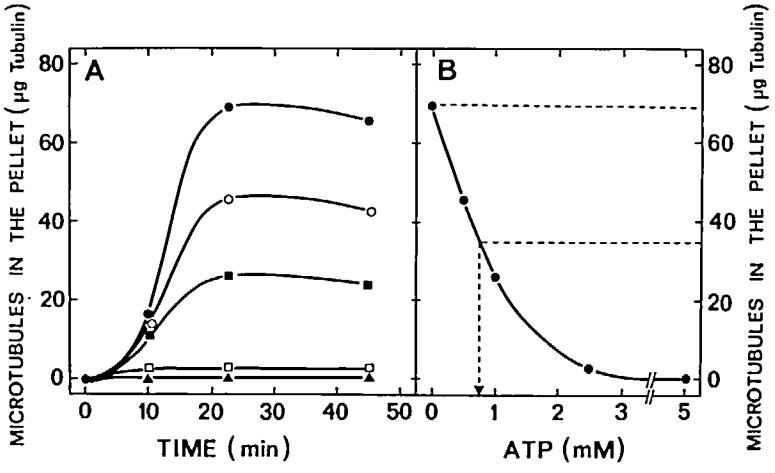

Fig. 3. Effect of ATP on the formation of microtubule-lysosome complexes. ^{125}I-labeled microtubules (280 µg, 90 cpm/µg of tubulin) were incubated with lysosomes at a concentration of 0.55 mg/ml. Panel A: kinetics of the formation of microtubule-lysosome complexes. Experiments were carried out in buffer A containing 5 mM EDTA and Mg-free ATP at the following concentrations: 0 (●); 0.5 mM (○); 1 mM (■); 2.5 mM (□) and 5 mM (▲). All determinations were made in duplicate. Panel B: plot of the amount of microtubules found in the complex at the plateau versus ATP concentration. The concentration of ATP inducing half-maximal inhibition is represented by the dashed lines.

activation was maximal at concentrations ranging from 2 to 4 mM total Mg^{2+} or 1.7 to 3.5 mM free Mg^{2+}. At a concentration higher than 4 mM, the Mg^{2+} effect declined so that the concentration-response curve exhibited a bell shape (Fig. 2B). Addition of 10 mM EDTA reversed the effects of 2 and 4 mM Mg^{2+} by 95% and 85%, respectively (Fig. 2B). Neither EDTA nor Mg^{2+} (whatever the concentration was, up to 8 mM) induced any modification of the proportion of assembled microtubules which represented about 50% of the total microtubule protein after 30 min of incubation with lysosomes; it was also checked that in the absence of lysosomes, Mg^{2+} did not induce any increase of microtubules in the $10000 \times g$ pellet.

Effect of adenosine 5'-triphosphate on the microtubule–lysosome interaction

In order to block the activation caused by Mg^{2+} present in buffer A, experiments were carried out in the presence of 5 mM EDTA. Mg-free ATP caused a concentration-dependent inhibition of the interaction (Fig. 3A). The plot of the amount of microtubules bound to lysosomes at the maximum of interaction versus ATP concentration is shown in Fig. 3B. The half-maximal inhibitory effect occurred at an ATP concentration of 0.83 ± 0.11 mM ($n = 7$), 0.75 mM in the experiment reported in Fig. 3. The effect of ATP was also observed when added at the plateau of the interaction; ATP induced the dissociation of preformed microtubule–lysosome complexes. This action of ATP was very rapid; it occurred in less than 5 min, and the amount of microtubules remaining bound to lysosomes was equal to that observed when ATP was added at the beginning of the incubation (data not shown).

