Planta (2000) 210: 723–731

Planta

Functional characterisation of LKT1, a K^+ uptake channel from tomato root hairs, and comparison with the closely related potato inwardly rectifying K^+ channel SKT1 after expression in *Xenopus* oocytes

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Received: 3 August 1999 / Accepted: 2 November 1999

Abstract. A cDNA encoding a novel inwardly rectifying potassium (K^+_{in}) channel, LKT1, was cloned from a root-hair-specific cDNA library of tomato (*Lycopersicon esculentum* Mill.). The *LKT1* mRNA was shown to be most strongly expressed in root hairs by Northern blot analysis. The LKT1 channel is a member of the AKT family of K^+_{in} channels previously identified in *Arabidopsis thaliana* (L.) Heynh. and potato (*Solanum tuberosum* L.). Moreover, LKT1 is closely related (97% identical amino acids) to potato SKT1. An electrophysiological comparison of the two channels should therefore assist the identification of possible molecular bases for functional differences. For this comparison, both channels were functionally expressed and electrophysiologically characterised within the same expression system, i.e. *Xenopus laevis* oocytes. Voltage-clamp measurements identified LKT1 as a K^+-selective inward rectifier which activates with slow kinetics upon hyperpolarising voltage pulses to potentials more negative than −50 mV. The activation potential of LKT1 is shifted towards positive potentials with respect to SKT1 which might be due to single amino acid exchanges in the rim of the channel's pore region or in the S4 domain. Like SKT1, LKT1 reversibly activated upon shifting the external pH from 6.6 to 5.5, which indicates a physiological role for pH-dependent regulation of AKT-type K^+_{in} channels. The pharmacological inhibitor Cs^+, applied externally, inhibited K^+_{in} currents mediated by LKT1 and SKT1 half-maximally with a concentration (IC_{50}) of 21 μM and 17 μM, respectively. In conclusion, LKT1 may serve as a low-affinity influx pathway for K^+ into root hair cells. Comparison of homologous K^+_{in} rectifiers from different plant species expressed in the same heterologous system allows conclusions to be drawn in respect to structure-function relationships.

Key words: *Lycopersicon* (K^+ channel) – pH regulation – Potassium inwardly rectifying channel – Root hair – *Solanum* (K^+ channel) – *Xenopus* oocytes

Introduction

Plant roots are major organs for the uptake of nutrients and water from the soil. At least two distinct systems, with low- and high-affinity transport properties, participate in K^+ uptake from the soil and differ in their energy requirements (Maathuis and Sanders 1996). The role of root hairs, substantially extending the root surface up to 20-fold (Glass 1989), in the process of nutrient uptake into plant roots remains controversial. It was shown, by injection of dyes into epidermal cells of *Arabidopsis thaliana* roots, that mature root hair cells are symplastically isolated (Duckett et al. 1994). It was suggested by Duckett et al. (1994) that root hairs act as a sensor for the nutrient concentration in the soil solution but are not directly involved in ion uptake. However, at least the uptake of phosphorus by root hairs has been demonstrated recently (Gahoonia and Nielsen 1998). Furthermore, direct electrophysiological measurements at plasma membranes from root hairs of wheat revealed the presence of voltage-dependent potassium channels, which may function as a major component of a low-affinity potassium uptake system at K^+ concentrations above 0.5 mM (Gassmann and Schroeder 1994). Aluminum, known to interfere with root growth (Ryan et al. 1993) and to inhibit cation uptake (Miyasaka et al. 1989), blocks potassium uptake channels in root hairs of Al^{3+}-sensitive wheat (Gassmann and Schroeder 1994).

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Abbreviations: AKT = *Arabidopsis* K^+ transporter; IC_{50} = inhibitor concentration which inhibits half-maximally; K^+_{in} = inwardly rectifying K^+ (channel, current); LKT = *Lycopersicon* K^+ transporter; SKT = *Solanum* K^+ transporter

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The gene coding for the *Arabidopsis thaliana* potassium channel AKT1, which was isolated by functional complementation of a potassium-uptake-deficient yeast mutant (Sentenac et al. 1992), is mainly expressed in roots but also in hydathodes and leaf primordia (Basset et al. 1995; Lagarde et al. 1996). Promoter-β-glucuronidase studies demonstrated that *AKT1* is expressed in various root cell types, preferentially in peripheral cell layers including root hairs (Lagarde et al. 1996). In Northern blot experiments, a similar expression pattern was observed for a homologous potato gene, *SKT1*, for which the strongest expression was observed in roots and in epidermal fragments that are highly enriched with guard cells (Zimmermann et al. 1998).

Both AKT1 and SKT1 belong to the family of *Shaker*-like plant K^+ inwardly rectifying channels, which on a structural basis can be divided into at least two subfamilies (Czempinski et al. 1999). All channels of this family have in common six transmembrane spans (S1–S6), a K^+-selective pore domain between S5 and S6, a voltage-sensor within S4, a putative cyclic nucleotide-binding domain as well as an interaction domain at the C-terminus. The presence of ankyrin repeats within the cytosolic C-terminus distinguishes AKT- from KAT-like channels, which lack these repeats. Functional diversity among members of the AKT-like subfamily has been recently discovered by the characterisation of AKT2/3 (Baizabal-Aguirre et al. 1999; Marten et al. 1999) as a weakly rectifying channel and by the identification of SKOR (Gaymard et al. 1998) as a plant *Shaker*-like outward rectifier. Structure-function studies using random or site-directed mutagenesis aim at the identification of structural elements within the peptide sequences responsible for certain functional properties of the K^+ channels (reviewed in Dreyer et al. 1999). For instance, mutations of amino acids within or at the rim of the pore domain have been found to influence selectivity and voltage dependence of the studied channels (Becker et al. 1996). Besides mutation analysis, the comparative analysis of homologous channels from closely related plant species may give further hints as to functional determinants within the ion channel structure.

