Structural Determinant for Assembly of Mammalian K^+ Channels

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ABSTRACT K^+ channel function is regulated through the assembly of channel subunit isoforms into either homo- or heterotetrameric structures each characterized by distinct pharmacologic and kinetic properties. In studying the molecular basis of subunit association in mammalian *Shaker*-like K^+ channels, we constructed deletion mutants of the inactivating K^+ channel hKv1.4 alone and in tandem with hKv1.5 and examined the functional properties electrophysiologically in *Xenopus* oocytes. Deletion of 255 amino acids in the amino-terminal domain of hKv1.4 prevented the formation of hybrid channels within the subfamily but had no effect on homomultimerization or voltage-dependent gating. The amino-terminal deletion mutant of Kv2.1, a noninactivating K^+ channel from a distantly related subfamily also forms functional homomultimeric channels. Although members of different K^+ channel subfamilies do not coassemble, coexpression of the amino-terminal deletion mutants of hKv1.4 and Kv2.1 resulted in the formation of functional hybrid channels. These results demonstrate that the amino-terminal region of mammalian K^+ channels subserves two functions. It provides a recognition site necessary for hetero- but not homomultimeric channel assembly within a subfamily and prevents coassembly between subfamilies.

INTRODUCTION

Although encoded by a single gene in *Drosophila* (Papazian et al., 1987), mammalian *Shaker*-type (hKv1.X) voltage-dependent K^+ channel subunits are encoded by seven unique genes (Kamb et al., 1989; Koren et al., 1990; McKinnon, 1989; Philipson et al., 1991; Philipson et al., 1993; Roberds and Tamkun, 1991; Sheng et al., 1993; Stuhmer et al., 1989; Swanson et al., 1990; Tempel et al., 1988; Wang et al., 1993). The diversity of mammalian K^+ channels arises in large part from the combinatorial assembly of subunits generated by multiple genes giving rise to an array of homo- and heterotetrameric structures. Although heteropolymerization is prevented between K^+ channel families (Covarrubias et al., 1991), functional hybrid channels may form through assembly of subunits within a single subfamily (Christie et al., 1990; Covarrubias et al., 1991; Isacoff et al., 1990; Ruppersberg et al., 1990). A highly conserved, self-tetramerizing structural element located in the amino-terminal domain of nonmammalian K^+ channels has recently been shown to prevent hybrid channel formation between families (Li et al., 1992). Deletion of this domain results in channels which fail to homomultimerize (Li et al., 1992; Shen et al., 1993). Our experiments demonstrate that a homologous structural domain in mammalian *Shaker*-like K^+ channels, while permissive for hybrid channel assembly within a subfamily is not essential for the assembly of homomultimeric channels.

Received for publication 13 September 1993 and in final form 10 December 1993.

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0006-3495/94/03/667/07 $2.00

MATERIALS AND METHODS

The wild-type hKv1.4 channel (Philipson et al., 1990) was prepared for expression in *Xenopus* oocytes by ligation of a 2.2-kb *NcoI*/*Hin*cII fragment of the 4-kb hKv1.4 cDNA with the pSP64T (Krieg and Melton, 1987) vector after addition of *Bgl*II linker adaptors. The deletion mutant, $hKv1.4_{\Delta 28-283}$ was constructed by digestion of wild-type hKv1.4 in pSP64t with *Apa*I. The 765-bp *Apa*I fragment was separated by agarose gel electrophoresis and the 4.7-kb channel/vector fragment was purified and recircularized with T4 DNA ligase. The deletion was verified by restriction enzyme digestion.

The chimeric dimer hKv1.4-hKv1.5 was constructed by first amplifying a 176-bp target in hKv1.4 using a 20-mer forward primer (bp 1802–1821, upstream of the unique hKv1.4 *Xba*I site) CTTCTTCTTCCCTGGGGGAC, and a 30-mer reverse primer, GAGGGCATGCTGGCTTCCCACAT-CAGTCTC, which was complementary to the last 16 bases of the hKv1.4 coding region. This primer introduced a mutation changing the stop codon TGA to a Gly residue (GGA) and included 14 bp of hKv1.5 5′-untranslated sequence, with its unique *Sph*I site. The amplified 176-mer was cloned into pBluescript KSII+, completely sequenced, excised by double digestion with *Xba*I and *Sph*I, ligated with *Sph*I-*Xba*I hKv1.5 excised from pSP64T-hKv1.5, and then ligated into pSP64T-hKv1.4 at the *Xba*I site. The resultant construction was confirmed by restriction mapping and sequencing across the junction. In summary, the dimeric cDNA contained all of the hKv1.4 coding region followed by sequences encoding a 10-residue linker (GSQHALCSRA), created primarily by 5′ flanking residues of hKv1.5, followed by the complete coding region of hKv1.5.

Two-microelectrode voltage clamp recordings were obtained from cRNA-injected oocytes as described previously (Philipson et al., 1991) using a Warner OC-725 voltage clamp. Currents were recorded in OR-2 solution (83 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM Na_2HPO_4, 5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.6). Currents were filtered at 500 Hz, digitized at 1 kHz, and stored on a IBM PC/AT compatible.

cRNA was transcribed in vitro using the mCAP RNA kit (Stratagene). Oocytes were injected with 50 nl of cRNA in water at concentrations of 0.01 μg/μl for hKv1.4, hKv1.5, and 1:1 coinjections of hKv1.4 and hKv1.5; 0.1 μg/μl for the wild-type tandem; 0.02 μg/μl for the $tandem_{\Delta 28-283}$; 0.1 μg/μl for $hKv1.4_{\Delta 28-283}$; and 0.1 μg/μl for a 1:48 coinjection of hKv1.5 and $hKv1.4_{\Delta 28-283}$.

