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Functional diversity of potassium channel voltage-sensing domains

León D. Islas
Departamento de Fisiología, Facultad de Medicina; National Autonomous University of Mexico (UNAM), Ciudad Universitaria, México City, México

ABSTRACT
Voltage-gated potassium channels or Kv’s are membrane proteins with fundamental physiological roles. They are composed of 2 main functional protein domains, the pore domain, which regulates ion permeation, and the voltage-sensing domain, which is in charge of sensing voltage and undergoing a conformational change that is later transduced into pore opening. The voltage-sensing domain or VSD is a highly conserved structural motif found in all voltage-gated ion channels and can also exist as an independent feature, giving rise to voltage sensitive enzymes and also sustaining proton fluxes in proton-permeable channels. In spite of the structural conservation of VSDs in potassium channels, there are several differences in the details of VSD function found across variants of Kvs. These differences are mainly reflected in variations in the electrostatic energy needed to open different potassium channels. In turn, the differences in detailed VSD functioning among voltage-gated potassium channels might have physiological consequences that have not been explored and which might reflect evolutionary adaptations to the different roles played by Kv channels in cell physiology.

KEYWORDS
gating charge; potassium channels; voltage-activated channels; voltage sensing domain

Introduction
Voltage-gated potassium channels are central to many physiological processes and have also played a fundamental role in our present understanding of the biophysical mechanisms of electrical excitability. Collectively referred to as Kv channels, they are responsible for a variety of electrical phenomena, including, but not limited to, the repolarization of the action potential, spike frequency adaptation, synaptic repolarization, smooth muscle contraction, etc. This diversity in the physiological roles of Kv channels is mirrored by functional diverse behaviors. Although generally activated by depolarization, this activation occurs over varying ranges of voltages and with diverse voltage-dependences. Also, their kinetics is highly variable, ranging from classical delayed rectifiers to rapidly inactivating A-type currents. In spite of this diversity, we now know that the central mechanism of detection of voltage changes by Kv channels can be localized to the first 4 transmembrane segments, which assemble into a well-defined domain called the voltage sensing domain or VSD.

One of the big surprises of the past decade is the discovery that these VSDs can adopt a stable conformation in the absence of the rest of the channel, having functional properties in the absence of the pore domain and can be used to gate pore domains of different channels. In fact, VSDs exist as independent gene products, coupled to other kinds of proteins, as demonstrated by the voltage-sensitive phosphatases and can even mediate proton transport, as shown in the voltage-sensitive proton channels. As diverse as the functions of VSDs may be, they all work by converting electrostatic energy into conformation changes and it seems that the basic mechanism of voltage sensing is the same. In the case of Kv channels, voltage sensing by VSDs is the initial step in channel function. Coupling of VSD function to gating of the pore domain is essential. However, this review will focus on the VSDs of voltage-gated potassium channels, in an attempt to portray a comparative picture.

Known structures of voltage sensing domains: a common theme
Voltage-dependent potassium channels (Kv) have been the most studied class of voltage-dependent
channels. Since the cloning of the Shaker channel, it was recognized that the functional channel is a tetramer and that each subunit is formed by 6 transmembrane domains.\textsuperscript{15-17} The first 4 where postulated to be responsible for voltage sensing, while the S5-P-S6 segments were rapidly identified to encompass the pore domain and regulate permeation.\textsuperscript{17} This organization has been widely confirmed not only for Kv channels, but also for many other related channel proteins that comprise a superfamily of Kv-like channels.\textsuperscript{18-20} There exists a very rich literature, mostly based on mutagenesis and functional studies, which has unveiled fascinating biophysical insight into these proteins.

The first voltage-sensing domain (VSD) of a Kv channel, and indeed the first VSD of any other channel, to be gleaned in molecular detail was part of the crystal structure of the KvAP potassium channel.\textsuperscript{21} This pioneering structure showed a rather unexpected arrangement of the VSD, where the S3b-S4 regions, dubbed the “paddle,” were oriented toward the intracellular region. It is now recognized that the first KvAP structure was likely distorted by crystallization conditions and its structure has been corrected and shown to be consistent with later structures of other VSDs.\textsuperscript{22,23}