To further substantiate an involvement of root hairs in K^+ uptake and to compare two K^+ channels from closely related species, the present work was directed towards the isolation of a K^+ channel expressed in tomato plant root hairs. Using a polymerase chain reaction (PCR)-generated cDNA fragment of *AKT1* as a heterologous probe, we cloned an inwardly rectifying K^+ channel (K^+_{in}) from a tomato (*Lycopersicon esculentum* Mill.) root hair cDNA library. Electrophysiological studies using the *Xenopus laevis* oocyte expression system identified LKT1, the tomato AKT1 homologue, as an influx pathway for potassium ions. Interestingly, comparison of LKT1 properties with the most homologous ion channel SKT1 from potato expressed in Baculovirus-infected insect cells as well as within the same expression system, i.e. *X. laevis* oocytes, revealed a shift in the activation potential. Because only a limited number of amino acids are exchanged in LKT1 in comparison to SKT1 these findings allow the residues that might be responsible for the different properties to be identified. Thus, the identification and analysis of homologous ion channels in different species differing only in few amino acids provide a tool with which to study structure-function relations.

Materials and methods

Enzymes and chemicals. Enzymes for restriction and modification of DNA were obtained from Boehringer Mannheim. Other chemicals were purchased from Sigma or Merck.

Plants and bacteria. Escherichia coli was cultivated at 37 °C in standard yeast tryptone medium supplemented with appropriate antibiotics using standard methods. *Escherichia coli* strain XL1-Blue (Stratagene, La Jolla, Calif. USA) was used for DNA cloning procedures.

Tomato (*Lycopersicon esculentum* Mill. cv. Moneymaker; Hilmar, Hild Samen, Marbach, Germany) plants were grown under greenhouse conditions (14 h light, 16 °C/10 h dark, 10 °C, 50–80% relative humidity). Tomato seedlings for preparation of a root-hair-specific cDNA library and for testing gene expression in root hairs were grown on a metal mesh placed on paper (No. 0858; Schleicher & Schuell, Dassel, Germany) which had been soaked in half-strength Hoagland solution (Hoagland and Arnon 1950), in large Petri dishes under sterile conditions as described by Bucher et al. (1997).

Isolation of the LKT1 cDNA. A λ ZAPII cDNA library prepared from tomato root hairs (Bucher et al. 1997) was screened under low stringency in polyethyleneglycol (PEG) buffer (Amasino 1986), using a PCR-generated 1.2-kb DNA fragment, which matched the 5′ region, including the highly conserved membrane-spanning domain of the *AKT1* cDNA (Sentenac et al. 1992), as a radioactively labelled hybridisation probe. Plaque-purified phage clones were converted to pBluescript SK derivatives by *in vivo* excision according to the manufacturer's protocol (Stratagene). Four cDNA inserts between 2.4 kb and 2.9 kb were recovered and the longest cDNA was sequenced on both strands using [α-^{35}S]dATP (Amersham Life Science, Braunschweig, Germany) and the T7 Sequencing Kit (Pharmacia, Freiburg, Germany). Sequence analysis was performed with the help of the programs of the Genetics Computer Group (GCG Package, version 8.1; Madison, Wis., USA).

Extraction of RNA and Northern blot analysis. Plant RNA was extracted from greenhouse-grown tomato plants according to the method of Logemann et al. (1987). Thirty micrograms of total RNA was used for Northern blot analysis as described by Landschütze et al. (1995), using the radioactively labelled *Xho*I/*Ssp*I-fragment from the 3′ end of the *LKT1* cDNA as a hybridisation probe. Autoradiography using intensifying screens was performed overnight at −70 °C.

Expression in Xenopus *oocytes and electrophysiological analysis.* For construction of the oocyte expression plasmid, the *LKT1* cDNA was cloned as an *Eco*RI fragment into the *Eco*RI site of the expression vector pGEMHE. The *SKT1* cDNA (accession number: X86021) was cloned blunt into pGEMHE. The RNA was transcribed with T7 RNA polymerase from *Nhe*I- or *Sph*I-digested DNA, respectively, using the mMessage mMachine transcription protocol (Ambion Inc., Austin, Tex., USA). The cRNA quality (amount and presence of full-length transcript) was checked by loading a denatured cRNA sample on a formaldehyde-agarose gel. The cRNA resulting from *in vitro* transcription as well as subsequent protein expression in oocytes may be negatively influenced by the presence of 3′ overhangs in the linearised template DNA (Schenborn and Mierendorf 1985).

To achieve functional expression of SKT1, the *Sph*I-linearised plasmid pGEMHE-SKT1 had to be blunted before *in vitro* transcription.

Xenopus laevis frogs were purchased from H. Kähler (Hamburg, Germany). Oocytes were isolated as described elsewhere (Cao et al. 1992) and injected with ca. 20–30 ng of cRNA per oocyte using a microinjector (World Precision Instruments, Sarasota, Fla., USA). Electrophysiological measurements were made 2–5 d after the injection with a two-microelectrode voltage-clamp amplifier (GeneClamp 500; Axon Instruments, Foster City, Calif., USA) together with a Digidata 1200 interface 200B (Axon Instruments) using 0.5 to 1-MΩ pipettes filled with 3 M KCl. Membrane currents were measured in standard solution (K^+ Ringer) that contained 119 mM KCl, 1.8 mM $CaCl_2$, 1 mM $NaHCO_3$, 10 mM Hepes, 1 mM $MgCl_2$, adjusted to pH 7.4 with KOH. Variations of experimental solutions were as described in legends to corresponding figures. To study selectivities, external K^+ was replaced by Na^+ (Na^+ Ringer). Osmolarities were adjusted with sorbitol to 240–260 mosmol/kg using a vapor pressure osmometer (Wescor 5500, Wescor, Logan, Utah, USA). When added, aluminum was introduced as $AlCl_3$ from a stock solution of 10 mM $AlCl_3$ in 1 mM HCl to a modified bath solution consisting of 10 mM KCl, 1.8 or 0.2 mM $CaCl_2$, 1 mM $NaHCO_3$, 1 mM $MgCl_2$, 200 mM sorbitol, 10 mM Mes, adjusted to pH 4.5 with HCl/KOH. Free Al^{3+} activities were calculated by use of the program "GEOCHEM" (D.R. Parker, University of California, Riverside, Calif., USA) considering a solid phase at higher Al^{3+} concentrations.