RESULTS

In this study, we used mammalian K^+ channels of the *Shaker* (hKv1.4 and hKv1.5) and *Shab* (Kv2.1) subfamilies to determine the contribution of the amino-terminal domain in

homo- versus heteromultimeric subunit assembly. A mutant of the fast inactivating hKv1.4 (hKv1.4$_{\Delta 28-283}$) was constructed in which amino acid residues 28 through 283 were deleted (Fig. 1 *a*). The eliminated region contained a 114-amino acid cytoplasmic domain that is highly conserved within the *Shaker* K^+ channel subfamily and that has been shown to be critical in homomultimeric as well as heteromultimeric channel assembly in *Shaker* B (ShB) K^+ channels (Li et al. 1992), leading to the prediction that the mutant would not form channels. In contrast to previous studies on ShB K^+ channels (Isacoff et al., 1990), deletion of this highly conserved fragment in hKv1.4 did not prevent homomultimeric K^+ channel current expression in *Xenopus* oocytes, and the voltage-dependent properties of the homomultimeric mutant channel current were not different from the wild-type current. The time course of hKv1.4$_{\Delta 28-283}$ inactivation (Fig. 1 *b*) was similar to that observed for wild-type (at +40 mV, τ_{inact} = 40 ± 6 ms (n = 6) for