The fact that ATP exerts its inhibitory effect of the presence of EDTA indicates that the ATP-induced inhibition was not related to ATP hydrolysis, but rather to a process of recognition without transformation of the molecule. In order to identify which part of the ATP molecule was essential in this process, we tested the effect of structural analogs, among which the non-hydrolyzable ATP analog: p[NH]ppA. Analogs were tested at two concentrations, 1 and 5 mM; at these concentrations, a molecule with the same potency as ATP should inhibit the formation of microtubule-lysosome complexes by about 50% and almost 100%. p[NH]ppA, ADP and GTP, although slightly less effective than ATP, induced a concentration-dependent inhibition of the microtubule–lysosome interaction measured at the plateau (Table I). Contrarily, AMP had a slight inhibitory effect which was not concentration-dependent. The ATP effect was thus obtained with nucleotides with at least two phosphates. The following step has been to test whether polyphosphate molecules related to ATP, tripolyphosphate (PPP_i); to ADP, pyrophosphate (PP_i) and to AMP, orthophosphate (P_i) could mimick the ATP effect. We have found that (1) PPP_i was as effective as ATP at a concentration of 5 mM and even more at 1 mM; (2) PP_i was as effective as ATP at 1 mM, but rather less so at 5 mM; (3) P_i was without effect (Table I). None of these molecules, except PPP_i (see legend Table I), induced a significant depolymerizing effect on microtubules in the conditions of the interaction.

TABLE I

EFFECT OF ATP ANALOGS AND POLYPHOSPHATES ON THE MICROTUBULE-LYSOSOME ASSOCIATION

Experiments were carried out under the same conditions as those described in the legend to Fig. 3. All measurements were made at the maximum of the interaction. Data are expressed as the mean ± S.D. of values obtained from two to five different experiments. In each case, the amount of microtubules bound to lysosomes under control conditions was standardized to 100%. Adenosine could not be tested, because it induced the lysis of lysosomes under the conditions used in this study. The proportion of microtubules (usually 50% of total microtubule protein at the maximum of interaction) was decreased by up to 25% in the presence of 5 mM PPP_i. This effect was taken into account in the calculations to obtain a true comparison with controls.

	Microtubules bound to lysosomes (% of control)	
	1 mM	5 mM
ATP	40 ± 5	2 ±2
p[NH]ppA	66.5± 5	28 ±2
GTP	68.5±10	15.5±1.5
ADP	74 ± 4	11 ±9
AMP	85 ± 2	82 ±3
PPP_i	22 ± 2	1.5±1
PP_i	34.5± 1	11 ±1.5
P_i	81 ± 2.5	79 ±0.5

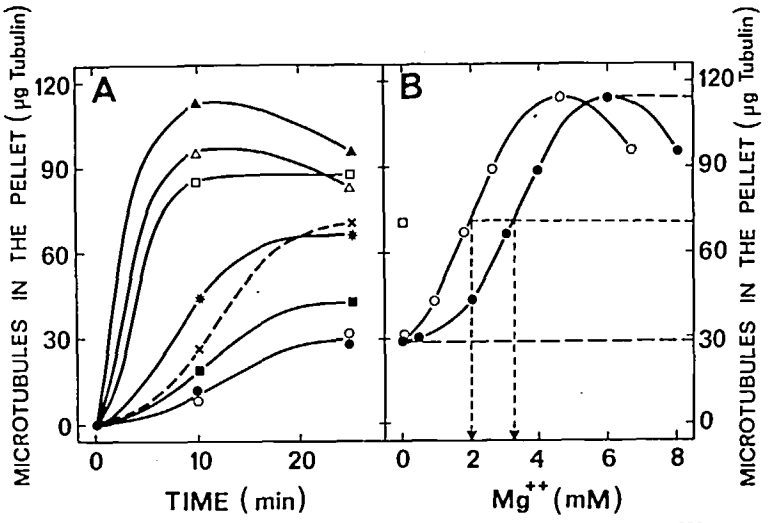

Fig. 4. Effect of Mg^{2+} on the microtubule–lysosome interaction in the presence of 1 mM ATP. ^{125}I-labeled microtubules (270 µg, 125 cpm/µg microtubule protein) were incubated with lysosomes at a concentration of 0.52 mg/ml. Panel A: time-course of the microtubule-lysosome interaction. Experimental conditions were: buffer A containing 1 mM ATP (○), buffer A containing 1 mM ATP and 5 mM EDTA (●), 1.5 mM $MgSO_4$ (■), 2.5 mM $MgSO_4$ (★), 3.5 mM $MgSO_4$ (△), 5.5 mM $MgSO_4$ (▲) or 7.5 mM $MgSO_4$ (□). The dotted line refers to a control experiment carried out in the absence of ATP and in the presence of 5 mM EDTA. The maximum of the interaction was reached in all cases after 25 min of incubation. Determinations were made in duplicate. Panel B: plot of the amount of microtubules as microtubule-lysosome complexes at the maximum of the interaction versus total Mg^{2+} concentration (●) or free Mg^{2+} concentration (○). □ refers to the experiment performed in the absence of ATP and the presence of EDTA. The dotted lines refer to the determination of the half-maximal activation effect of Mg^{2+}.