Voltage-pulse protocols, data acquisition and analysis were performed with the pClamp 6.0.3 program suite (Axon Instruments). The filter frequency was set to 1 kHz; the acquisition time of data points was in the range of 2–10 ms dependent on the voltage protocols (usually 5 ms). Voltage protocols used are described in the figure legends. Tail currents were measured at the beginning of the second voltage pulse. The reversal potentials (E_{rev}) of these tail currents were determined from relationships of current transients versus potential. Permeability ratios were calculated according to Goldman-Hodgkin-Katz $\Delta E_{rev} = E_{rev,K} - E_{rev,Na} = R \cdot T/F \cdot \ln\{P_K \cdot [K^+]_o/(P_{Na} \cdot [Na^+]_o)\}$, where P_K and P_{Na} are the permeability constants for K^+ and Na^+; $[K^+]_o$, $[Na^+]_o$ are the external concentrations; *F*, *R*, and *T* have their usual meanings. To determine the current-voltage relations, leak subtractions were applied. Unless otherwise indicated, figures are shown for one representative cell, and statistics are given as mean ± SD (*n* indicates the number of cells tested). All experiments were performed at room temperature (20–22 °C). Dose-response curves of the Cs^+-inhibition were fitted by a Michaelis-Menten equation [$y = (Imax \cdot [Cs])/(IC50 + [Cs])$].

Analysis of the relative open probability. The dependence of the open probability on the membrane potential was derived from the I–V relationship of LKT1 and SKT1 deactivating currents. The value of the tail-current relaxation (i_t) when stepping back to the holding potential (−20 mV) after a 4-s prepulse was plotted against the membrane potential value (E, ranging from −160 mV to +40 mV). This I-V relationship was assumed to obey a Boltzmann law:

$$i_t = i_{tmax}(1/\{1 + \exp[z_g F(E - E_{a50})/(RT)]\}) \quad \text{(Eq. 1)}$$

All symbols are constants or experimental data except i_{tmax}, z_g, and E_{a50} which are the adjustable parameters. The constants *F*, *R*, and *T* have their usual meanings; i_{tmax} is the value of i_t which could be obtained following a full activation, E_{a50} is the potential of half-activation, z_g is the equivalent gating charge. The relative open probability P_0, with $P_0 = i_t/i_{tmax}$, of LKT1 ($n = 8$) and SKT1 ($n = 7$) currents was plotted against the membrane potential (E) and could be described by a second Boltzmann equation:

$$P_0 = 1/(1 + \exp\{z_g F[E - E_{af50}]/[RT]\}) \quad \text{(Eq. 2)}$$

E_{af50} is the potential of half-maximal activation derived from Eq. 2.

Results

Cloning of the LKT1 cDNA from tomato root hairs. To isolate an AKT1-homologous potassium channel from root hairs we used a root-hair-specific cDNA library of *Lycopersicon esculentum*. Screening of this library with an *AKT1* probe (see *Materials and methods*) resulted in the identification of four cross-hybridising phage clones with cDNA insert sizes ranging from 2.4 to 2.9 kb. Sequence analysis of the four cDNA inserts and comparison to known plant potassium channels revealed that the longest clone harboured a full-length cDNA coding for an 883-amino-acid polypeptide of 99.5 kDa. The complete cDNA sequence is available under GenBank accession No. X96390. Sequence comparison showed a high degree of homology between the novel protein and other plant *Shaker*-like potassium channels such as AKT1 (73% identical and 85% similar amino acids) from *Arabidopsis*, or SKT1 (97% identical and 98% similar amino acids) from potato (Fig. 1A). We named the newly identified protein LKT1 (for *Lycopersicon esculentum* homologue of AKT1). The hydrophobicity profile of LKT1 predicts six membrane-spanning domains (S1 to S6) and a hydrophilic C-terminus as presented in Fig. 1B. Like its counterparts in *Arabidopsis* and potato, LKT1 possesses a highly basic region in the fourth transmembrane domain (S4), a putative K^+-selective pore-forming region (P) between the fifth and sixth membrane span, and a putative cyclic nucleotide-binding domain (cNBD) within its C-terminal half (amino acids 402–485). Single amino acid exchanges compared to the most homologous inward rectifier SKT1 are located at the N- and C-termini, within the S1–S2 and S2–S3 linker regions, and interestingly within the S4 domain (Fig. 1A,B). A further difference in amino acid sequence occurs in the outer rim of the pore region. At its very C-terminus, LKT1 possesses the interaction domain K_{HA}, which has previously been described as a general feature of plant *Shaker*-type K^+ channels (Ehrhardt et al. 1997). Such a domain structure is typical of all other previously isolated plant *Shaker*-like potassium channels belonging either to the KAT or AKT subfamily (Czempinski et al. 1999). The two subfamilies differ by the absence (KAT-like proteins) or presence (AKT-like proteins) of ankyrin repeats within the cytosolic C-terminal region. In the LKT1 polypeptide, six ankyrin repeats, which have been proposed to bind cytoskeletal proteins (Cao et al. 1995), are present. Evolutionary relationships between the various cloned plant *Shaker*-type K^+ channels from different species are presented in a dendrogram (Fig. 1C). Within the AKT1 branch, LKT1 and SKT1 are most closely related. Although the presence of the ankyrin repeats structurally distinguishes AKT- and KAT-like channels, sequence homologies group AKT1-like channels and AtKC1 more closely to the KAT branch than to AKT2 or SKT2, respectively.

