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Biochemistry

Cooperative binding of ATP and MgADP in the sulfonylurea receptor is modulated by glibenclamide

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ABSTRACT **The ATP-sensitive potassium (K_{ATP}) channels in pancreatic β cells are critical in the regulation of glucose-induced insulin secretion. Although electrophysiological studies provide clues to the complex control of K_{ATP} channels by ATP, MgADP, and pharmacological agents, the molecular mechanism of K_{ATP}-channel regulation remains unclear. The K_{ATP} channel is a heterooligomeric complex of SUR1 subunits of the ATP-binding-cassette superfamily with two nucleotide-binding folds (NBF1 and NBF2) and the pore-forming Kir6.2 subunits. Here, we report that MgATP and MgADP, but not the Mg salt of γ-thio-ATP, stabilize the binding of prebound 8-azido-[α-^{32}P]ATP to SUR1. Mutation in the Walker A and B motifs of NBF2 of SUR1 abolished this stabilizing effect of MgADP. These results suggest that SUR1 binds 8-azido-ATP strongly at NBF1 and that MgADP, either by direct binding to NBF2 or by hydrolysis of bound MgATP at NBF2, stabilizes prebound 8-azido-ATP binding at NBF1. The sulfonylurea glibenclamide caused release of prebound 8-azido-[α-^{32}P]ATP from SUR1 in the presence of MgADP or MgATP in a concentration-dependent manner. This direct biochemical evidence of cooperative interaction in nucleotide binding of the two NBFs of SUR1 suggests that glibenclamide both blocks this cooperative binding of ATP and MgADP and, in cooperation with the MgADP bound at NBF2, causes ATP to be released from NBF1.**

ATP-sensitive potassium (K_{ATP}) channels play many important roles in various tissues by linking the cell metabolic state to the membrane potential (1, 2). The K_{ATP} channels in pancreatic β cells are critical in the regulation of glucose-induced insulin secretion (3–6). Electrophysiological studies of the complex control of K_{ATP} channels by ATP and MgADP provide the current model of glucose-induced insulin secretion in which the subsequent metabolism of glucose transported through glucose transporters increases ATP and decreases MgADP in the pancreatic β cells. The increase in the ATP:ADP ratio closes the K_{ATP} channels, thereby depolarizing the β cell membrane, and leads to the opening of voltage-dependent Ca^{2+} channels, allowing Ca^{2+} influx into the β cells. The resultant rise in the intracellular concentration of Ca^{2+} triggers insulin release. The pancreatic β cell K_{ATP} channels are also the site of action of important therapeutic pharmacological agents such as sulfonylureas and K^+-channel openers. Sulfonylureas, commonly used in the treatment of non-insulin-dependent diabetes mellitus, stimulate insulin secretion by closing the K_{ATP} channels, whereas K^+-channel openers inhibit insulin secretion by opening the K_{ATP} channels (7).

The pancreatic β cell K_{ATP} channel is a complex of four SUR1 subunits of the ATP-binding cassette (ABC) superfamily with two nucleotide-binding folds (NBF1 and NBF2) and four Kir6.2 subunits of the inwardly rectifying K^+-channel family (8, 9). SUR1 is thought to mediate the stimulatory effect of MgADP and is the primary target for pharmacological agents, such as the sulfonylurea glibenclamide and the K^+-channel opener diazoxide. The primary site of ATP inhibition of K_{ATP}-channel activity seems to be in Kir6.2 (10, 11). However, the regulation of the β cell K_{ATP} channels by adenine nucleotides and pharmacological agents is complex. In addition to the inhibitory effect, MgATP enhances β cell K_{ATP}-channel activity (12, 13). ADP also has both stimulatory and inhibitory effects (14, 15). Although the interaction of sulfonylureas with SUR1 abolishes the stimulatory effect of MgADP on K_{ATP} channels (10), the sensitivity of the K_{ATP} channel to sulfonylureas is increased in the presence of MgADP (16–18).