Action of Mg^{2+} on the ATP-induced inhibition of the microtubule-lysosome association

We have examined whether Mg^{2+} could reverse the ATP inhibitory action. In order to do so we studied the effects of increasing concentrations of Mg^{2+} on the response to 1 mM ATP, a concentration of physiological relevance in numerous cells. The results are reported in Fig. 4. At concentrations up to 2 mM (about 1 mM free), Mg^{2+} had little or no effect on both the rate or the extent of the interaction measured in the presence of 1 mM ATP. Between 2 and 6 mM total Mg^{2+}, i.e., 1 and 4.6 mM free Mg^{2+}, the ion exerted a concentration-dependent effect on the two parameters. The half-maximal effect of Mg^{2+} on the formation of microtubule-lysosome complexes in the presence of ATP was obtained at about 3.2 mM total Mg^{2+}, corresponding to a concentration of free Mg^{2+} of about 2 mM (Fig. 4B). Under these conditions, the amount of microtubules bound to lysosomes was similar to that obtained under basal conditions (no ATP, no Mg^{2+}). Therefore, a total Mg^{2+} concentration of 3 mM was able to surpass the inhibition caused by 1 mM ATP. The same type of experiment has been performed with 2 mM ATP instead of 1 mM, very comparable results were obtained except that the total concentration of Mg^{2+} required to cancel the ATP inhibition was between 5 and 6 mM (data not shown).

Discussion

In our preceding paper [23], we reported that purified thyroid lysosomes bind to microtubules in a complete acellular system, the rate of the reaction and the amount of complex formed being analyzed by a sedimentation assay using ^{125}I-labeled microtubules. In the present study, using the same method, we present evidence for a distinct and opposite regulatory role of ATP and Mg^{2+}.

ATP exhibits a concentration-dependent inhibitory effect on the microtubule–lysosome interaction. This effect is not related to a hydrolysis phenomenon. Several observations support this

statement. First, ATP exerts its inhibitory effect in the absence of Mg^{2+} (experiments performed in the presence of 5 mM EDTA). Therefore, ATP inhibits the formation of microtubule-lysosome complexes under conditions where ATP is in a non-hydrolyzable form. Second, the ATP effect is reproduced by structural analogs which are not substrates of ATPases, i.e., p[NH]ppA, ADP, PP_i or PPP_i. These findings, together with the fact that AMP and P_i do not mimick the ATP action, indicate that a molecule with binding site(s) for diphosphate residues plays a key role in the microtubule–lysosome interaction. In accordance with this proposal, a limited structural modification of the terminal diphosphate of ATP such as that occurring in 5′-adenylyl imidodiphosphate (p[NH]ppA) alters the potency of the molecule. It is worth noting that the ATP concentration which induces half-maximal inhibition of the reaction is very close to the ATP concentration, about 1 mM, which is observed in numerous cells, among which is the thyroid cell [29].

Unexpectedly, Mg^{2+} activates the microtubule–lysosome interaction both in the absence and in the presence of ATP. Results from experiments carried out at different Mg^{2+}/ATP ratios show that the concentration of Mg^{2+} necessary to obtain a given amount of complexes is higher when ATP is present. The difference is not solely due to the reduction of the free Mg^{2+} concentration as the result of the complexation of Mg^{2+} by ATP. Indeed, in the absence of ATP, the half-maximum effect of Mg^{2+} was obtained at 0.5 mM free Mg^{2+}, whereas in the presence of 1 mM ATP, the half-maximum effect of Mg^{2+} occurred at 2 mM free Mg^{2+}, a concentration yielding the maximum activation in the absence of ATP. It seems difficult to estimate the respective activating or inhibiting power of free Mg^{2+}, free ATP and Mg-ATP, since the three species coexist in all cases, however we may think that the ratio of Mg^{2+} to ATP could play a regulatory role in the association of lysosomes to microtubules. As mentioned for ATP, Mg^{2+} concentrations exerting a regulatory role, under our experimental conditions, and the expected intracellular Mg^{2+} concentrations are comparable. The intracellular concentration of Mg^{2+} in thyroid cells should be close to 4 mM [30]. Consequently, small physiological fluctuations of the Mg^{2+} and ATP concentrations are expected to modulate the association of lysosomes to microtubules in a very efficient manner.