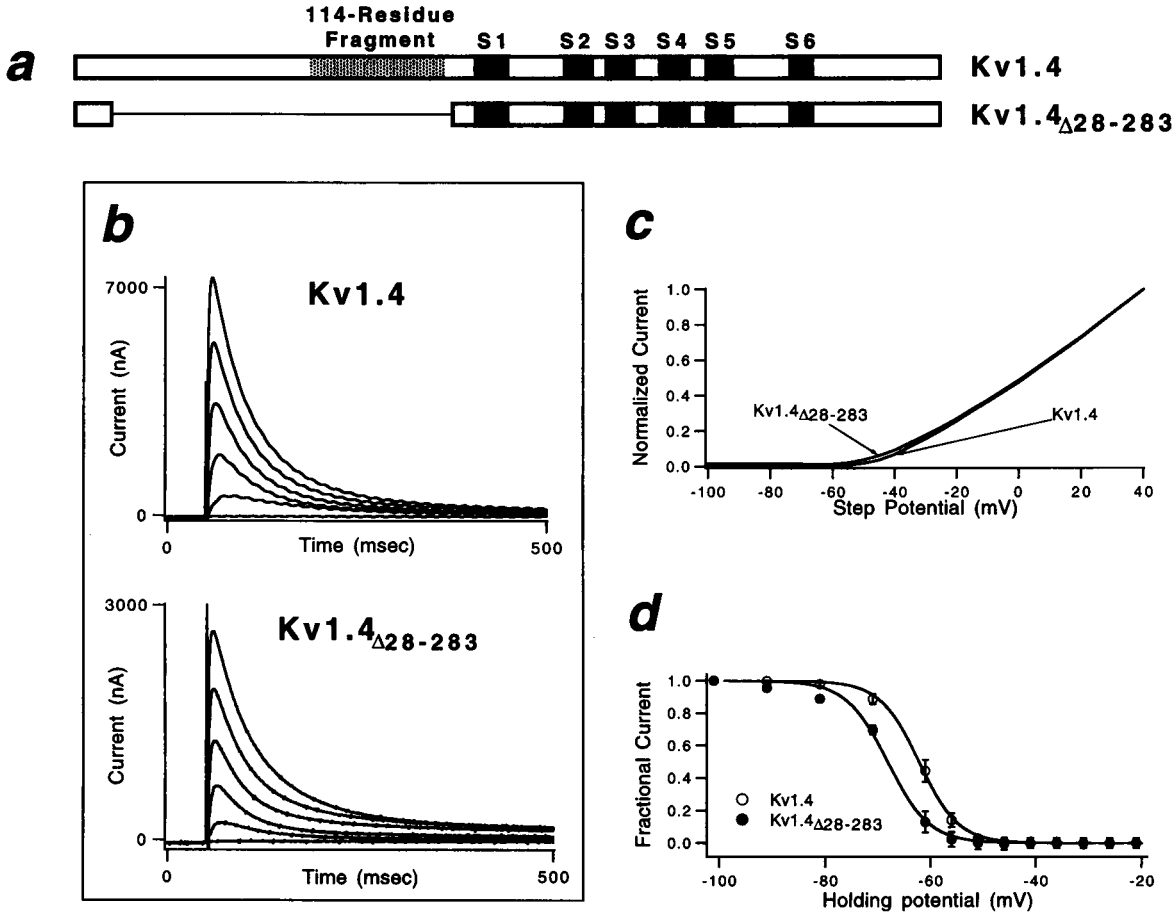

FIGURE 1 The voltage-dependent properties of hKv1.4 are not changed by a deletion of 255 amino acids in the amino terminus. (*a*) (*Top*) diagram of the full-length hKv1.4 polypeptide, darkened segments represent the six putative transmembrane domains. The shaded box located in the hydrophilic amino-terminal domain represents the 114 amino-acid stretch highly conserved within the *Shaker* K^+ channel subfamily shown to be involved in homophilic subunit interactions in ShB. (*Bottom*) diagram of the mutant hKv1.4 (hKv1.4$_{\Delta 28-283}$) in which amino acids 28 through 283 were deleted. (*b*) Representative current records from *Xenopus* oocytes injected with mRNA encoding either wild-type hKv1.4 or hKv1.4$_{\Delta 28-283}$. The major time constant of current inactivation for the mutant channels was 40 ± 6 ms (n = 6) and 43 ± 6 ms (n = 6) for wild-type channels in a step to +40 mV. Currents were elicited by step depolarizations from a holding potential of −80 mV to test potentials from −60 to +40 mV in +20 mV increments; interpulse interval was 10 s. (*c*) Voltage dependence of activation for wild-type hKv1.4 or hKv1.4$_{\Delta 28-283}$ current. Currents were measured in step depolarizations from a holding potential of −80 mV to test potentials from −120 to +40 mV; interpulse interval was 10 s. Leak current estimated, in test potentials to −110 and −90 mV, was scaled and subtracted from the test potential records. Activation threshold was determined as the potential at which the peak current was 1% of the peak current measured during a voltage step to +40 mV. Activation threshold was −53 ± 4 mV (n = 3) for currents measured in oocytes injected with hKv1.4$_{\Delta 28-283}$ and −51 ± 1 mV (n = 3) for currents recorded from oocytes injected with wild-type hKv1.4. Activation curves are averages of peak currents, normalized to the peak current measured at a voltage step to +40 mV. (*d*) Steady-state inactivation curves for currents obtained from oocytes injected with either wild-type hKv1.4 (*open circles*) or the mutant channel hKv1.4$_{\Delta 28-283}$ (*filled circles*). Curves displayed are the average of five normalized curves measured for hKv1.4 and 4 normalized curves measured for hKv1.4$_{\Delta 28-283}$, error bars indicate SEM. Steady-state inactivation was measured from currents evoked in voltage pulses to +40 mV after the membrane was held at the indicated voltages for 1 minute. Midpoints of steady-state inactivation were determined by fitting averaged points with a single Boltzmann isotherm of the form: $g_k(v) = G_{kmax}/\{1 + \exp[(V - V_{1/2})/k]\}$, where $V_{½}$ is the voltage at the midpoint of steady-state inactivation. The midpoint of the single curve was not significantly different from the midpoints derived from the fitted curves for each cell. Deletion of the amino-terminal region of hKv1.4 shifted the midpoint of steady-state inactivation by 6 mV in the hyperpolarizing direction, from −62 ± 1 mV (n = 5) for wild-type hKv1.4, to −68 ± 1 mV (n = 4) for hKv1.4$_{\Delta 28-283}$.

$hKv1.4_{\Delta 28-283}$ versus τ_{inact} = 43 ± 6 ms (n = 6) for wild-type). The threshold for current activation (Fig. 1 *c*) was indistinguishable between the mutant and wild-type channels (−53 ± 4 mV (n = 3) versus −51 ± 2 mV (n = 3)). The midpoint of steady-state inactivation for the mutant channel (Fig. 1 *d*) was shifted to slightly more hyperpolarized potentials (−68 ± 1 mV (n = 4) versus −62 ± 1 mV (n = 5)).

Expression of hKv1.5 in *Xenopus* oocytes is characterized by a noninactivating current, with an activation threshold of −25 mV and midpoint of steady-state inactivation (−25.3 ± 0.4 (n = 8)) which is depolarized by 37 mV relative to that for hKv1.4 (Philipson et al., 1991, 1990).

The homomultimeric channels hKv1.4 and hKv1.5 differ in their kinetics of inactivation as well as midpoints for steady-state inactivation, and heteromultimers can be identified by the presence of voltage-dependent properties intermediate between the two homomultimeric forms. Steady-state inactivation curves for wild-type hKv1.4:hKv1.5 heteromultimeric current in coinjected oocytes were well-fit with a single Boltzmann isotherm with a midpoint intermediate between that obtained for the steady-state inactivation curves for hKv1.5 and hKv1.4 homomultimeric current (Fig. 2 *a*). Steady-state availability curves for wild-type hKv1.4:hKv1.5 heteromultimeric current could not be fit by the arithmetic sum of the steady-state inactivation curves for hKv1.5 and hKv1.4 homomultimeric current (Fig. 2 *b*). Additional evidence for the formation of heterotetramers came from the kinetics of the wild-type heteromultimeric current available at a holding potential of −50 mV that exhibited a fast-inactivating component that was not predicted by the arithmetic sum of the two wild-type current types (see *insert* in Fig. 2 *b*). Taken together, this data supported the conclusion that hKv1.4 and hKv1.5 formed functional heteromultimers and that the predominant current type in the coinjection experiments could not be explained by the arithmetic sum of currents contributed by a varying ratio of the homomultimeric forms of hKv1.4 or hKv1.5.

