Assembly, Maturation, and Turnover of $K_{ATP}$ Channel Subunits*

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ATP-sensitive $K^+$, or $K_{ATP}$, channels are comprised of $K_{IR6.6}$ and sulfonylurea receptor (SUR) subunits that assemble as octamers, $(K_{IR}/SUR)_8$. The assembly pathway is unknown. Pulse-labeling studies show that when $K_{IR6.2}$ is expressed individually, its turnover is biphasic; $60\%$ is lost with $t_{1/2} \approx 36$ min. The remainder converts to a long-lived species ($t_{1/2} \approx 26$ h) with an estimated half-time of 1.2 h. Expressed alone, SUR1 has a long half-life, $25.5$ h. When $K_{IR6.2}$ and SUR1 are co-expressed, they associate rapidly and the fast degradation of $K_{IR6.2}$ is eliminated. Based on changes in the glycosylation state of SUR1, the half-time for the maturation of $K_{ATP}$ channels, including completion of assembly, transit to the Golgi, and glycosylation, is $2.2$ h. Estimation of the turnover rates of mature, fully glycosylated SUR1 associated with $K_{IR6.2}$ and of $K_{IR6.2}$ associated with Myc-tagged SUR1 gave similar values for the half-life of $K_{ATP}$ channels, a mean value of $7.3$ h. $K_{ATP}$ channel subunits in INS-1 $\beta$-cells displayed qualitatively similar kinetics. The results imply the octameric channels are stable. Two mutations, $K_{IR6.2}$ W91R and SUR1 $\Delta F1388$, identified in patients with the severe form of familial hyperinsulinemic hypoglycemia (16, 17). Nonsense and splice-site mutations that truncate SUR1 result in loss of functional channels at the cell surface in patient $\beta$-cells and retention of truncated SUR1 in the ER (4, 18). Missense mutations have been identified that result in loss of stimulation by MgADP (19–22), and other mutations are reported to affect trafficking to the cell surface as a consequence of improper folding or possibly by altering interactions with the ER retention motifs (23–25). One general suggestion has been that retention in the ER provides additional time for completion of channel assembly. $K_{IR6.2}$ subunits lacking the -RKR- motif can assemble channels in the absence of a SUR, and these will reach the surface indicating SUR is not obligatory for assembly of the channel pore. The assembly pathway is unknown, and at what stage SUR interacts with $K_{IR}$ is a matter of conjecture. Similarly, the lifetimes of the individual subunits, mutant subunits, and of the channel complex have not been reported.

We have used $^{35}$S[Met/Cys] pulse-labeling methods to determine the lifetimes of SUR1 and $K_{HR6.2}$ alone, to examine the effect of two severe hyperinsulinemic mutations on subunit lifetime, and to follow the maturation of channels by monitoring changes in the glycosylation state of SUR1. The results show that when SUR1 is expressed in the absence of the inward rectifier it is long-lived in contrast, for example, to CFTR, which has a half-life of $\approx 30$ min in the ER (26, 27). When $K_{HR6.2}$ is expressed in the absence of SUR1, a fraction ($\approx 60\%$) degrades rapidly, but the remainder is converted to a long-lived species. When $K_{HR6.2}$ and SUR1 are co-expressed, complexes form quickly and the rapid degradation of $K_{HR}$ is eliminated. In the complex, SUR1 undergoes a time-dependent change in its glycosylation state as the channel transits to the cell surface. Two loss-of-function mutations, $\Delta F1388$ in SUR1 and W91R in $K_{HR6.2}$, both drastically decrease subunit lifetime irrespective of whether they are co-assembled with their partner subunit.

EXPERIMENTAL PROCEDURES

Molecular Biology and Plasmid Construction—$K_{HR6.2}$ and SUR1 proteins were modified by addition of extracellular Myc tags. The details of

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¶ The abbreviations used are: ER, endoplasmic reticulum; ABC, ATP-binding cassette; SUR, sulfonylurea receptor; CFTR, cystic fibrosis transmembrane regulator.
construction of the hamster pECE SUR1, pECE SUR1Myc, and human pECE K_{IR}6.2 have been described (24, 25).