Expression of the LKT1 gene. The presence of *LKT1* mRNA was analysed by Northern blot experiments. Total RNA isolated from various tissues of greenhouse-

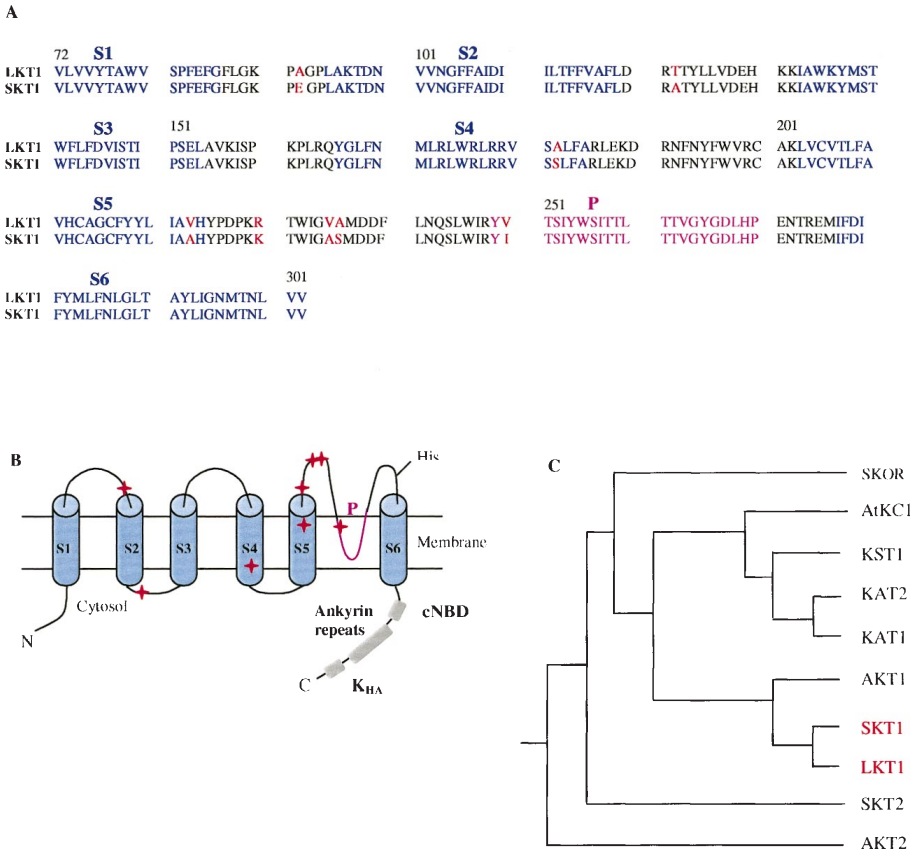

Fig. 1A–C. Relationship between tomato LKT1 and potato SKT1. **A** Partial sequence comparison between LKT1 and SKT1 in the range of transmembrane spans. *Blue*, transmembrane domains S1 to S6; *pink*, pore domain; *red*, amino acid exchanges. Accession numbers: LKT1, X96390; SKT1, X86021. **B** Structural model of the LKT1 protein. *S1* to *S6*, transmembrane segments; *P*, pore region; *cNBD*, putative cyclic-nucleotide-binding domain; ankyrin-like repeats; K_{HA}, C-terminal homology domain; *His*, conserved His residue at the outer pore domain. Amino acids diverging between LKT1 and SKT1 are marked by *red crosses*. Further differences, which are not indicated, occur within the cytosolic N- and C-terminus. **C** Phylogenetic tree of cloned plant potassium channels. The dendrogram (unrooted) was calculated with the help of the Phylip program suite (Department of Genetics, University of Washington, USA) using the implemented DNA parsimony algorithm (version 3.573C). Open reading frames of cloned *Shaker*-like K^+ channels from *Arabidopsis*, potato, and tomato were used for the comparison. Accession numbers: SKOR (AJ 223357); AtKC1 (U81239); KST1 (X 79779); KAT2 (U25694); KAT1 (M86990); AKT1 (X62907); SKT2 (Y09699); AKT2 (U40154)

grown tomato plants was hybridised to an *LKT1*-specific fragment from the 3′-end of the cDNA. Figure 2A shows that the highest *LKT1* transcript level was present in roots. A much weaker expression was detected in leaves, whereas in buds and stems of tomato almost no *LKT1* mRNA was measurable. This result is in agreement with the previous observation that *AKT1* and *SKT1* are expressed in roots of *Arabidopsis* and potato, respectively (Basset et al. 1995; Lagarde et al. 1996; Zimmermann et al. 1998). Because the *LKT1* cDNA was isolated from a root-hair-specific library, we analysed *LKT1* expression in root hairs of tomato seedlings (Fig. 2B). For the preparation of root hairs we used the freeze-fracture method in which roots of seedlings are stirred in liquid nitrogen (Röhm and Werner 1987). This procedure allows a very pure root hair fraction to be sampled from the surface of the liquid nitrogen; the remaining roots, contaminated with only a small amount of root hairs, concentrate at the bottom of the nitrogen container. A clear enrichment of *LKT1* transcript was found in the root hair fraction in comparison to the root fraction.

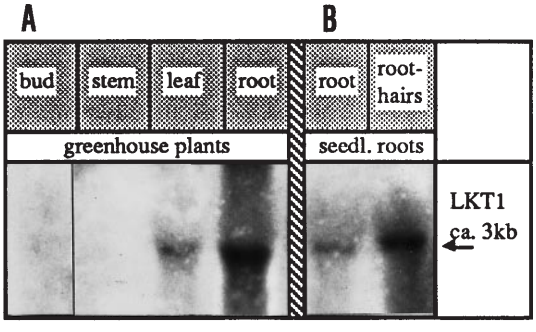

Fig. 2A,B. RNA blot analysis of *LKT1* gene expression in different *Lycopersicon esculentum* tissues. Total RNA (30 µg per lane) extracted from the various tissues was separated on formaldehyde-agarose gels and transferred to nylon membranes. The Northern blots were hybridized with an *LKT1*-specific 390-bp *Xho*I/*Ssp*I-fragment from the 3′ end of the cDNA. **A** Expression of *LKT1* in different tissues of greenhouse-grown plants. **B** Expression of *LKT1* in root hairs and the remaining part of the roots of tomato seedlings

Functional expression of LKT1 in Xenopus laevis *oocytes.* The *LKT1* cDNA was found not to functionally complement a yeast mutant (CY162; Ko and Gaber 1991) deficient in K^+ uptake (data not shown). For electrophysiological studies, oocytes of *X. laevis* were tested as a heterologous expression system enabling the comparison of the LKT1 channel with other heterologously expressed plant ion channels. Notably, comparison of two ion channels differing in only very few amino acids, as is the case for tomato LKT1 and potato SKT1, might provide clues on functional determinants arising from naturally occurring sequence variations.