We investigated the importance of the amino-terminal domain in heteromultimeric channel formation among mammalian K^+ channels within the *Shaker*-like (hKv1.X) subfamily, by coinjecting *Xenopus* oocytes with either wild-type hKv1.4 or $hKv1.4_{\Delta 28-283}$ and hKv1.5 cRNA. Analysis of steady-state availability curves for current expressed in oocytes coinjected with hKv1.5 and $hKv1.4_{\Delta 28-283}$, in contrast to coinjection experiments performed with the wild-type channels, failed to show any heteromultimeric current. Steady-state inactivation curves obtained from oocytes coinjected with hKv1.5 and the mutant channel could not be fit with a single Boltzmann isotherm (Fig. 3 *a*). In contrast to wild-type heteromultimeric hKv1.4:hKv1.5 current, steady-state inactivation curves for $hKv1.5{:}hKv1.4_{\Delta 28-283}$ current were well-fit with an algebraic sum of the steady-state availability curves for hKv1.5 and $hKv1.4_{\Delta 28-283}$ homomultimeric current (Fig. 3 *b*), evidence that the mutant channel was not capable of forming functional heteromultimers. Furthermore, the inactivation kinetics of the $hKv1.5{:}hKv1.4_{\Delta 28-283}$ current available for activation at the depolarized holding potential of −55 mV lacked the fast-inactivating kinetics indicative of heteromultimer formation between the wild-type channels, kinetics which

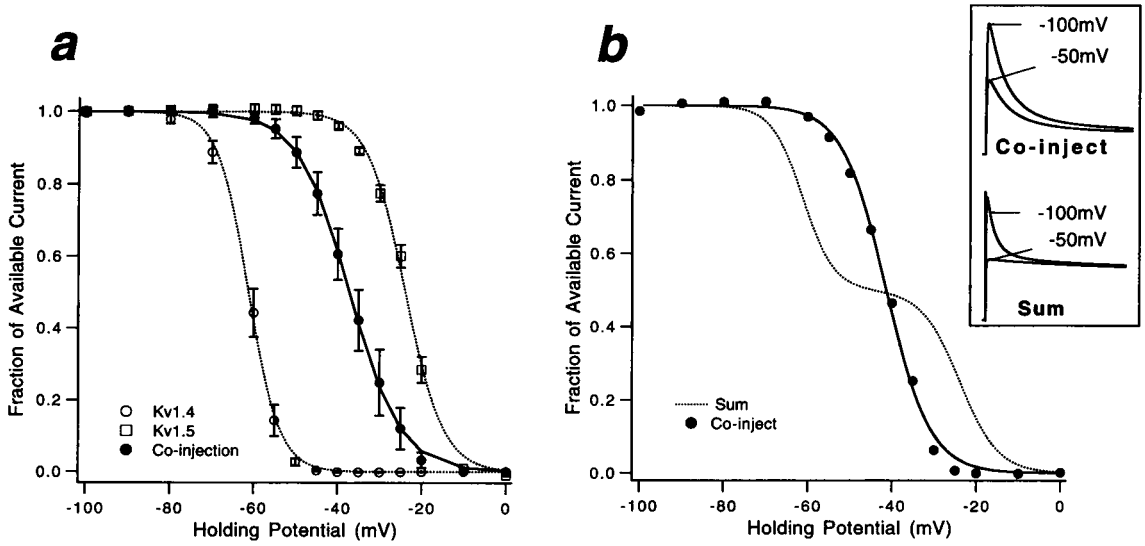

FIGURE 2 Current records and steady-state inactivation curves from heteromultimeric K^+ channels in *Xenopus* oocytes upon coinjection of hKv1.5 and wild-type hKv1.4 cRNA. (*a*) Steady-state inactivation curves for currents recorded from oocytes coinjected with hKv1.5 and wild-type hKv1.4 cRNA fit with a single Boltzmann isotherm (*solid line*), $V_{½}$ for the hKv1.5:hKv1.4 (*closed circles*) current was intermediate between that determined for hKv1.5 (*open squares*) and hKv1.4 (*open circles*) current. Steady-state inactivation protocols were identical to those described in Fig. 1. Symbols represent averages of normalized currents (n = 6 for coinjected, n = 5 for hKv1.5, n = 4 for hKv1.4), error bars represent SE. (*b*) Steady-state inactivation curve for current from an oocyte coinjected with hKv1.5 and wild-type hKv1.4 cRNA (*closed circles*) as compared to a waveform obtained by the digital addition of normalized steady-state curves from currents measured in oocytes injected with either hKv1.5 or hKv1.4 cRNA (*dashed line*). Insert: (*Top*) currents obtained from the oocyte in *b* coinjected with hKv1.5 and wild-type hKv1.4. (*Bottom*) current waveforms obtained by the digital addition of normalized hKv1.5 and hKv1.4 currents in a 1:1 ratio.