Cell Culture and Transient Transfection—COS m6 cells were cultured in Dulbecco’s modified Eagle’s medium, 4.5 g/liter glucose, supplemented with 10% fetal bovine serum. Approximately 7 × 10^{6} cells were transfected with 8 μg of the pECE SUR1 and/or 1 μg of the pECE K_{IR}6.2 plasmids by electroporation (BioRad Laboratories, Inc.) following the manufacturer’s directions (950 μF, 2.20 V in 0.4-mm cuvettes) in RPMI medium supplemented with 10% fetal bovine serum and 1.25% MeSO. The average efficiency of transfection was 7—10%, estimated by co-transfection with a green fluorescent protein-marker plasmid. There was no significant difference in transfection efficiency when multiple plasmids were used. Pulse-chase experiments were carried out 18 h after transfection.

INS-1 β-cells, line 832/13 (29) (a kind gift from Dr. Christopher Newgard), were grown in RPMI 1640 with 11 mm glucose, supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 10 μM HEPES, 2 mM t-glutamine, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol.

Pulse-Chase and Immunoprecipitation Protocol—Cells were transfected and seeded in 6-well plates in duplicate at 70—80% confluence. The following day cells were incubated for 2 h in t-methionine/t-cysteine-free media (Invitrogen) at 37 °C with 5% CO_{2}. Subsequently, cells were labeled for 60 min with a mixture of [^{35}S]methionine/cysteine (200 μCi/ml; EaseTag™ mix, PerkinElmer Life Sciences) and then incubated with pre-warmed Dulbecco’s modified Eagle’s medium supplemented with 5 mM unlabeled t-methionine/t-cysteine. At the indicated times, wells were washed twice with cold phosphate-buffered saline, collected, and then lysed on a rotating wheel for 6 h at 20 °C in 1 ml of 1% digitonin (Sigma) in phosphate-buffered saline plus cysteine and serine protease inhibitors (Roche Applied Science). Lysates were clarified at 9000 × g for 15 min at 4 °C. The supernatants were pre-absorbed for 1 h with protein-G plus and then incubated with anti-Myc or anti-K_{IR}6.2 (N-18) antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4 °C followed by a 3-h incubation with protein-G-agarose (Santa Cruz Biotechnology). The agarose beads were washed five times with modified lysis buffer (2.5% SDS, 0.1 M dithiothreitol, 0.06 M Tris base, 20% glycerol, 0.008 M EDTA, pH 6.8) added to the beads. Proteins were separated on 7.5% or 10% SDS-polyacrylamide gels (30). Gels were stained, fixed in 50% methanol, 10% acetic acid, then treated with Fluorenhance (Amersham Biosciences) for 30 min, dried, and exposed to either x-ray film (Hyperfilm; Eastman Kodak Co.) at 80 °C for 17 h or for 30 min (26, 27), with only 20–40% estimated to leave the ER. The mature, fully glycosylated CFTR at the cell surface is longer-lived, reported t_{1/2} values of 7.5–16 h (26, 27). P-glycoprotein (MDR1), another ABC protein, has reported half-lives of >24 h, whereas mutants have been described with t_{1/2} values of ~3 h (33–35).

Note on Sample Preparation—The mobility of SUR1 on SDS-polyacrylamide gels is dependent on its glycosylation state; the immature or unglycosylated form displays an apparent molecular mass of 150–160 kDa, whereas the mature, fully glycosylated form of SUR1 has a molecular mass of 170 kDa (Fig. 1, A and B). Because maturation of membrane glycoproteins occurs in the medial Golgi apparatus (reviewed in Ref. 31), the mobility difference is a marker for trafficking of SUR1 (4, 8). The immature receptor turns over slowly in the ER (Fig. 2, A and B). The turnover of K_{IR}6.2 expressed alone is markedly different from SUR1. Turnover is biphasic with ~60% of the [^{35}S]Met/Cys-labeled subunits being rapidly degraded with a half-life ~35.7 ± 11.8 min. The remaining 40% turns over slowly with a half-life ~26.1 ± 8.2 h (n = 3). K_{IR}6.2 can assemble functional tetrameric pores in the absence of SUR that will reach the cell surface if their ER retention sequences are altered or deleted (36–38). Thus we propose that the biphasic decay of the K_{IR}6.2 reflects a difference in the rates of degradation of unassembled subunits versus assembled pores. We used this two-state assumption and the estimated degradation rates to model the turnover assembly process. The estimated half-life for conversion of a K_{IR}6.2 monomer into a stable species, assumed to be a tetramer, is ~1.2 h. The solid line in Fig. 2B was calculated using these parameters.