Following the structure of KvAP, the next VSD to be defined at atomic level and considered a native, non-perturbed structure was part of the now paradigmatic structure of the Kv1.2 mammalian potassium channel.\textsuperscript{24} This structure showed that the VSDs interact with the pore domain of an adjacent subunit and stand at the periphery of the whole channel. The resolution and usefulness of this structure was soon improved when the structure of a chimaera between Kv1.2 and the Shab-like mammalian Kv2.1 channel was also solved by X-ray diffraction.\textsuperscript{25} This chimera is comprised of most of the Kv1.2 sequence and it has a replacement of a turn-helix structure from Kv2.1, effectively replacing the S4 segment. In this structure, often referred to as the “paddle chimaera,” the VSD is thought to be in the activated position, while the pore domain is likely in an open-desensitized state.\textsuperscript{26}

The structure of the paddle chimaera has allowed rationalization of much of the functional information obtained in several other Kv channels and also is a framework for modeling efforts that make use of molecular dynamics simulations. This has pushed forward the construction of models of the open and closed states and calculations of the possible molecular motions involved in channel gating. There have been several efforts aimed to this end\textsuperscript{27-30} and we will touch upon them further into the text.

Apart from the 2 structures derived from Kv1.2, other voltage sensors from related proteins have been recently solved by X-ray crystallography and cryo-electron microscopy. Almost simultaneously reported, the structures of the proton channel Hv1 and the voltage-sensitive phosphatase, were solved by X-ray diffraction.\textsuperscript{8,31,32} Remarkably, the structure of CiVSP was obtained in putative activated and resting states, while the structure of Hv1 seems to be of a resting state.

Recently, the structure of 2 TRP channels, TRPV1 and TRPA1 were solved by cryo-electron microscopy, and although there is ambiguity as to the assignment of amino acid side chains and the state of the structure solved, the arrangement of both VSDs is extremely similar to that of the paddle chimera.\textsuperscript{33,34}

The voltage-sensing domains discussed up to here, even though fulfilling very different physiological roles, are structurally very similar. In Figure 1, I compare these structures and as can be seen, it is possible to align all the VSDs to the structure of the paddle chimera. This structural alignment shows that the 4 transmembrane segments occupy essentially the same relative positions. A large portion of the VSD, at least as seen in the high-resolution paddle chimera, is in contact with the lipid membrane. In fact, the paddle chimera shows specific interactions between lipids and amino acid residues in the S4 and other parts of the VSD.

Apart from the common 4-transmembrane domain architecture, one of the main structural themes in the design of VSDs is their appearance as an hourglass. In this description of the VSD, the upper and lower parts of the hourglass represent large vestibules that are likely occupied by several water molecules, as illustrated in Figure 2. Such structural motif has very important repercussions for the mechanism of voltage sensing, as will be discussed shortly. The presence of water in these vestibules has been postulated on the basis of continuum electrostatic calculations in Shaker channels,\textsuperscript{35,36} and the observation that waters occupy these regions in molecular dynamics calculations of Kv1.2 channels.\textsuperscript{28,29,37} Water occupancy of the vestibules of VSDs is also implied by the proposed mechanism of proton permeation via water wires in Hv1 channels\textsuperscript{38} and the existence of proton and alkali ion currents in certain mutations of the VSD of Shaker.
Figure 1. Structures of known voltage sensing domains from voltage-activated proteins. Ribbon representation of the VSD domains formed by the S1 to S4 transmembrane segments from voltage-gated proteins whose atomic structure has been solved by X-ray crystallography. The structures represented and corresponding PDB accession numbers are: Kv1.2 (2A79), Kv1.2–2.1 chimera (2R9R), Kv1.2 model (available at: http://www.ks.uiuc.edu/Research/kvchannel/), CiVSP (4G7V), Hv1-CiVSP chimera (3WKV). All these structures are thought to represent activated states. The lower right panel is a structural alignment of all 5 VSDs and it shows that the 4 transmembrane segments occupy the same relative positions and that the S4 helices (illustrated as ribbons) in the activated state have undergone a similar conformational change.

Figure 2. Schematic representation of the VSD domain and its electrostatic properties. (A) Cartoon of the transverse section (normal to the membrane) of an idealized VSD showing its hourglass like shape. Regions marked as 3 and 1 represent the water-filled cavities that allow focusing of the electric field. This focusing occurs mostly at the septum region, identified by 2. The membrane potential is indicated by the values of $\phi$. (B) The distribution of the membrane potential across the VSD as calculated by computationally solving the linearized Poisson-Boltzmann equation. In this example, the different color isopotential lines go from zero mV in the intracellular solution to 250 mV-applied potential across the membrane. Focusing of the field at the septum region is indicated by an increased slope in the graph at right, which plots the potential, $\phi$ as a function of axial distance normal to the membrane.
and Na channels, specifically of the charged residues in S4 and S2. \(^{39-42}\)

The large degree of structural conservation suggest that the mechanism of activation of all voltage sensors is also shared among them, and important clues have been obtained from studies of all of these VSDs.