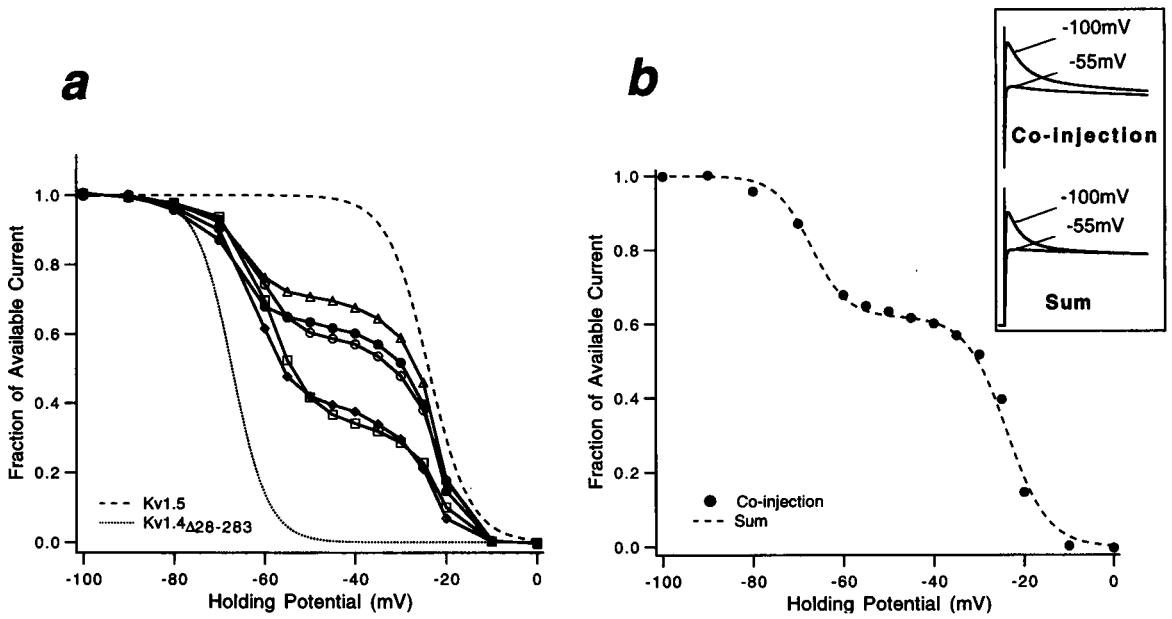

FIGURE 3 Heteromultimeric K^+ channels do not form when hKv1.5 and $hKv1.4_{\Delta 28-283}$ cRNA are coinjected in *Xenopus* oocytes. (*a*) Steady-state inactivation curves from five oocytes coinjected with hKv1.5 and $hKv1.4_{\Delta 28-283}$ cRNA (*solid lines*). Note that curves show considerable variability from oocyte to oocyte and cannot be fit with a single Boltzmann isotherm. Steady-state inactivation curves for hKv1.5 and $hKv1.4_{\Delta 28-283}$ are included for reference (*dotted lines*). Steady-state inactivation protocols as in Fig. 1. (*b*) Steady-state inactivation curves for currents recorded in one oocyte (*filled circle*, also shown in Fig. 1 *A*) can be fit well by the algebraic sum of the steady-state inactivation curves for hKv1.5 and $hKv1.4_{\Delta 28-283}$ (*dashed lines*). Insert: (*Top*) current measured from an oocyte coinjected with hKv1.5 and $hKv1.4_{\Delta 23-283}$ cRNA. (*Bottom*) current waveform obtained by the digital addition of normalized hKv1.5 and $hKv1.4_{\Delta 28-283}$ current in a 1:1 ratio.

would have been expected if hKv1.5 and $hKv1.4_{\Delta 28-283}$ subunits had formed functional heteromultimers (see *insert* in Fig. 3 *b*).

In order to test the hypothesis that removal of the amino-terminal domain prevented heterotetrameric interaction between hKv1.5 and $hKv1.4_{\Delta 28-283}$ by the removal of the amino-terminal recognition site permissive for heterophilic subunit interactions and not by the creation of subunit incompatibility, we constructed a fusion gene linking one copy of $hKv1.4_{\Delta 28-283}$ with hKv1.5 in a single open reading frame. In the tandem dimer, a linker of 10 residues (GSQHALCSRA) connected the last residue of $hKv1.4_{\Delta 28-283}$ with the initial residue of hKv1.5. Constraining the stoichiometry of the heteromultimers in the tandem construct thus eliminated a recognition step in heterotetramer formation. We compared the voltage-dependent properties of the current expressed from a tandem composed of hKv1.5 and $hKv1.4_{\Delta 28-283}$ to current induced from a tandem construct of wild-type polypeptides. The wild-type hKv1.5-hKv1.4 tandem construct produced heteromultimeric current which was fast inactivating (Fig. 4 *a*) (at +40 mV, τ_{inact} = 78 ± 6 ms (n = 8)). The threshold of activation and midpoint of steady-state inactivation for the wild-type tandem construct was intermediate between that observed for hKv1.5 and hKv1.4 homomultimeric current (Fig. 4 *b* and 4 *c*), as would be expected if heteromultimers consisting of equal numbers of hKv1.5 and hKv1.4 subunits were the predominate K^+ channel formed. The threshold for current activation for the wild-type tandem was −37 mV and the midpoint of steady-state inactivation was −46.5 for the fit to the data averaged from seven cells.

Expression of the mutant tandem construct made from $hKv1.4_{\Delta 28-283}$ and hKv1.5 also gave rise to fast inactivating currents that were kinetically similar to currents expressed with the wild-type tandem (Fig. 4 *a*) (at +40 mV, τ_{inact} = 59 ± 6 ms [n = 4])). The activation threshold of the mutant tandem construct (−45 mV), while similar to that observed for the wild-type tandem, was significantly less negative than that observed for $hKv1.4_{\Delta 28-283}$ as can be seen in Fig. 4 *b*. The midpoint for steady-state inactivation ($V_{½}$ = −55 mV for the fit to the data averaged from five cells) for the mutant tandem construct was, however, shifted to a value negative from that observed for the wild-type tandem as illustrated in Fig. 4 *c*. The fast-inactivating current expressed in oocytes injected with cRNA for the tandem formed from mutant $hKv1.4_{\Delta 28-283}$ was not due to $hKv1.4_{\Delta 28-283}$ homomultimers, since a substantial component of fast inactivating current remained available for activation at a holding potential of −55 mV where 100% of the $hKv1.4_{\Delta 28-283}$ homomultimeric current would be inactivated (see *insert* in Fig. 4 *c*).