Oocytes injected with *LKT1* cRNA were assayed for K^+ inward currents using the two-electrode voltage-clamp method 2–5 d after injection. In frog K^+-Ringer solution, in which Na^+ is replaced by K^+, hyperpolarising pulses elicited slowly activating negative currents

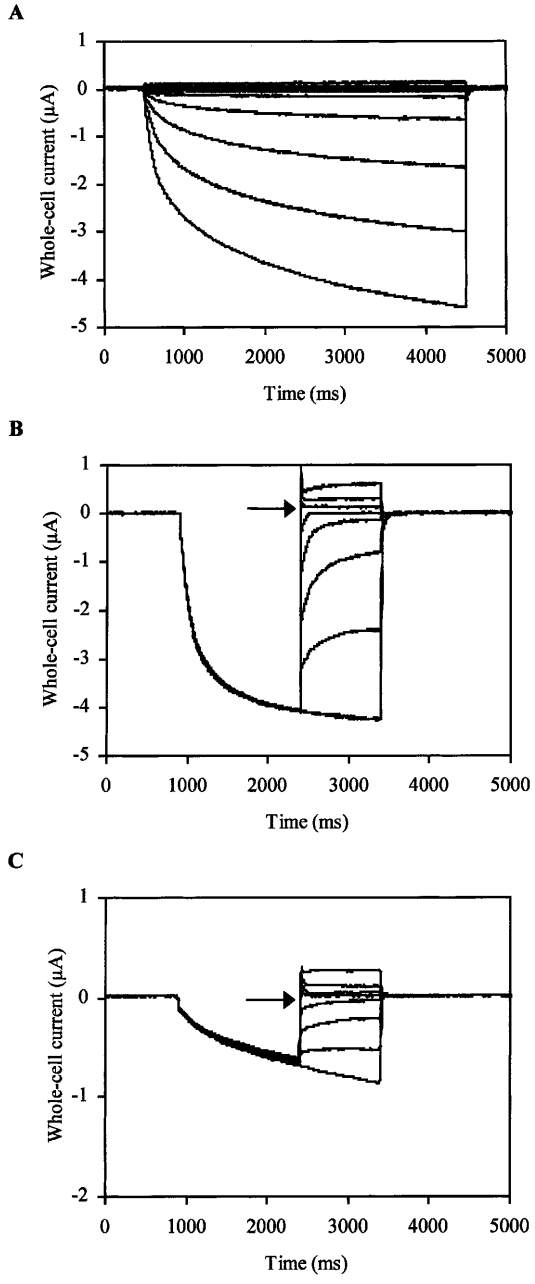

Fig. 3A–C. Electrophysiological characteristics of LKT1 expressed in *Xenopus* oocytes. The cells were measured by means of the two-electrode voltage-clamp technique 2–5 d after injection of cRNA. **A** Hyperpolarisation-activated LKT1 currents shown for one representative oocyte injected with approx. 25 ng of *LKT1* cRNA. Voltage pulses (4 s) were applied every 10 s from +40 to −160 mV in 20-mV increments from a holding potential of −20 mV. **B** LKT1 tail currents elicited by double-voltage pulses. Inward currents were activated at −140 mV (1.5 s) from a holding potential of −20 mV. Current transients in response to a second voltage pulse (1 s) from −140 to +70 mV in 30-mV increments were recorded. The *arrow* marks the reversal potential in 119 mM external potassium. **C** LKT1 tail currents elicited as in **B** in 12 mM external potassium

(Fig. 3A). To analyse the selectivity of LKT1 we measured the current in two different external K^+ solutions (119 mM and 12 mM) using double-pulse experiments (Fig. 3B,C). The reversal potential E_{rev} shifted towards more negative potentials in the low external K^+ concentration according to the expected direction of the shift of the Nernst potential. However, the absolute value of the E_{rev} shift (−49 ± 10 mV, n = 12) did not always reach −58 mV, suggesting an influence of other endogenous permeabilities or permeation of other ions. Therefore, we measured the permeability of LKT1 to externally applied sodium. The current was strongly reduced in the K^+-free solution (Na^+ Ringer) with a permeability ratio P_{Na}/P_K of 0.035 ± 0.02 (n = 7, two different oocyte batches), demonstrating that LKT1 is a highly selective K^+ channel.

Functional comparison of LKT1 and SKT1, upon expression in Xenopus *oocytes.* Despite the high degree of homology between the two channels, the biophysical properties of LKT1 (such as current activation and sensitivity towards pH or Cs^+) were apparently different from those originally determined for SKT1 after expression in Baculovirus-infected insect cells (Zimmermann et al. 1998). To clarify whether these differences are due to expression systems (for review, see Dreyer et al. 1999) or whether they represent functional differences arising from variations in protein sequence we tried to express SKT1 in *Xenopus* oocytes. Although SKT1 was previously found not to be functionally active in oocytes (Dreyer et al. 1997; Zimmermann et al. 1998), as is also the case for the *Arabidopsis* homologue AKT1 (Gaymard et al. 1996), we were recently able to measure SKT1-mediated K^+_{in} currents in oocytes, after slightly modifying the protocol for preparing the injected cRNA (see *Materials and methods*). Functional expression of SKT1 in oocytes therefore made it possible to directly (i.e. in the same expression system) compare its properties with the characteristics of LKT1 from tomato.

Current-voltage relations of *LKT1*- and *SKT1*-injected oocytes displayed inward rectification of the resulting currents upon hyperpolarising pulses (Fig. 4A). However, the activation potential of the LKT1-mediated current at around −50 mV was found to be shifted by 20 mV to more positive voltages when compared with potato SKT1. The SKT1 channel activated at −70 mV when expressed in oocytes (at −60 mV after expression in insect cells; Zimmermann et al. 1998). Analysis of the relative open probability P_0 revealed that the potential of the half-maximal open probability is shifted by 18 mV to more-positive values in LKT1 (E_{a50} = −99.4 ± 6.2 mV, n = 8) in comparison to SKT1 (E_{a50} = −117 ± 10.2 mV, n = 7). The gating charge z_g of both channels was found to be in the same range with 1.84 ± 0.29 for LKT1 and 1.91 ± 0.37 for SKT1. The P_0-V relationship of both channels could be described by a Boltzmann equation (see *Materials and methods*) revealing similar values (see Fig. 4B) for E_{a50} and z_g. The positive shift of E_{af50} in LKT1 (15 mV) was similarly pronounced after fitting the data points, whereas the calculated value of the gating charge seems to be the same for both channels. The activation potential E_a was not appreciably dependent on the external K^+ concentrations (data not shown).