**Molecular mechanism of voltage sensing**

Electrostatic energy is stored in the transmembrane voltage difference that is common to all cells. In neurons, this potential difference at rest is in the order of 60 mV, the intracellular side being negative with respect to the extracellular solution, and during an action potential can be larger than +40 mV. This means that a positive test charge placed in the homogeneous electric field that correspond to these voltages, will experience a potential energy of \(e_C V 9.6 \times 10^{-21}\) Joule or 2 \(k_B T\). This energy is large and can be used to perform work that manifests as a change in conformation of the channel protein.

A key characteristic of the design of the VSD module is the presence of several charged amino acid residues. Most striking are the positive arginines and lysines of the S4 segment that in all VSD appear as repeating motives of one positive charge separated by 2 hydrophobic residues. The total number of positive charges in S4 is variable, going from 7 in Shaker and some Shaker related channels, to 2 or 3 in Hv1 proton channels and voltage-sensitive phosphatases. Moreover, each of these positive charges makes a contribution to the total electrostatic energy that is dependent on their physical position within the membrane electric field. Both the field and the location of the S4 charges should be different in each VSD.

It was proposed early on that the S4 charges interact with the membrane electric field and thus contribute to the work of producing a conformational change. \(^{43,44}\) Experimentally this contribution has been demonstrated for only a handful of ion channels. \(^{45-47}\) As the transmembrane voltage changes polarity during a depolarization and the cytoplasmic side becomes positive, the energy on the S4 charges changes and thus the whole S4 undergoes movement, which is directed in the outward direction. This conformational change alters the molecular interactions of the S4 with other parts of the VSDs and of these with the rest of the channel, leading to another conformational change that opens access to the conduction pore.

How big is the movement of the S4 segment? This is a question that for some time received several, sometimes divergent, answers. Early demonstration of voltage-dependent S4 movements came from cysteine accessibility measurements in Na channels. \(^{48}\) Replication of these results in K channels showed that accessibility of introduced cysteine residues in the Shaker S4 was a function of voltage and occurred in a manner consistent with a large outward displacement of the \(\alpha\) helix upon depolarization. \(^{49,50}\)

Later measurements using spectroscopic techniques, particularly the LRET and another variation of FRET involving transfer between dyes and hydrophobic quenchers, suggested that the magnitude of movement was at most a couple of Angstroms (Å). \(^{51,52}\) In contrast, a proposal based on the KvAP structure and state-dependent avidin/biotin accessibility experiments suggested that the S3-S4, therein named the paddle, might traverse most of the membrane thickness in a rigid body motion. Although there is not a consensus, most models of voltage-dependent gating seem to suggest that the S4 segment translates several angstroms and undergoes a ~120 degree counter clockwise rotation. \(^{49,54}\) This protein motion can be exemplified by comparing the open state of the paddle chimera with models of the closed state. \(^{30}\) As can be seen in Figure 3, the displacement of the S4 helix in the closed and active states proposed by \(^{37}\) is in the order of 10 Å. Comparison of closed and open models of the paddle chimera used in the very long 200 \(\mu\)s simulations by Jensen, \(^{27}\) shows a displacement of up to 15 Å. Remarkably, after more than a decade of research into this particular question, we still do not have a definitive answer at hand.

In the absence of a convincing measurement of S4 displacement, different models of the VSD conformational change have been proposed to account for charge movement. A large body of experimental evidence has revealed some of the dynamic structural changes occurring during a cycle of voltage sensor movement.

At rest, the c-terminal portion of the S4 transmembrane domain is accessible to the intracellular solution. Several experiments have demonstrated that thiol compounds can modify introduced cysteines and that the rate of this reaction can be modulated by voltage. \(^{55,56}\) As a depolarization happens, these residues become less accessible and begin to be accessible from the extracellular solution. The net effect of this
movement is that the positively charged residues move with the rest of S4 to an external position. This is the main evidence for the existence of outward movement of the S4 of potassium channels\(^49,57-61\). The rest of the transmembrane domains seem to remain mostly static as the S4 moves, providing a physical and electrostatic scaffold\(^62,63\).