Previously, we found that SUR1 strongly binds 8-azido-ATP at NBF1, whereas MgADP is bound at NBF2, and that preincubation of SUR1 with MgADP efficiently inhibits 8-azido-ATP binding to SUR1 (ref. 19; Fig. 1*A*, preincubation procedure). Because this inhibitory effect of MgADP was reduced by mutations in NBF2, we thought the MgADP bound at NBF2 might facilitate MgADP binding at NBF1 and thereby prevent 8-azido-ATP binding to NBF1. The strong 8-azido-ATP binding to SUR1 made it possible to investigate the biochemical basis of such cooperative interaction. Here, we provide direct biochemical evidence, obtained by postincubation procedure, of the cooperative interaction in nucleotide binding of the two NBFs of SUR1. In addition, we show that the sulfonylurea glibenclamide modulates this cooperative interaction in nucleotide binding of the two NBFs of SUR1.

MATERIALS AND METHODS

Materials. The 8-azido-[α-^{32}P]ATP and 8-azido-[γ-^{32}P]ATP were purchased from ICN.

Reaction of SUR1 with Nucleotides (Postincubation Procedure). Membrane proteins from COS-7 cells expressing SUR1, prepared as described (19), were incubated with 10 μM 8-azido-[α-^{32}P]ATP or 8-azido-[γ-^{32}P]ATP as described in the legend for Fig. 1*B*.

RESULTS

Effects of MgADP and MgATP on the Prebound 8-Azido-[^{32}P]ATP. When SUR1 was incubated with 8-azido-[α-^{32}P]ATP in the presence of Mg^{2+} (Fig. 1*B*, postincubation procedure), 8-azido-[α-^{32}P]ATP continued to bind to SUR1 for 15 min at 0°C, but they dissociated gradually at 37°C (Fig. 2). In the presence of MgADP, 8-azido-[α-^{32}P]ATP remained tightly bound to SUR1 for 15 min at 37°C. When SUR1 was incubated with ADP or ATP in the absence of Mg^{2+} after

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Abbreviations: ABC, ATP-binding cassette; ATPγS, γ-thio-ATP; CFTR, cystic fibrosis transmembrane conductance regulator; K_{ATP}, ATP-sensitive potassium; NBF, nucleotide-binding fold.

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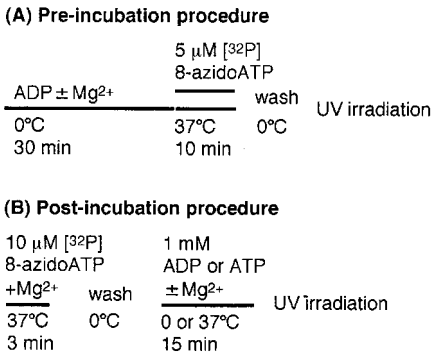

FIG. 1. Schematic diagrams of the reaction of SUR1 with nucleotides. (*A*) Preincubation procedure (19). Membrane proteins from COS-7 cells expressing hamster SUR1 were first incubated with 10–500 μM ADP in 5 μl of buffer containing 40 mM Tris·HCl (pH 7.5), 3 mM $MgSO_4$, and 0.1 mM EGTA (+Mg TE) or in 5 μl of the same buffer without $MgSO_4$ (−Mg TE) for 30 min at 0°C. Next, 8-azido-[α-^{32}P] ATP was added to the mixture to a final concentration of 5 μM, and the mixture was incubated for 10 min at 37°C. The reactions were stopped by the addition of 500 μl of ice-cold +Mg or −Mg TE, and free 8-azido-[^{32}P]ATP was removed after centrifugation (15,000 × *g* for 5 min at 2°C). Pellets were washed in the same buffer and resuspended in 10 μl of ice-cold +Mg TE. The mixture was irradiated for 5 min (at 254 nm, 5.5 mW/cm^2) on ice. (*B*) Postincubation procedure. Membrane proteins from COS-7 cells expressing SUR1 were incubated with 10 μM 8-azido-[α-^{32}P]ATP or 8-azido-[γ-^{32}P]ATP in 2.5 μl of +Mg TE containing 2 mM ouabain for 3 min at 37°C. The reactions were stopped, and free 8-azido-[^{32}P]ATP was removed as above. Pellets were resuspended in 5 μl of +Mg or −Mg TE containing 2 mM ouabain. Membrane proteins were then mixed with 5 μl of +Mg or −Mg TE containing 2 mM ADP or ATP. The mixture was incubated for 15 min at 0°C or 37°C and irradiated on ice. Samples were electrophoresed on an SDS/7% polyacrylamide gel and autoradiographed. The trapped 8-azido-[^{32}P]ATP in SUR1 was measured by scanning with a radioimaging analyzer (BAS2000, Fuji). Experiments were done at least in triplicate.