If the amino-terminal region functions as an intrafamily recognition element, then removal of this region might allow subunit coassembly from divergent subfamilies. To test this hypothesis, we performed coexpression experiments with $hKv1.4_{\Delta 28-283}$ and an amino-terminal deletion mutant of the

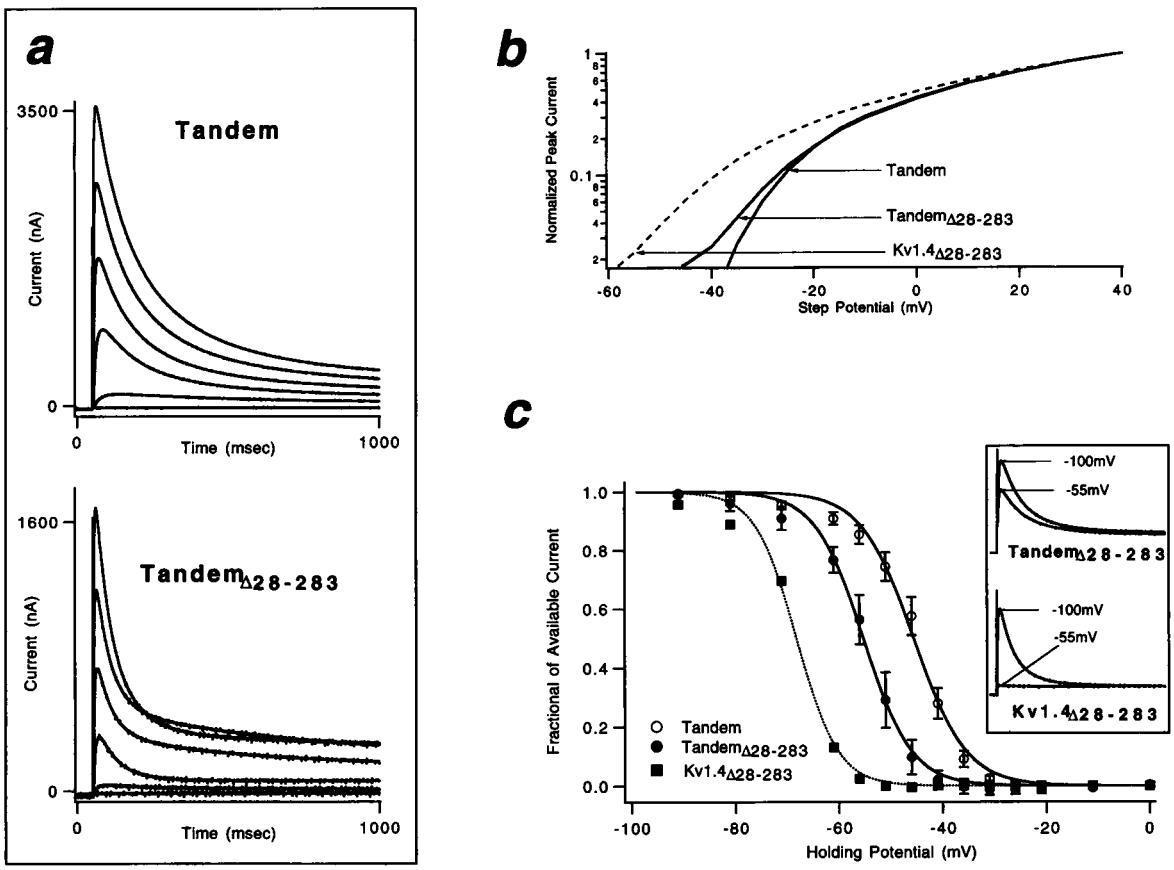

FIGURE 4 hKv1.4$_{\Delta 28-283}$ and hKv1.5 polypeptides form heteromultimic K^+ channels when expressed as a tandem dimer construct. (*a*) Current records from *Xenopus* oocytes injected with either the wild-type hKv1.4-hKv1.5 tandem construct cRNA (*top*) or the mutant hKv1.4$_{\Delta 28-283}$-hKv1.5 tandem construct cRNA (*bottom*). Currents were elicited during voltage pulses from −60 to +40 mV in 20-mV steps, holding potential was −80 mV, and interpulse interval was 10 s. (*b*) Comparison of the voltage dependence of current activation for the mutant hKv1.4$_{\Delta 28-283}$-hKv1.5 tandem construct and the wild-type hKv1.4-hKv1.5 tandem construct (*solid lines*). Threshold of activation for the mutant tandem construct was shifted to a more hyperpolarized potential as compared to that observed for the wild-type tandem construct but substantially depolarized with respect to the threshold of activation observed for hKv1.4$_{\Delta 28-283}$ (*dashed line*). Activation protocols were the same as those described in Fig. 1. (*c*) Steady-state inactivation curves for both tandem constructs as well as the mutant hKv1.4$_{\Delta 28-283}$ channel. Steady-state inactivation protocols are identical to those described in Fig. 1. The midpoint of steady-state inactivation for the mutant tandem construct (*filled circles*) was shifted to a more hyperpolarized potential from that measured for the wild-type tandem (*open circles*); however, it was substantially depolarized from that measured for hKv1.4$_{\Delta 28-283}$ current (*closed squares*). Data points represent averages obtained from five oocytes for the mutant hKv1.4$_{\Delta 28-283}$-hKv1.5 tandem and seven oocytes for wild-type hKv1.4-hKv1.5 tandem. The smooth line through the points represents the fit to the averaged values. Steady-state inactivation data for hKv1.4$_{\Delta 28-283}$ was taken from Fig. 1 *D*. Insert: (*Top*) current from an oocyte injected with the mutant tandem cRNA. (*Bottom*) current from an oocyte injected with the mutant channel cRNA.