The simplest interpretation of the effects of ATP and Mg^{2+} and of the counterbalance effect of increasing the Mg^{2+} concentration in the presence of ATP would be the positive charges of the magnesium ions and the negative charges on phosphate residues of ATP respectively enhance or prevent an electrostatic-mediated interaction between microtubules and lysosomes. However, several observations do not fit with this explanation: (i) p[NH]ppA was not as potent as ATP, although exhibiting the same negative charges; (ii) AMP and P_i were ineffective even though a proportion of about 60% exhibits two negative charges at pH 6.4 and (iii) equimolar amounts of ATP and Mg^{2+} had the same effect as Mg^{2+}-free ATP, whilst, under these conditions, at least 50% of the charges on ATP molecules are cancelled by Mg^{2+} complexation. It is thus probable that mechanisms other than charge effects contribute to the observed effects of ATP and Mg^{2+}.

Considering the concentration-dependent effects of Mg^{2+} and nucleotides on one hand, and the concentrations of Mg^{2+} and GTP of buffer A on the other, it is apparent that the initial conditions under which the formation of microtubule-lysosome complexes were evidenced [23] were rather favorable. The concentration of GTP, which is partly hydrolyzed before the interaction (by the microtubule assembly process) varied from 0.2 to 0.3 mM at time zero. Therefore, GDP should be present at a concentration close to 0.2–0.3 mM. At these concentrations, GTP and GDP had no measurable inhibiting effect on the interaction. The calculated concentration of free Mg^{2+} under these conditions is about 0.3 mM. This concentration of free Mg^{2+} induces a significant activation of the microtubule-lysosome association.

There are now numerous examples of ATP-induced dissociation of complexes between microtubules and soluble molecular species [31–33], between vesicles and soluble factors [34,35], between microtubules and secretory granules [36] and between microtubule-vesicle complexes and soluble polypeptides [37]. It seems difficult to compare our results with those obtained in other reconstituted systems, because of the variety of

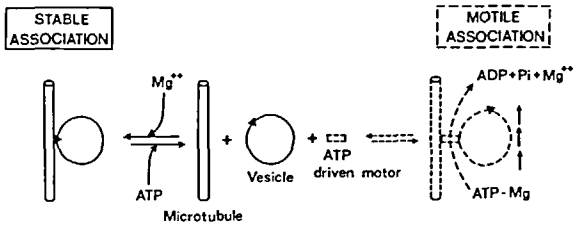

Fig. 5. Hypothetical scheme integrating an ATP/Mg^{2+} modulation of a stable microtubule–vesicle interaction and an ATP-driven microtubule based translocation. The drawings in solid lines are directly derived from this study, those in dashed lines are hypothesized from the literature.

organelles and tissues studied and of the presence or the absence of cytoplasmic elements. However, considering that the interaction of microtubules with lysosomes takes place without microtubule-associated proteins and cytosolic components [23], one may postulate that some membrane proteins have the capacity to bind directly to microtubules to form stable microtubule-lysosome complexes and that a regulatory binding site(s) for ATP or other nucleotides exists either on the same protein or on another protein complexed to it.

We have tried to reconcile our findings with the very numerous data related to the microtubule-based translocation of vesicles. According to Fig. 5, we would like to propose that a given vesicle could be subjected to two different types of interaction with microtubules: 'stable' association or motile association depending on the intracellular concentration of regulatory components such as ATP, Mg^{2+}, ATP/Mg^{2+} ratio.... Considering the ATP/Mg^{2+} ratio as a regulation parameter, the formation of stable microtubule-vesicle complexes would be favored at a low ATP/Mg^{2+} ratio. Conversely, an increase in the ATP/Mg^{2+} ratio would lead to the dissociation of stable-microtubule-vesicle complexes and promote motile association mediated by the translocation machinery requiring ATP hydrolysis for energy supply [38]. Finally, our work has shed some light on a particular aspect of microtubule functions which is to determine the positioning of organelles inside the cells. The clustering of lysosomes [19] close to the centrosome, a region characterized by a high density of microtubules, could be dependent upon the formation of 'stable' lysosome-microtubule complexes.

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