preincubation with 8-azido-[α-^{32}P]ATP, 8-azido-[α-^{32}P]ATP dissociated from SUR1 in 15 min. About 80% of prebound 8-azido-[α-^{32}P]ATP dissociated from SUR1 in 5 min in the absence of Mg at 37°C (data not shown), showing that 8-azido-[α-^{32}P]ATP readily dissociates from SUR1 in the absence of Mg^{2+} at 37°C. Accordingly, MgADP, by binding to one NBF, stabilizes prebound 8-azido-[α-^{32}P]ATP binding to the other NBF of SUR1. MgATP stabilizes prebound 8-azido-[α-^{32}P]ATP binding to SUR1 with slightly less efficiency, compared with MgADP. In contrast, an ATP analogue that hydrolyzes slowly, ATPγS, had no greater stabilizing effect than Mg^{2+} alone. The effects of MgADP and MgATP on the stabilization of prebound 8-azido-[α-^{32}P]ATP binding to SUR1 were concentration-dependent (maximal effects at 0.5 mM for both; Fig. 3), suggesting that MgADP, either by direct binding or by hydrolysis of bound MgATP, stabilizes 8-azido-ATP binding. When SUR1 was incubated with MgADP or MgATP after preincubation with 8-azido-[γ-^{32}P]ATP, SUR1 was photoaffinity-labeled as efficiently as it was with 8-azido-[α-^{32}P]ATP (Fig. 4), indicating that prebound 8-azido-[γ-^{32}P]ATP is not hydrolyzed during incubation with MgADP or MgATP.

Effects of Mutations in NBF2 on Photoaffinity Labeling. K_{ATP}-channel activation requires both NBFs of SUR1 to be functional (20, 21). To clarify the role of NBF2 in the stabilization of nucleotide binding, the effects of mutations in NBF2 on photoaffinity labeling were examined by a postincubation procedure. Mutations in the Walker A and B motifs, K1385M and D1506N, abolished the stabilizing effects of MgADP on 8-azido-ATP binding, although they had almost no effect on 8-azido-ATP (10 μM) binding overall (Fig. 5). Mutations at the corresponding sites in other ABC proteins reduce MgATP