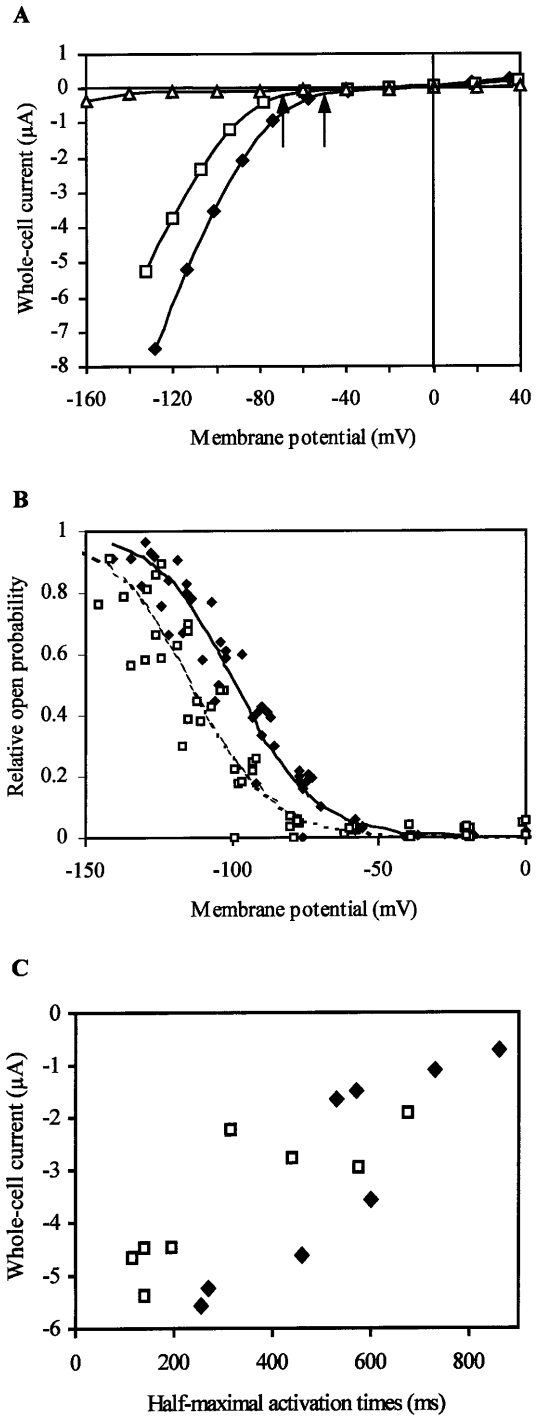

Fig. 4A–C. Mean currents in *LKT1*- and *SKT1*-injected oocytes. **A** Current-voltage relation of the mean 'quasi' steady-state currents at the end of 4-s voltage pulses derived from control oocytes (n = 10; water-injected; △), oocytes injected with *LKT1* cRNA (n = 8; ♦), or with *SKT1* cRNA (n = 8; □), respectively. The activation potentials (E_a) of LKT1 and SKT1 were around −50 mV and −70 mV, respectively (*arrows*). **B** Boltzmann fit of the relative open probabilities (P_0) for LKT1 (n = 8; *solid line*) and SKT1 (n = 7; *broken line*). The symbols represent the calculated P_0 values from tail-current measurements of LKT1 (♦) and SKT1 (□). Parameters for the Boltzmann fit were: E_{af50} = −99 mV (LKT1) and −114.5 mV (SKT1); z_g = 1.75 (LKT1) and 1.72 (SKT1). **C** Dependence on current amplitudes of half-maximal activation times ($t_{1/2}$) of LKT1 (♦) and SKT1 (□) currents at −140 mV

Activation kinetics of LKT1- and SKT1-mediated currents could be fitted by a multi-exponential function. Half-activation times, representing the time in which half-maximal amplitudes of the 'quasi'-steady-state currents at the end of the 4-s pulse are reached, were determined at −140 mV. These half-activation times were dependent on the amplitudes as shown in Fig. 4C and ranged from 115 to 860 ms. A similar dependence of activation kinetics was shown for KAT1, depending on the amount of injected RNA (Véry et al. 1994). No current inactivation was observed for pulses lasting as long as 9 s (data not shown).

A basic pharmacological property of plant *Shaker*-like K^+_{in} channels of the KAT and AKT subfamily is their inhibition by externally applied Cs^+ (Hedrich et al. 1995; Müller-Röber et al. 1995; Véry et al. 1995) which also had been found to inhibit K^+_{in} currents *in planta* (Tester 1990). Application of Cs^+ to the bath solution decreased the inward currents of LKT1 (Fig. 5A) and SKT1 within a few minutes. Tail current experiments demonstrated that the inhibition was dependent on the applied potentials (Fig. 5B) as previously observed for KAT1 (Véry et al. 1994) and KST1 expressed in oocytes (Müller-Röber et al. 1995), or SKT1 expressed in insect cells (Zimmermann et al. 1998). The degree of inhibition was determined at −130 mV at different external Cs^+ concentrations and the dose-response curves revealed closely similar values for half-maximal inhibition of LKT1 (IC_{50} = 21 μM) and SKT1 (IC_{50} = 17 μM), respectively, when expressed in oocytes (Fig. 5C). We reported previously, that the IC_{50} for Cs^+ inhibition of SKT1 was moderately higher, 105 μM, when SKT1 was expressed in insect cells (Zimmermann et al. 1998).

Because aluminum often inhibits root growth and K^+ uptake, as well as K^+ channel activity in root hairs of Al^{3+}-sensitive wheat (Gassmann and Schroeder 1994), we tested the effect of different aluminum concentrations on the LKT1-mediated current. With external free Al^{3+} activity of up to 4 μM no inhibition of the K^+ current could be observed for LKT1 (n = 6) or for potato SKT1 (n = 3; data not shown). The insensitivity of SKT1 towards aluminum was confirmed in Baculovirus-infected insect cells (n = 3, data not shown).