Given that the rest of the VSD remains static, as the charged residues in S4 move, they traverse its central portion, which contains an aromatic residue that has been dubbed the charge transfer center\(^64\). This residue is located at the region of the waist of the hourglass, and is the thinnest portion of the VSD structure, separating the outer and inner vestibules and keeping water and ions from traversing it.

During a depolarization, the conformational change seems to happen in discrete steps. This has been known from detailed kinetic analysis of the activation of several voltage-dependent potassium channels\(^65,66\) and is also reflected in some molecular dynamics simulations\(^27,29\) and in experiments tracking the movement of S4 with metal-ion bridges\(^67\).

Apart of a structural scaffold, the non-S4 segments of the VSD provide specific electrostatic interactions that stabilize the charged arginine and lysine residues. These charge-charge interactions are necessary to reduce the energy of charged residues in a low dielectric constant environment such as the lipid bilayer. Apart from the intra protein salt bridges, positive charges are stabilized by interactions with phospholipid head groups\(^68,69\) and hydration waters present in the inner and outer vestibules\(^28\).

It is worth mentioning that other voltage-gated potassium channels such as the cardiac potassium channel hERG, the sperm SpIH inward rectifier, a member of the HCN type of channels and the Slo maxi-K channels, seem to share the same mechanism of activation of the VSD domain described above\(^70-72\) possibly implying that the VSDs from these channels preserve the canonical structure described previously for Kv channels.

**Magnitude of the charge movement**

During S4 movement, positive charge is repositioned from the intracellular side of the VSD to the outside. When this charge displacement is measured from a large number of channels, it gives rise to a small transient current known as the gating current\(^73,74\) or sensing current in the voltage-sensitive phosphatases\(^12,75\).

The magnitude and time course of gating currents is determined by how much charge is translocated in the opening process and the rates of transitions that move said charges.\(^73\) Since the size of the gating current is also proportional to the number of channels contributing to the measurement, a more interesting measurement is how much charge each channel mobilizes.

It is currently impossible to measure the gating charge of a single channel because even in the best patch-clamp recording conditions, the current noise is several orders of magnitude larger than the expected single-channel gating current. The first calibrated measurement of the charge per channel was carried out in Shaker channels by normalizing the charge, obtained from the integral of the gating current by the number of channels, which was measured from the ionic current measured in the same patch. This pioneering experiment indicated that each channel moves at least 12.3 elementary charges (\(e_o\))\(^76\) Given that Shaker potassium channels are tetramers, this means...
that the activating conformational change of a single VSD contributes at least 3 e₀.

This calibrated measurement, with different methodologies to measure the number of channels, has been later repeated for Shaker by at least 4 groups, resulting in values of 13.6 e₀, 12.9 e₀, 13.6 e₀, and 13.3. The only other channel, for which a calibrated measurement of the gating charge is available, is the Kv1.2 rat potassium channel.

Charge per channel can also be estimated from the voltage dependence of channel opening. In this measurement, and only if the channel has a single open state and all the states previous to opening have to be traversed, the open probability of the channel is estimated when the probability of occupying the open state (Pₒ) is very low. This is usually achieved by measuring the rare openings occurring at very negative voltages in membrane patches containing a large number of channels. In these conditions, the voltage-dependence of Pₒ is exponential and can be estimated in a straightforward manner. For this estimate to be a good approximation to the gating charge, Pₒ has to be lower than 10⁻⁴ and preferably between 10⁻⁷ – 10⁻⁶.

Limiting-slope estimates of the charge per channel have been obtained for a number of voltage dependent potassium channels. Table 1 lists the gating charge estimates for those Kv channels for which the conditions for limiting-slope measurements mentioned above have been experimentally justified.

Other estimates of the gating charge from limiting slope experiments have been reported apart from those presented in Table 1. These vary widely, presumably due to the fact that the open probability reached during the experiments was not low enough. So for Shaker some groups have reported 10.6 e₀ while others have measured 16 e₀. For Kv1.2 and Kv2.1, the gating charge was estimated at 7.42 e₀ and 2.61 e₀, respectively. Other estimates obtained from whole-oocyte voltage-clamp (TEVC) recordings have been 6.2 e₀ for Kv1.1 and 3.6 e₀ for Shab. These last ones are probably in error due to voltage control problems in TEVC as well as the open probability not being low enough.