mammalian DRK1 from the Shab subfamily (Kv2.1$_{\Delta N139}$) missing the corresponding conserved amino-terminal region (Vandogen et al., 1990). Fig. 5 compares hKv1.4$_{\Delta 28-283}$ and Kv2.1$_{\Delta N139}$ homomultimeric current with current obtained from oocytes coinjected with both amino-terminal deletion mutant cRNAs. The Kv2.1$_{\Delta N139}$-induced current was noninactivating with 100% availability at a holding potential of −50 mV. In contast, hKv1.4$_{\Delta 28-283}$-induced current was unavailable at the same holding potential. Heteromultimeric hKv1.4$_{\Delta 28-283}$-Kv2.1$_{\Delta N139}$ current measured in coinjected oocytes could be identified as an inactivating current available at a holding potential of −50 mV where hKv1.4$_{\Delta 28-283}$-induced current would be expected to be unavailable. The kinetic character of the homomultimeric and heteromultimeric currents is compared in Fig. 5. The heteromultimeric current could not be approximated by the sum of the two homomultimeric amino-terminal deletion mutant currents.

DISCUSSION

In this study, we set out to determine whether the amino-terminal domain was involved in the formation of hetero- as well as homomultimer formation in *Shaker*-like mammalian K^+ channels. Functional channels were obtained after 255 residues of the amino-terminal domain in hKv1.4 were eliminated. This result demonstrates that interactions between amino-terminal domains are not necessary for the formation of functional mammalian hKv1.X homotetramers and that subunit contacts critical for homotetramer formation are