It has previously been shown that extracellular acidification enhances K^+_{in} channel activities of the guard-cell channels KAT1 from *Arabidopsis* (Véry et al. 1995) and KST1 from potato (Müller-Röber et al. 1995). A conserved external histidine residue contributing to the pH sensitivity was identified in KST1 (Hoth et al. 1997) although other amino acids were recently shown to be involved in pH regulation in KAT1 (Hoth and Hedrich 1999). The histidine residue is also present in members of the AKT subfamily of K_{in} channels, including SKT1 for which pH-dependent current amplitudes were observed after expression in Baculovirus-infected insect cells: a current reduction of 56% at −140 mV was determined upon increasing the external pH from 5.5 to 6.6 (Zimmermann et al. 1998). The current reduction was found to be less pronounced, i.e. 13 ± 7% (n = 6), when SKT1 was measured in *Xenopus* oocytes. The same pH change in the external

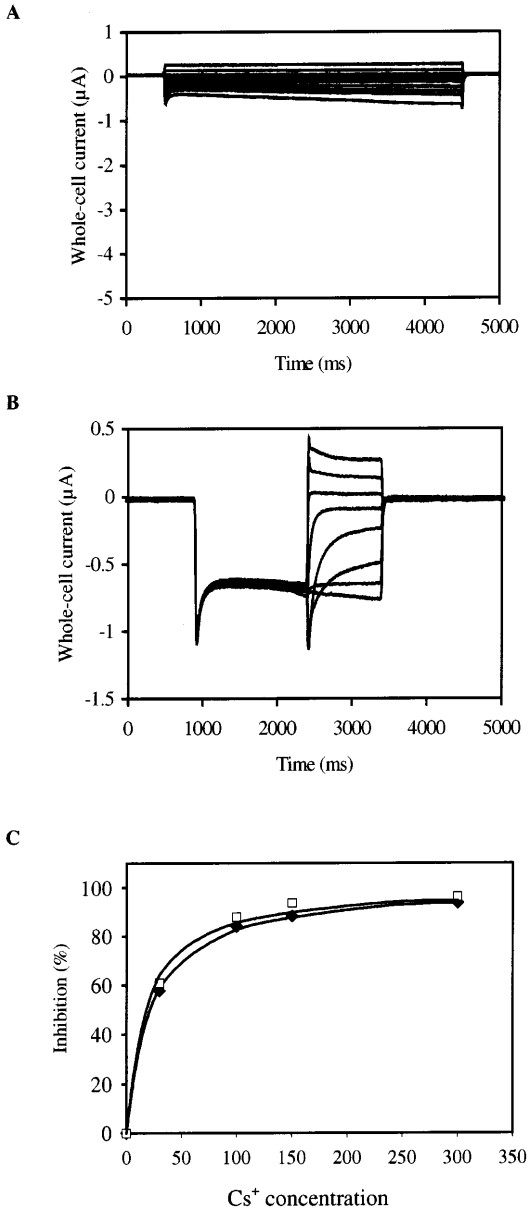

Fig. 5A–C. Cesium-inhibition of LKT1-mediated K^+_{in} currents. **A** Inhibition of hyperpolarisation-activated LKT1 current by 300 µM Cs^+ shown for one representative oocyte. The voltage protocol was as described in Fig. 3A. **B** Voltage-dependent block of LKT1 tail currents in the presence of 300 µM Cs^+ elicited by double-voltage pulses. Inward currents were activated at −140 mV (1500 ms) from a holding potential of −20 mV. Current transients in response to a second voltage pulse (1000 ms) from −140 to 70 mV in 30-mV increments were recorded. **C** Dose-response curve of current inhibition by externally applied Cs^+ of LKT1 (♦) in comparison to SKT1 (□). Percent inhibition was determined for 'quasi' steady-state currents at −130 mV and fitted by a Michaelis-Menten equation (see *Materials and methods*)

solution reduced currents at −140 mV by 18 ± 11% (n = 9) in oocytes expressing LKT1 (Fig. 6). The pH regulation was found to be reversible (data not shown) as previously seen for SKT1 in insect cells.

Taken together, we were able to functionally characterise LKT1 in *Xenopus* oocytes as a K^+ uptake channel

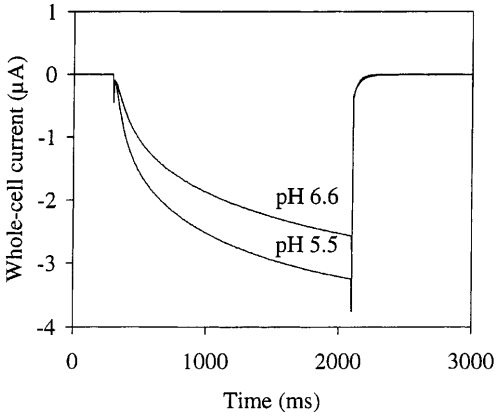

Fig. 6. Dependence of LKT1 currents on external pH. Decrease of current amplitude for a representative cell as a result of alkalinisation of the external pH from 5.5 to 6.6. Currents were elicited by 1.5-s voltage pulses to −140 mV from a holding potential of −20 mV. pH changes were achieved by titrating the standard solution with HCl/KOH to pH 5.5 and 6.6, respectively

from tomato root hairs. The biophysical features of LKT1-mediated currents were compared with those of the most homologous ion channel SKT1 from potato within the same expression system, revealing that very few diverging amino acids might influence sensitivity to voltage.

Discussion

A considerable number of ion channel genes, most notably of K^+ channels, have been identified from higher plants in recent years (for review, see Czempinski et al. 1999) and it is almost certain that numerous additional channels will soon be uncovered on the molecular level. Novel channels from various plant species, including crops, need to be carefully characterised with respect to their electrophysiological and biochemical properties through expression in heterologous systems, such as *Xenopus* oocytes or insect cells. So far, functional expression of plant K^+ channels in heterologous systems has mainly been demonstrated for K^+ channels from the genetic model plant *Arabidopsis thaliana* (for review, see Czempinski et al. 1999; Dreyer et al. 1999) as well as from potato (Müller-Röber et al. 1995; Zimmermann et al. 1998). The *LKT1* gene was cloned from a tomato root hair cDNA library via homology to *AKT1*. On the amino acid level it is very similar to *Arabidopsis* AKT1 (Sentenac et al. 1992) but even more similar to potato SKT1 (Zimmermann et al. 1998). The LKT1 channel was characterised by heterologous expression in *Xenopus* oocytes as an inwardly rectifying K^+-selective channel which is activated with slow kinetics by hyperpolarising voltage pulses. Similarly, AKT1 and SKT1 were previously shown to be inwardly rectifying K^+ channels when expressed in insect cells.