FIG. 1. Heating in SDS alters the mobility of SUR1. COS m6 cells were transfected with Myc-tagged SUR1 with or without K_{IR}6.2, photolabeled with [^{35}S]labeled glibenclamide, and subjected to immunoprecipitation as described. The immunoprecipitates were solubilized in a modified SDS sample buffer and either heated (95 °C, 5 min) or held at room temperature. Fig. 1, left panel shows the effect of heating solubilized immunoprecipitates containing Myc-tagged SUR1. The right panel shows a similar effect for fully glycosylated SUR1 present when Myc-tagged SUR and K_{IR}6.2 subunits are co-expressed. Additionally, the lower right panel confirms an earlier report on the reduced recovery of K_{IR}6.2 following heating (32). The appearance of the heated Myc-tagged SUR1 samples is not affected by treatment with endoglycosidases. These samples were identified by photo labeling with [^{35}S]labeled glibenclamide, but equivalent results were obtained using [^{35}S]Met/Cys-labeled subunits and wild type SUR1. The addition of 0.1 μM unlabeled glibenclamide (glib) eliminates photolabeling.

was done using the non-linear routines in Origin Pro (OriginLabs Corp., Northampton, MA). The results are given as mean ± S.D.
Fig. 2. Turnover of individual K<sub>ATP</sub> channel subunits. A, in the absence of K<sub>ir</sub>6.2, SUR1 is long-lived. COS m6 cells transfected with Myc-tagged SUR1 were pulse-labeled with [35S]methionine/cysteine for 60 min and then processed as described under “Experimental Procedures” using anti-Myc antibodies. A single exponential function was fit to the data, given as the fraction remaining. The $t_{1/2} = 25.5 \pm 4.4$ h ($n = 6$). B, the same protocol, with anti-K<sub>ir</sub>6.2 antibodies, was used to analyze the turnover of K<sub>ir</sub> subunits in the absence of SUR. The results show that turnover is biphasic with ~60% of the subunits being rapidly lost. A double exponential function was fit to the data. The $t_{1/2}$ values were 35.7 ± 11.8 min and 26.1 ± 8.2 h, respectively ($n = 3$). The dotted lines are the calculated values for the rapid and slow turnover fractions, respectively. The solid line was calculated using the estimated half-lives and a half-life for conversion of monomer-to-tetramer of 1.2 h obtained from a two-state simulation. The gels are representative figures; the $t_{1/2}$ values are averages from the indicated numbers of experiments. The times shown are 0, 0.5, 1, 2, 5, 10, 21, 24, and 30 h. Values are mean ± S.D.
assembly and maturation of K_{ATP} channels in COS m6 cells co-transfected with both SUR1 and K_{IR}6.2. In principle, using two antibodies should provide information on the assembly of SUR1 with K_{IR}6.2 complexes and vice versa. The results using the anti-K_{IR} antibodies are shown in Fig. 3A. Co-expression with SUR1 markedly affects the turnover of K_{IR}6.2. The rapid decay, evident in Fig. 2B when K_{IR}6.2 is expressed alone, is missing. The amount of K_{IR}6.2 in the immunoprecipitates increases over the first 60 min and then decays with a half-life of $-3.5 \pm 4$ h, providing one measure of channel lifetime. The result implies SUR1 must rapidly associate with and stabilize the early assembly intermediates (Fig. 3A). This interpretation is supported by the observation that K_{IR}6.2 and SUR1 co-immunoprecipitate at the earliest time point, increasing in amount for $-2$ h (compare first bands in Fig. 3, A and B). In addition, K_{IR}6.2-SUR1 complexes were detectable after brief (10 min) pulse-labeling periods (data not shown). As shown in Fig. 3B, there is essentially no detectable fully glycosylated SUR1 present at the earliest times, but the mature receptor becomes evident within 2 h, peaking at $-10$ h. Because the immunoprecipitation was carried out with anti-K_{IR}6.2 antibodies, SUR1 must be associated with K_{IR}6.2. The appearance of the mature receptor as the core species disappears is consistent with a precursor-product relationship. The estimated half-life for conversion to mature SUR1 is $-2.2 \pm 0.14$ h, based on fitting a single exponential (Fig. 3B, dotted line) to thedisappearance of the immature form of the receptor beginning with the 2-h time point. The estimated half-life of the channel complex, based on fitting a single exponential to the mature SUR1 data, beginning with the 10-h time point, is $-5.9 \pm 1$ h. This is in reasonable agreement with the $-8.5 \pm 4$ h estimate for K_{IR}6.2 because the K_{IR} number will include contributions from any subunits not assembled with SUR1.