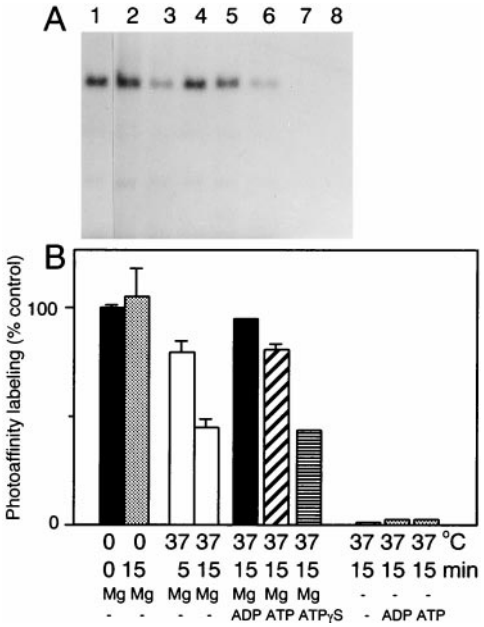

FIG. 2. Cooperative binding of MgADP and 8-azido-[α-^{32}P]ATP. Membrane proteins from COS-7 cells expressing SUR1 were preincubated with 10 μM 8-azido-[α-^{32}P]ATP. (*A*) After free 8-azido-[α-^{32}P]ATP was removed, proteins were photoaffinity-labeled immediately (lane 1) or after incubation with 3 mM $MgSO_4$ for 15 min at 0°C (lane 2), with $MgSO_4$ for 15 min at 37°C (lane 3), with $MgSO_4$ and 1 mM ADP (lane 4), with $MgSO_4$ and 1 mM ATP (lane 5), with $MgSO_4$ and 1 mM γ-thio-ATP (ATPγS) (lane 6), without $MgSO_4$ or nucleotide (lane 7), or with 1 mM ADP (lane 8) for 15 min at 37°C as described for Fig. 1*B* (postincubation procedure). (*B*) Photoaffinity labeling is expressed as the percentage of control (photoaffinity-labeled immediately) of those shown in *A*, lane 1.

binding at low concentrations (<10 μM), but not at high concentrations (≈1 mM), and abolish ATP hydrolysis (22, 23). These results suggest that SUR1 binds 8-azido-ATP strongly at NBF1 and that MgADP, by binding at NBF2, stabilizes prebound 8-azido-ATP binding at NBF1. Because MgADP had maximal effect on wild-type SUR1, it is unlikely that ATP hydrolysis is required to stabilize 8-azido-ATP binding. MgADP binding to NBF2 more likely induces a conforma-

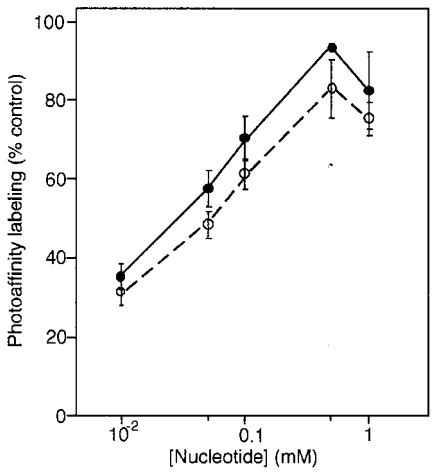

FIG. 3. Effects of ADP and ATP on photoaffinity labeling. Membrane proteins from COS-7 cells expressing SUR1 were preincubated with 10 μM 8-azido-[α-^{32}P]ATP. After free 8-azido-[α-^{32}P]ATP was removed, proteins were photoaffinity-labeled after incubation with 3 mM $MgSO_4$ and ADP (●) or with $MgSO_4$ and ATP (○) for 15 min at 37°C. Photoaffinity labeling is expressed as percentage of control (photoaffinity-labeled immediately).

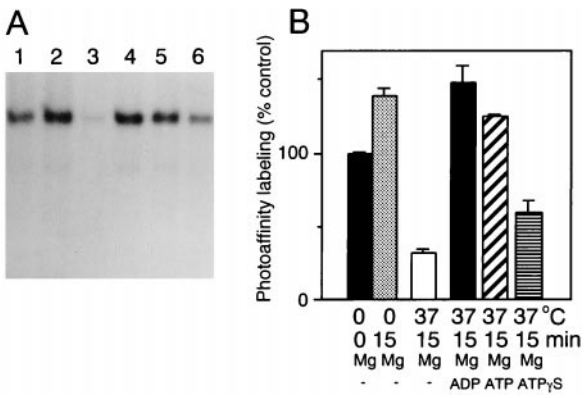

FIG. 4. Cooperative binding of MgADP and 8-azido-[γ-^{32}P]ATP. Membrane proteins from COS-7 cells expressing SUR1 were preincubated with 10 μM 8-azido-[γ-^{32}P]ATP. (*A*) After free 8-azido-[γ-^{32}P]ATP was removed, proteins were photoaffinity-labeled immediately (lane 1) or after incubation with 3 mM $MgSO_4$ for 15 min at 0°C (lane 2), with $MgSO_4$ for 15 min at 37°C (lane 3), with $MgSO_4$ and 1 mM ADP (lane 4), with $MgSO_4$ and 1 mM ATP (lane 5), or with $MgSO_4$ and 1 mM ATPγS (lane 6) for 15 min at 37°C as described for Fig. 1*B* (postincubation procedure). (*B*) Photoaffinity labeling is expressed as percentage of control (photoaffinity-labeled immediately) of those shown in *A*, lane 1.

tional change at NBF2 that transduces another conformational change in NBF1 to stabilize ATP binding at NBF1. Mutations in NBF2 could well impede these changes.