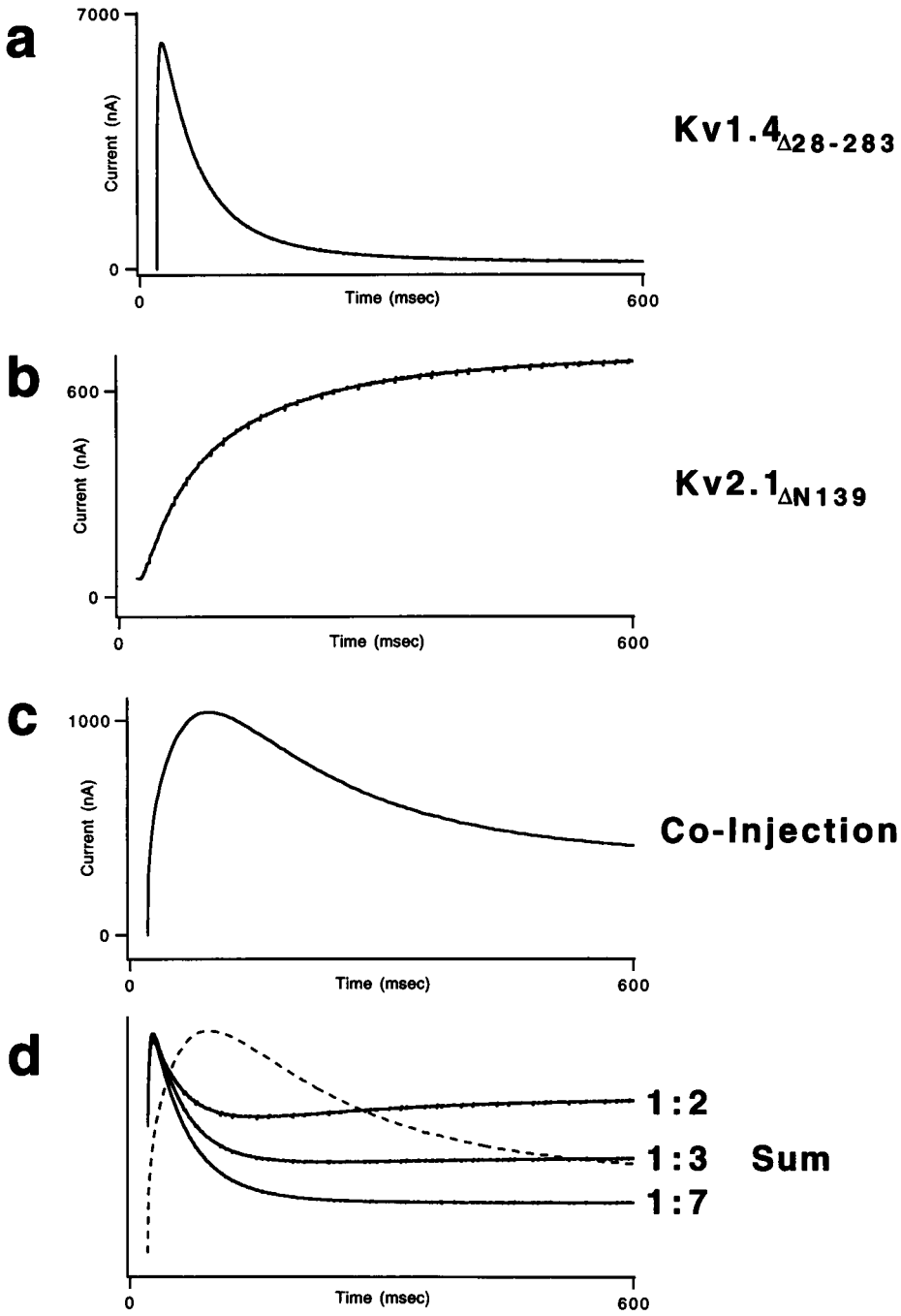

FIGURE 5 Formation of functional heteromultimeric channels between hKv1.4$_{\Delta 28-283}$ and Kv2.1$_{\Delta N139}$. (*a*) hKv1.4$_{\Delta 28-283}$ current during a voltage step to +40 mV from a holding potential of −100 mV. (*b*) Kv2.1$_{\Delta N139}$ current during a voltage step to +40 mV from a holding potential of −50 mV. (*c*) Heteromultimeric hKv1.4$_{\Delta 28-283}$-Kv2.1$_{\Delta N139}$ heteromultimeric current during a voltage step to +40 mV from a holding potential of −50 mV, where none of the homomultimeric hKv1.4$_{\Delta 28-283}$ current would be available for activation (see Fig. 1). (*d*) Arithmetic sum of the homomultimeric hKv1.4$_{\Delta 28-283}$ current at a holding potential of −100 mV and homomultimeric Kv2.1$_{\Delta N139}$ current measured at −50 mV (*solid line*) digitally added in the ratios noted to the right of each trace. The heteromultimeric current from *c* is shown as a superimposed dashed line for comparison. If hKv1.4$_{\Delta 28-283}$ current were to be available at −50 mV, the inactivation kinetics of the current measured in the coinjection experiments cannot be explained by the arithmetic sum of the independent expression of hKv1.4$_{\Delta 28-283}$ and Kv2.1$_{\Delta N139}$ channel currents. In all experiments, oocytes were held at the specified holding potential for 1 min prior to the depolarizing step to +40 mV.

made between sites located in the central core of the channel polypeptides as was found for the *Shab* subfamily (Kv2.X) (Vandogen et al., 1990).

Elimination of the conserved amino-terminal domain had little effect on the rate of inactivation of homomultimeric hKv1.4 current. Elimination of the remaining 28 residues (hKv1.4$_{\Delta 3-283}$) abolished current inactivation (Lee, Philipson and Nelson, 1993, unpublished observations) indicating that hKv1.4 exhibits amino-terminal inactivation.

The amino-terminal domain, while acting as a barrier to the formation of hybrid channels between members of divergent subfamilies, appears to provide additional adhesive stability that is necessary for intrafamily heteromultimerization of mammalian channels. Functional heteromultimers across divergent subfamilies can form, however, if they share a common amino-terminal recognition site (Li et al., 1992) or if the amino-terminal region of all interacting subunits is removed. The formation of functional heteromultimers between the deletion mutants of hKv1.4 and Kv2.1 suggests that subunit contacts within the central core region are secondary sites involved in stabilizing subunit interactions and are conserved across subfamilies of K^+ channels.

Data in this study supports a model in which the amino terminus functions as a critical subunit recognition-discrimination element providing additional thermodynamic stability in intrafamily heteromultimer assembly of mam-

malian *Shaker*-like K^+ channels. Channel assembly appears to involve interactions in the hydrophobic core as well as the amino-terminal domain. Removal of the amino-terminal domain from one of the interacting subunits decreases the thermodynamic stability of heteromultimeric channels relative to the stability of homomultimeric channels as observed in the coinjection experiments with $hKv1.4_{\Delta 28-283}$ and hKv1.5. Removal of the amino-terminal domain from both interacting subunits as in the coinjection experiments with the mutant hKv1.4 and Kv2.1 permits the functional expression of hybrids even between divergent families.

The failure to detect intrafamily heteromultimers between $hKv1.4_{\Delta 28-283}$ and hKv1.5 could be explained, alternatively, on the basis of a change in the kinetics of channel assembly rather than a mismatch in subunit structure. Since the amino-terminal domain is synthesized first and, based on the invertebrate studies, is capable of self-assembly (Li et al., 1992), it is possible that the wild-type subunits are assembled cotranslationally and the amino-terminal deletion mutants are assembled post-translationally. Thus, the absence of intrafamily heteromultimerization between the deletion mutant and the wild-type could be due to the possibility that the two channel types are assembled at different times.

We thank Drs. R. Joho and R. Zühlke for the generous gift of DRK1 and DRK1ΔN139 as well as Drs. A. M. Brown and J. A. Drewe for the gift of DRK1. We gratefully acknowledge Drs. H. Fozzard, D. Hanck, and R. Aldrich for many helpful discussions and their critical review of the manuscript.

Funding was provided by The Marilyn M. Simpson Charitable Trust (to L. H. P.), National Institutes of Health Grant P01 DK44840 (to L. H. P. and D. J. N.), ADA Research and Development Award (to L. H. P.), and R01 GM36823 and P01 NS24575 (to D. J. N.).

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