The results for co-immunoprecipitation of Myc-tagged SUR1 and K_{IR}6.2 with anti-Myc antibodies are shown in Fig. 4. Consistent with the anti-K_{IR}6.2 immunoprecipitation result (Fig. 3A), K_{IR}6.2 does not turn over rapidly when associated with SUR1. There is a progressive increase in the amount of K_{IR}6.2 precipitating with SUR1, with a peak at $-5$ h, consistent with slow sequential assembly. The estimated channel half-life, based on the lifetime for K_{IR}6.2 associated with SUR1, is $7.8 \pm 1.1$ h. The precursor-product relation between immature and mature SUR1 is apparent with nearly the same timing (half-life for conversion of core-to-complex SUR1 is $-2.6 \pm 0.6$ h). The estimated channel half-life, based on fitting a single exponential to the mature SUR1 data, is $6.9 \pm 0.2$ h. The ratio of immature SUR1-to-K_{IR}6.2 at the end of the $35^S$Met/Cys pulse ($t = 0$) is higher than that observed for immunoprecipitation with anti-K_{IR}6.2 (9.5 versus 2.9, respectively), implying SUR1 is in excess under these transfection conditions. This idea is supported by comparison of the later time points in the SUR1 intensity data in Figs. 3B and 4B. The maturation of SUR1, marked by conversion to the mature form, is essentially complete in less than 20 h for SUR1 associated with K_{IR}6.2 (Fig. 3B), whereas the immature form remains readily detectable after 30 h in the anti-Myc immunoprecipitates (Fig. 4B). The result is consistent with there being non-K_{IR}6.2-associated SUR1 in the ER that turns over slowly as shown in Fig. 1A.

**Increased Rates of Turnover for Two K_{ATP} Channel Mutants**—A number of mutations in either SUR1 or K_{IR}6.2 are known to cause familial hyperinsulinism (10, 39, 40); several have been shown to affect trafficking, reportedly as a consequence of improper folding. We have used the $35^S$Met/Cys pulse-chase immunoprecipitation protocol to examine the turnover of two mutant subunits, SUR1 $\Delta F1388$ and K_{IR}6.2 W91R, expressed alone or with their respective partner. The $\Delta F1388$ mutation is in the second nucleotide binding domain of SUR1 and has been identified as a common cause of familial hyperinsulinism in Ashkenazi populations (41). The W91R mutation substitutes an arginine for the second in a pair of tryptophan residues near the top of K_{IR}6.2 (10). In KcaA, this tryptophan pair is in the pore helix and is thought to contribute significantly to the stability of the tetramer (42). Both mutations are associated with severe forms of hyperinsulinism.

Fig. 5 compares the turnover of K_{IR}6.2 W91R versus wild type K_{IR}6.2 subunits. K_{IR}6.2 W91R is degraded more rapidly in either the presence or absence of SUR1. The conversion to a long-lived species is missing in the mutant. Co-immunoprecipitation experiments show that K_{IR}6.2 W91R subunits do associate with SUR1, but this association does not appear to slow degradation significantly. The estimated half-lives for the K_{IR}6.2 W91R subunits in the absence and presence of SUR1 are 26.4 $\pm 2.4$ min ($n = 2$) versus 34.0 $\pm 2.6$ min ($n = 2$). These values are not significantly different from those determined for the fastest component observed when wild type K_{IR}6.2 is expressed in the absence of SUR1 (35.7 $\pm 11.8$ min; Fig. 2B). The results are consistent with the hypothesis that the W91R subunits are unable to assemble into stable tetramers and turn over rapidly as free monomers. To support this idea, we expressed Myc-tagged K_{IR}6.2 with either K_{IR}6.2 W91R or wild type K_{IR}6.2 and then determined the levels of W91R versus wild type K_{IR}6.2 in anti-Myc immunoprecipitates (Fig. 6). The Myc tag does not substantially alter the properties of K_{IR}6.2-SUR1 channels but has a greater molecular mass and serves to identify the “carrier” subunits in the immunoprecipitates. K_{IR}6.2 W91R is degraded more rapidly than wild type K_{IR}6.2, being nearly undetectable after 5 h, whereas the turnover of wild type K_{IR}6.2 parallels that of the Myc-tagged K_{IR}6.2 subunits (Fig. 6).