Effects of Glibenclamide on Photoaffinity Labeling. Although glibenclamide in the presence of Mg^{2+} alone did not reduce photoaffinity labeling, glibenclamide reduced it in the presence of MgADP or MgATP in a concentration-dependent manner (Fig. 6). Because glibenclamide does not affect 8-azido-[α-^{32}P]ATP binding to NBF1 directly in the preincubation procedure (data not shown), it could block interaction between ATP binding at NBF1 and MgADP binding at NBF2 and thereby dissociate 8-azido-ATP from NBF1. Interestingly, 1 μM glibenclamide dissociated 8-azido-ATP from NBF1 more efficiently in the presence of MgATP or MgADP than in the presence of Mg^{2+} alone. Accordingly, glibenclamide may not only block the transduction that stabilizes ATP binding at NBF1 but also, in cooperation with MgADP at NBF2, may cause the release of ATP from NBF1.

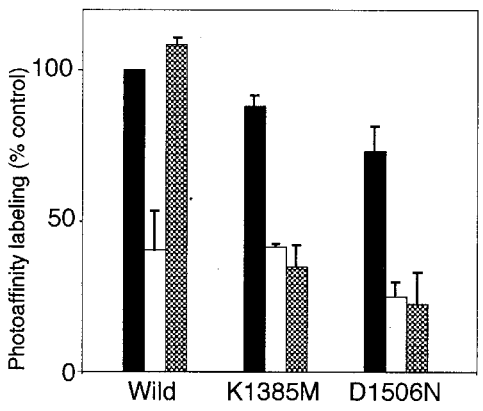

FIG. 5. Photoaffinity labeling of the wild-type SUR1 and the K1385M and D1506N mutants of SUR1. Membrane proteins from COS-7 cells expressing SUR1 were preincubated with 10 μM 8-azido-[α-^{32}P]ATP. After free 8-azido-[α-^{32}P]ATP was removed, proteins were photoaffinity-labeled immediately (black bars), after incubation with 3 mM $MgSO_4$ (white bars), or with $MgSO_4$ and 0.5 mM ADP (shaded bars) for 15 min at 37°C. Photoaffinity labeling is expressed as percentage of control (photoaffinity-labeled immediately) of the wild-type SUR1.

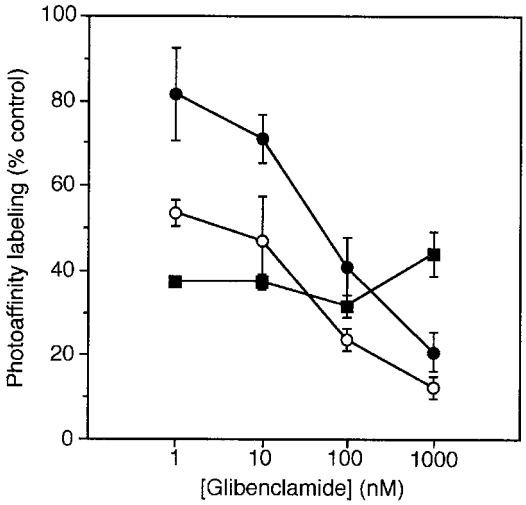

FIG. 6. Interaction between glibenclamide and MgADP or MgATP on the photoaffinity labeling of SUR1. Membrane proteins from COS-7 cells expressing SUR1 were preincubated with 10 μM 8-azido-[α-^{32}P]ATP. After free 8-azido-[α-^{32}P]ATP was removed, proteins were incubated with 3 mM $MgSO_4$ (□), with $MgSO_4$ and 0.5 mM ADP (●), or with $MgSO_4$ and 0.5 mM ATP (○) in the presence of various concentrations of glibenclamide for 15 min at 37°C. Proteins were then photoaffinity-labeled. Photoaffinity labeling is expressed as percentage of control (photoaffinity-labeled immediately).