Clues about cell-type-specific functions of ion channels in relation to turgor regulation, nutrient uptake and nutrient transport can be expected from the determina-

tion of gene expression patterns. Dominant expression of *LKT1* in root hairs was confirmed by Northern blot analysis. All three homologous channel genes, i.e. *AKT1*, *SKT1* and *LKT1*, are expressed in root cells consistent with the hypothesis of a role in K^+ uptake from the soil. The role of the plant K^+ inward rectifiers in nutrient uptake has been recently emphasised by the finding that disruption of the *AKT1* gene in an *Arabidopsis thaliana* T-DNA-tagged mutant led to poor growth on low potassium concentrations in the presence of NH_4^+, as well as to reduced Rb^+ uptake (Hirsch et al. 1998). Electrophysiological measurements of ion channel activities at the plasma membrane of root cells and root hair cells have identified up to 10 different types of K^+-conducting channel as the dominant channel species (for review, see Maathuis and Sanders 1996). In root-hair protoplasts of wheat, Gassmann and Schroeder (1994) identified large, time-dependent inward currents highly selective for K^+ in a majority of cells. The absence of channel inactivation over a period of 10 min ensures sustained K^+ uptake. Inward currents activating at potentials more negative than −75 mV described for the wheat root-hair protoplasts are quite reminiscent of the properties of LKT1 expressed in oocytes, suggesting that LKT1 functions as a transport system for potassium uptake from the soil. Additionally, we tested the sensitivity towards aluminum, which often inhibits the growth of primary roots and root hairs at low pH, thereby limiting crop production in acid soils. Although Gassmann and Schroeder (1994) showed that K^+ inwardly rectifying currents of wheat root hairs were blocked by Al^{3+} at micromolar concentrations, we found that both cloned channels LKT1 and SKT1 were *not* blocked by Al^{3+}, indicating that they are not direct targets of phytotoxic aluminum.

The SKT1 channel was also found to be functionally expressed in oocytes (this study), basically confirming the data obtained with the insect cells (Zimmermann et al. 1998). The expression of LKT1, as well as of SKT1, in *Xenopus* oocytes makes it possible to directly compare these two highly homologous AKT-type channels within the same expression system. Sequence comparison revealed only a rather limited number of amino acid exchanges, especially within the voltage-sensor S4 and in the region near the rim of the pore domain, as well as in the N- and C-terminus and in two linker regions between membrane spans S1–S2 and S2–S3. These naturally occurring sequence variations are obviously responsible for differences in the biophysical properties of the two channels. The activation potential of LKT1 compared to SKT1 is shifted by 20 mV towards more positive voltages, pointing to the amino acid exchanges near the pore region as well as possibly within the S4 segment. Structure-function studies with KAT1 demonstrated that mutations close to the rim of the pore affect activation potentials, indicating an interaction of these amino acids with the voltage sensor (Becker et al. 1996). Interestingly, the amino acid L251 within the pore region of KAT1 is conserved within KST1, and mutation of this amino acid in KAT1 to isoleucine (L251I) shifted the half-activation potential to more positive potentials. The corresponding leucine residue is replaced by isoleucine (I253) in LKT1 and SKT1, and the channel's activation is shifted to more-positive potentials when compared with KST1 or KAT1. Furthermore, the difference in activation seen for LKT1 and SKT1 might be explained by an amino acid variation (V250 in LKT1, I250 in SKT1) in the same region. Besides that, it is also probable that further exchanges between S5 and the pore domain influence activation of the channels.

Regulation of LKT1 and SKT1 by extracellular pH might indicate a combination of membrane hyperpolarisation and extracellular acidification for stimulation of K^+ uptake. The observed difference in pH-sensitivity for SKT1 when expressed in insect cells versus *Xenopus* oocytes indicates that the heterologous expression system influences the functional behaviour of the channel.

Plant growth and activity of K^+ channels are extremely sensitive to extracellular Cs^+ (e.g. Tester 1990; Sheahan et al. 1993). Mutation of KAT1 within the pore domain reduced the sensitivity of this channel towards external Cs^+ and, after expression in transgenic plants, led to Cs^+-resistant stomatal opening (Ichida et al. 1997). Here we showed that the tomato-root hair K^+ inward rectifier LKT1 exhibits a voltage-dependent inhibition with an IC_{50} of 21 μM at −130 mV when expressed in *Xenopus* oocytes. A very similar value (17 μM) was found for potato SKT1 when expressed in oocytes, whereas the IC_{50} value previously determined in insect cells was higher (around 100 μM), which probably resulted from fitting to a more sigmoidal dose-response curve (cf. Zimmermann et al. 1998). For AKT1 expressed in yeast, a Cs^+ block with an IC_{50} in the range of 20 μM was found by Bertl et al. (1997). Therefore, it appears that the Cs^+ sensitivity is highly conserved between channels of the AKT-type.

Taken together, we have shown that LKT1, a K^+-selective ion channel cloned from a root-hair-specific cDNA library and heterologously expressed in *Xenopus* oocytes, may account for a potassium influx pathway into root hairs of tomato. Tomato LKT1 and potato SKT1 were found to differ in voltage dependence, suggesting that the limited number of exchanged amino acids between the two channels plays a role in controlling functional behaviour.

We thank J. Riesmeier (PlantTec Biotechnologie, Potsdam-Hermannswerder, Germany) for his contribution during the initial phase of this project. We also thank G. Plesch for critical comments on the manuscript, D.R. Parker (Dep. Soil Environ. Sci., University of California, Riverside, USA) for helpful advice on the GEOCHEM program, and Sebastian Kloska for his help with the Phylip program suite.

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