The turnover of SUR1 $\Delta F1388$ is dramatically faster than the wild type receptor (Fig. 7). The half-lives for SUR1 $\Delta F1388$ versus wild type SUR1, expressed alone, are 3 $\pm 0.4$ (n = 2) versus 25.5 $\pm 4.4$ (n = 3) hours, respectively. The SUR1 $\Delta F1388$ subunits are able to assemble with K_{IR}6.2 (Fig. 7B), but this association does not affect turnover of the mutant receptor significantly. We see no mature, fully glycosylated mutant receptors, consistent with their not leaving the ER. The estimated half-life for the K_{IR}6.2-SUR1 $\Delta F1388$ complex is 2.1 $\pm 0.6$ h, not significantly different from the mutant receptor alone. The association with SUR1 $\Delta F1388$ does significantly slow the degradation of K_{IR}6.2 (Fig. 7B). We assume there is a constantly changing mixture of SUR1-K_{IR}6.2 complexes in this experiment but have fit a single exponential function, $t_{1/2}$ = 5.6 $\pm 2.3$ h, to the data for purposes of comparison. It is noteworthy that both immunoprecipitations show enrichment of K_{IR}6.2 subunits versus SUR1 $\Delta F1388$ at later times, consistent with dissociation of $\Delta F1388$ from the complexes and subsequent degradation.

**Turnover of K_{ATP} Channel Subunits in the INS-1 β-cell line**—The kinetics of turnover and maturation of K_{ATP} channel subunits in INS-1 β-cells, determined by immunoprecipitation with anti-K_{IR} antibodies, were similar to what was observed in transfected COS m6 cells (Fig. 8). The level of expression of K_{ATP} channel subunits is lower than in transfected cells, but mature SUR1 was detectable between two and three hours after a 1-h $35^S$methionine/cysteine pulse. Based on the disappearance of immature SUR1, the half-life for maturation was 2.2 $\pm 0.5$ h. The average half-life of fully assembled channels, the mean of the values determined for K_{IR}6.2 (6.2 $\pm 2.0$ h) and mature SUR1 (6.8 $\pm 2.4$ h), is 6.5 $\pm 2.0$ h (n = 3).
**FIG. 3.** Turnover of SUR1 associated with Kᵢ₆.2. COS m6 cells were transfected with Kᵢ₆.2 and SUR1, pulse-labeled, and then processed as described under “Experimental Procedures” using anti-Kᵢ₆.2 antibodies. The plotted data are the relative densities of the bands shown; the $t_{1/2}$ values are averages for three experiments. The times shown are 0, 0.5, 1, 2, 3, 5, 10, 21, 25, and 30 h. A, Kᵢ₆.2 turns over with a half-life of 8.5 ± 4 h. B, turnover of immature and mature forms of SUR1 associated with Kᵢ₆.2. The dotted line is a single exponential function with a $t_{1/2} = 2.2 ± 0.14$ h, fit to the densities of immature, core glycosylated SUR1 (open squares); the solid line is a single exponential function with a $t_{1/2} = 5.9 ± 1$ h, fit to the densities of the mature, fully glycosylated receptor (filled squares). Values are mean ± S.D.