DISCUSSION

We previously reported that SUR1 strongly binds 8-azido-ATP at NBF1, whereas MgADP is bound at NBF2, and that preincubation of SUR1 with MgADP efficiently inhibits 8-azido-ATP binding to SUR1 (19). In the present study, we determine that MgADP, by binding at NBF2, stabilizes prebound 8-azido-ATP at NBF1. These findings suggest both that SUR1 strongly and stably binds ATP at NBF1 and that there is positive interaction between the two NBFs of SUR1. In our previous study, SUR1 was preincubated with MgADP (Fig. 1*A*); in this study, the MgADP bound at NBF2 may have facilitated MgADP binding at NBF1 and thereby inhibited 8-azido-[^{32}P]ATP binding to NBF1. In the present study, SUR1 was first incubated with 8-azido-[^{32}P]ATP (Fig. 1*B*). SUR1 was then postincubated with MgADP, after the removal of free 8-azido-[^{32}P]ATP. By this procedure, SUR1 was photoaffinity-labeled with only the 8-azido-[^{32}P]ATP prebound at NBF1 and continuously bound during postincubation; otherwise, the free unlabeled nucleotides would compete with the dissociated and diluted 8-azido-[^{32}P]ATP.

The interaction of sulfonylureas with SUR1 abolishes the stimulatory effect of MgADP on K_{ATP} channels (10). However, the sensitivity of the K_{ATP} channel to sulfonylureas is increased more in the presence of MgADP than in its absence (16–18). MgATP, like MgADP, is able to stimulate the activity of K_{ATP} channels containing a mutation (R50G) in Kir6.2 that impairs ATP inhibition (13). The degree of MgADP activation of K_{ATP} channels is greater than that of MgATP activation, which occurs over the same concentration range (0.1–1 mM). Both of these effects are abolished, however, when mutations are made in the NBFs of SUR1 (13). Our biochemical findings that glibenclamide abolishes the potentiating action of MgADP on photoaffinity labeling and that the inhibitory action of glibenclamide is increased in the presence of MgADP are consistent with these electrophysiological findings.

The present study thus shows that positive interaction between the two NBFs of SUR1 is important for K_{ATP}-channel regulation. Cooperative interaction of the two NBFs of ABC proteins has been shown for P-glycoprotein (22–24) and for the cystic fibrosis transmembrane conductance regulator (CFTR;

refs. 25 and 26). Both of the NBFs of P-glycoprotein and of CFTR seem to hydrolyze ATP and alternate in catalysis (25–27). For P-glycoprotein, the roles of the two NBFs may be equivalent (27), but the cooperativity between the two NBFs is very strong; a mutation or modification in either of the two NBFs in P-glycoprotein blocks ATP hydrolysis by the other, intact NBF (23). However, in CFTR, the roles of the two NBFs may not be equivalent; NBF1 is thought to be involved in channel opening, whereas NBF2 is thought to be involved in channel closing (25, 26). Cooperativity between the two NBFs is induced by phosphorylation of the regulatory domain by protein kinase A, which is also necessary for channel opening (26). However, CFTRs bearing a mutation in either of the two NBFs still function as channels even though the gating kinetics are altered (25, 26).