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**FIG. 4. Turnover of $K_{IR6.2}$ associated with Myc-tagged SUR1.** COS m6 cells were transfected with $K_{IR6.2}$ and Myc-tagged SUR1, pulse-labeled, and then processed as described under “Experimental Procedures” using anti-Myc antibodies. The plotted data are the relative densities of the bands shown; the $t_{1/2}$ values are averages for three experiments. The times shown are 0, 0.5, 1, 2, 3, 5, 10, 21, 25, and 30 h. A, $K_{IR6.2}$ turns over with a half-life of $7.8 \pm 1.1$ hr. B, turnover of core and complex glycosylated forms of SUR1 associated with $K_{IR6.2}$. The single exponential function fit to the densities of the immature form of SUR1 (open squares) has a $t_{1/2} = 2.6 \pm 0.6$ hr. A single exponential function fit to the densities of the mature receptor, beginning with the 10-h time point (filled squares), has a $t_{1/2} = 6.9 \pm 0.2$ hr. Values are mean ± S.D.
illustrates the turnover of wild type K IR6.2 subunits expressed in the presence or absence of Myc-tagged SUR1, behavior of unaccompanied KIR6.2 is more closely akin to the Refs. 34 and 43). Whereas SUR1 is long-lived in the ER, the extended half-life of SUR1 and the rapid turnover of the related ABCC protein, CFTR, is noteworthy (reviewed in 34 and 43). The formation of KIR6.2-SUR1 heteromers is rapid. We have observed that octameric KATP channels, once formed, do not dissociate readily and have estimated channel half-life by determining the rates of loss of KIR6.2 or SUR1 from immunoprecipitates obtained using either anti-KIR6.2 or anti-Myc antibodies, respectively. The assumption of channel stability is supported by the similar half-life values determined for the accompanying partner subunit. The four values range from 5.9–8.5 h, with a mean value of 7.3 ± 1.6 h. The pattern of maturation and turnover of KIR6.2 and SUR1 in INS-1 β-cells is similar with an estimated channel half-life of 6.5 ± 2.2 h, implying the data obtained with reconstituted channels reflects subunit processing in β-cells.

The precursor-product relation between the immature and mature receptors was used to estimate the timing of channel maturation. Subunit synthesis and initial glycosylation of SUR1 take place concurrently in the ER, whereas mature glycosylation takes place in the medial Golgi. We observe core glycosylated SUR1-KIR6.2 complexes at the earliest times and can follow their conversion to the fully glycosylated species. Based on the loss of core glycosylated SUR1 from the complex (Figs. 3B and 4B), the estimated half-time for maturation of the KIR6.2-SUR1 complexes is −2.2 h. What maturation entails is not well understood but must involve subunit folding, assembly into a complete channel, transit through the Golgi, addition of sialic acid residues in the Golgi, and transit to the cell surface.

**FIG. 5.** KIR6.2 W91R turns over rapidly. COS m6 cells expressing KIR6.2 W91R subunits, in the presence or absence of Myc-tagged SUR1, were pulse-labeled and then processed as described under “Experimental Procedures” using either anti-KIR6.2 or anti-Myc antibodies. Single exponential functions were fit to the data. The t1/2 values, with (filled squares) or without (open squares) SUR1, are 34.0 ± 2.6 (solid line) and 26.4 ± 2.4 (dashed line) minutes (n = 2), respectively. The dotted line illustrates the turnover of wild type KIR6.2 subunits expressed in the absence of SUR (taken from Fig. 2A). The gel illustrates KIR6.2 W91R turnover in the absence of SUR1, but the results were equivalent when Myc-tagged SUR1 was present and anti-Myc antibodies were used. The times shown are 0, 0.5, 1, 2, 3, 5, 10, 21, 25, and 30 h. Values are mean ± S.D.

**DISCUSSION**

Pulse-chase methods were employed to determine the turnover rates of KATP channel subunits expressed alone or together. Expressed individually, both subunits have long-lived species, half lives near 24 h. This stability is consistent with the idea that these channels assemble slowly. The difference between the extended half-life of SUR1 and the rapid turnover of the related ABCC protein, CFTR, is noteworthy (reviewed in Refs. 34 and 43). Whereas SUR1 is long-lived in the ER, the behavior of unaccompanied KIR6.2 is more closely akin to the turnover observed for CFTR, with ~60% of the KIR6.2 subunits being degraded before they can convert to a stable long-lived species. The means of stabilization are different; CFTR even-
Fig. 7. SUR1 ΔF1388 turns over rapidly. COS m6 cells were transfected with Myc-tagged SUR1 ΔF1388 with and without KIR6.2, pulse-labeled, and then processed as described under “Experimental Procedures” using either anti-Myc antibodies (A) or anti-KIR6.2 antibodies (B). A, the turnover of SUR1 ΔF1388 is essentially the same in the presence or absence of KIR6.2, 2.1 ± 0.6 versus 3 ± 0.4 h, open versus filled circles (n = 3), respectively. The dotted line illustrates the turnover of Myc-tagged SUR1 in the absence of KIR6.2 (from Fig. 2A). Only the immature form of ΔF1388 subunits is present, indicating failure to transit to the Golgi apparatus. The top gels illustrate the turnover of both KIR6.2 and SUR1 ΔF1388. Note that although the Myc-tagged ΔF1388 is barely detectable, KIR6.2 is enriched. The ΔF1388 bands are intentionally overexposed. The times shown are 0, 0.5, 1, 2, 3, 5, 10, 21, 25, and 30 h. B, assembly with SUR1 ΔF1388 slows the turnover of KIR6.2 subunits associated with SUR1 ΔF1388. A single exponential function, \( t_{1/2} = 5.6 ± 2.3 \) h (n = 2), was fit to the data, although multiple populations are presumably present. The dotted line illustrates the turnover of wild type KIR6.2 subunits expressed in the absence of SUR (from Fig. 2B). The lower gels illustrate subunit turnover using anti-KIR6.2 immunoprecipitation. The times shown are 0, 0.5, 1, 2, 3, 5, 10, 21, and 25 h. KIR6.2 is enriched at the later time points. Values are mean ± S.D.
The 2.2-h figure is an estimate of the average time between synthesis and the final addition of sialic acid residues. Estimates for the transit time of monomeric proteins between ER and Golgi vary widely, but most are shorter than 2.2 h. GLUT1 and GLUT4 are reported to take 5 versus 20 min, respectively, in 3T3-L1 adipocytes (44). The mean residence time of a green fluorescent protein-tagged mutant of the vesicular-stomatitis virus protein in both the ER and Golgi is estimated to be ~40 min (45, 46). If the residence time(s) for complete K_{IR}6.2-SUR1 channels in the ER and Golgi compartments are similar, the estimated maturation time of 2.2 h is consistent with the idea that although K_{IR}6.2 and SUR1 subunits rapidly associate, the rate-limiting step is the slow assembly of these heteromeric complexes into complete octameric channels. This idea is supported by the slow formation of homomeric K_{IR}6.2 pores ($t_{1/2}$ ~1.2 h), by the slow maturation of K_{ATP} channel subunits in

Fig. 8. Turnover of K_{ATP} channel subunits in INS-1 β-cells. INS-1 β-cells were pulse-labeled and then processed as described under "Experimental Procedures" using the anti-K_{IR}6.2 antibodies. The values are mean ± S.D. (n = 3). The data points are for the gels shown.

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INS-1 β-cells, and by the relatively slow maturation of other K⁺ channels, e.g. 80% conversion of immature Shaker subunits to the mature form in 1.5 h (47), independently of the presence of the Kvβ2 subunit, and a $t_{1/2}$ ~3 h for conversion of core glycosylated Kv1.4 to the fully glycosylated form (48).

Subunit Mutations and Degradation—Multiple mutations in both SUR1 and K_{IR}6.2 have been identified in patients with congenital hyperinsulinism (10, 39, 40). Truncations of the C terminus of SUR1 have been reported to affect trafficking to the cell surface (4). A number of point mutations have been identified that exhibit channel activity in isolated patches and display normal inhibition by ATP but fail to be stimulated by MgADP (19, 20, 49, 50). The F1388 mutation in SUR1 was one of the first mutations to be identified and is a frequent mutation in Ashkenazi populations (41). This is a severe mutation, and no channel activity was detectable when K_{IR}6.2/F1388 subunits were expressed with wild type K_{IR}6.2, although binding/labeling studies showed the mutant receptor was expressed (41). Interestingly, SUR1 F1388 was later shown to associate with K_{IR}6.2 subunits and protect them against rapid degradation. Although we fit a single exponential to the K_{IR} decay data, there are almost certainly multiple populations present because we are able to precipitate K_{IR}6.2 free of SUR1 ΔF1388 at the later time points (Fig. 7, >10 h).

The W91R mutation was identified by our group in a patient of Palestinian origin with severe hypoglycemia but has
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These mutations are recessive; patients homozygous for either mutation lack functional Kir6.2-SUR1 KATP channels at the β-cell surface. Heterozygous carriers would be expected to have a reduced complement of normal channels because the defective subunits would be weeded out by virtue of their increased tendency to dissociate from channels and be degraded.

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