We propose the following model of nucleotide activation of the K_{ATP} channel through the SUR1 subunit (Fig. 7). Channel activation is induced when SUR1 binds ATP in NBF1 and MgADP in NBF2 (state 1). When the intracellular MgADP concentration decreases, MgADP dissociates from NBF2 (state 2). MgADP interacts with NBF2 either by direct binding or by hydrolysis of bound MgATP (state 3). States 1 and 2 are states of equilibrium, and most of the SUR1 subunits will be in state 1 at high MgADP concentrations and in state 2 at low MgADP concentrations. MgADP dissociation from NBF2 leads to instability of ATP binding at NBF1, allowing the release of ATP (state 4). This dissociation of ATP from NBF1 may be involved in channel inactivation of the K_{ATP} channels seen in excised patches. Under physiological conditions, the intracellular ATP concentration is high enough, and ATP bound to NBF2 is hydrolyzed readily to ADP. Consequently, SUR1 is in states 1, 2, or 3. The amount of the photoaffinity-labeled SUR1 (probably in state 1) was directly proportional to the concentration of MgADP from 10 μM to 1 mM (Fig. 3). These results suggest that the intracellular concentration of MgADP is the primary factor determining the alteration between the active state (state 1) and the inactive state (state 2) of SUR1. ATP binding at NBF1 is required for the action of SUR1, and the intracellular concentration of ATP also could affect the equilibrium. SUR1 in state 2 or 3 cannot induce channel activation and allows the Kir6.2 subunits to close. ATP is also predicted to bind preferentially to Kir6.2 in the closed state (28). Importantly, glibenclamide may well convert an active state of SUR1 directly into the inactive state by dissociating ATP from NBF1 (state 5). Glibenclamide causes the release of ATP from NBF1, in cooperation with MgADP at NBF2. The active state of SUR1 (state 1) has similarities to that proposed for CFTR (26, 29), although MgADP binds at NBF1 whereas ATP binds at NBF2.

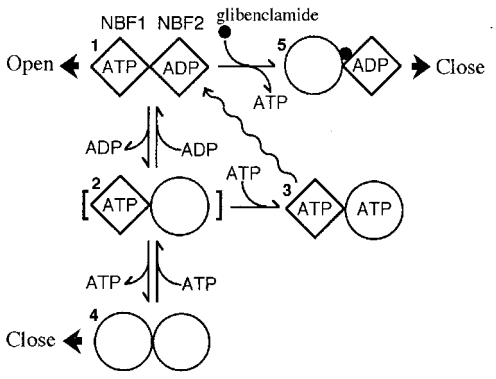

FIG. 7. Model for nucleotide activation of the K_{ATP} channel by SUR1 subunit. NBFs in the inactive state are depicted as circles; those with bound nucleotide and altered conformation are depicted as diamonds. State 1 is the active state in which SUR1 potentiates channel activity. States 2 and 3 are transient, and states 4 and 5 are inactive states in which SUR1 inhibits channel activity. The straight arrows indicate binding or unbinding reactions, and the wavy arrows indicate a hydrolytic reaction. The Mg^{2+} ion has been omitted for clarity. This model is modified from one in ref. 6.

When membrane was incubated for 15 min in the presence of Mg^{2+} at 0°C or in the presence of MgADP or MgATP at 37°C, the efficiency of photoaffinity labeling with 8-azido-[γ-^{32}P]ATP was increased significantly (Fig. 4). This result suggests that the conformational change in NBF1 transduced by MgADP binding to NBF2 could stabilize ATP binding at NBF1. Mg^{2+} binding to NBF2 might also transduce a conformational change in NBF1 to some extent, because Mg^{2+} alone only weakly stabilizes 8-azido-ATP binding at NBF1 (Fig. 2) and because photoaffinity labeling was increased significantly in the presence of Mg^{2+} at 0°C (Fig. 4).

In conclusion, the two NBFs of SUR1 show strong cooperativity in nucleotide binding. Indeed, K_{ATP}-channel activation may be induced primarily by the cooperative interaction of ATP binding at NBF1 and MgADP binding at NBF2. The intracellular concentration of MgADP is the primary factor in the determination of the active state of SUR1, although ATP is required for the action of SUR1. The direct biochemical investigation of the mechanism of action of glibenclamide on SUR1 also shows that sulfonylureas act in cooperation with MgADP at NBF2. Such cooperative interaction of the two NBFs is also important in the function of other ABC proteins, such as P-glycoprotein and CFTR (22–26), and thus deserve investigation as an appropriate target for various therapeutic drugs. The K_{ATP} channels in other tissues, which comprise Kir6.2 subunits and other SUR subunits, have differing sensitivities to sulfonylureas and K^+-channel-opening drugs (30, 31). Other ABC proteins may also be involved in the regulation of certain K^+ channels (32, 33). Variations in the cooperativity of the two NBFs in ABC proteins could account for the differences in function of the various ABC proteins as transporters, ion channels, or ion-channel regulators.

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