**Contribution of charged residues to the gating charge**

Since the arginines and lysines in the S4 are the main candidates for gating charges, they were studied by single-point mutagenesis. Two laboratories confirmed that neutralization of these residues in Shaker, which presumably eliminated movement of a positive charge, produced channels with reduced gating charge. Their results where consistent with basic amino acids contributing to gating charge, although the individual contributions sometimes are not what can be expected from a single positive charge traversing a fraction of the membrane electric field. For example, neutralization of the 3rd arginine, R368N reduced the charge to less than half of that of the WT channel.

In the Kv1.2 channel, neutralization of the first arginine also produces a reduction of the gating charge of more than half of the WT channel, while neutralization of other positive charges produces channels with reduced voltage dependence but less than expected for a neutralized charge per subunit. These results seem to imply that neutralization of S4 charges has a more profound effect on the structure of the VSD than a simple reduction of charge.

Finally, one study explored the role of negative countercharges in the S2 and S3 of Shaker, showing that one of the negative charges (E293) contributes to the gating charge. It is not clear if this effect is due to an inward movement of this residue, or a mutation-induced alteration of the structure of the VSD that reduces the magnitude of physical displacement of S4.

Experiments show that the magnitude of gating charge among potassium channels is variable. There is possibly a very good evolutionary reason for this variability that has to do with the specific function that channel subtypes play in cell physiology. Early on the description of cellular excitability, Hodgkin and then Adrian noted that the conduction velocity of action potentials in the squid axon would be a function of the number of channels, increasing with an augmented density of sodium channels and would be decreased by the increased capacitance contributed by

| Channel type | Charge per channel (LS), (e₀) | Charge per channel (Q/N), (e₀) |
|--------------|-------------------------------|------------------------------|
| Shaker       | 13(4) 12.1                    | 13.6, 12.9, 13.6, 13.3, 12.3 |
| Kv1.2        | 9.6                           | 9.9                          |
| Kv1.1        | 11.5(4)                       | NA                           |
| Kv2.1        | 12.5(4)                       | NA                           |
| Shab         | 7.5(4)                        | NA                           |

Notes: (4)55, (6)36, (45), (6)46, (47), (77), (8)76, (9)47, NA = Not available.
the charge movement of the VSD. This relationship implies the existence of a maximum conduction velocity as a function of total axon capacitance. The added capacitance contributed by a potassium channel with a large gating charge should produce a reduction of the conductance velocity. This possibility needs to be explored experimentally and theoretically.

Another consequence of the existence of varying amounts of gating charge will be described in the following section.

The electric potential profile and conformational energy

As seen in Figure 2A, the VSD structure is shaped as an hourglass. The waist region that separates the outside from the inside has been termed the septum and has recently been identified as the region that contains the charge transfer center. 64 This structure is remarkable, because it allows for the membrane electric field to be highly focused, 35,54,85,86 in a manner that echoes the focused fields of active sites in metaloenzymes. 87,88 Continuum electrostatic calculations that solve the Poisson-Boltzmann equation to find the membrane potential as a function of spatial coordinates, show that the magnitude of the field across the septum can be very high, in the order of 10–200 mV/Å at 100 mV (Fig. 2B). 35,36 For comparison, the field across the thickness of the whole lipid bilayer would be 3 mV/Å. One experimental determination is in general agreement with these theoretical estimates. 86 A major consequence of the focused electric field is that S4 displacement can be relatively small, not involving a movement across the whole thickness of the membrane, to accomplish the observed gating charge movement.

After the demonstration that the field is focused through the VSD, some models of charge movement proposed that the field distribution and thus the shape of the crevices should be different between the activated and deactivated states. Experimentally this is a difficult problem, but computational methods allow for an evaluation of this problem in models. Recently, through the analysis of several published structures of VSDs, and closed state models, it was proposed that the electrostatic structure of the VSD remains the same through the activation transition, and as a consequence, the distribution of the electric field is not altered 89 as the S4 moves outward and rotates. This result rules out an activation model that proposes field reshaping as the main conformational change involved in activation. 52

In normal conditions, the septum of the VSD has to remain impermeable to water and ions. Otherwise the transmembrane voltage difference would be short-circuited. Certain mutations, especially those that introduce a small amino acid residue in place of the first arginine in the Shaker S4, produce channels with a new conductance that allows non-specific permeation of alkali cations 39,42 and protons. 85 This remarkable conductance, called the omega (ω) current, seems to correspond to ions permeating a new pore formed though the middle of the VSD and is thus known as the ω-pore. These VSD currents have begun to show up in pathological conditions, especially in voltage-activated Na+ channels, where they are responsible for diseases like periodic paralysis and others. 41

Moreover, it was recently shown that a voltage-gated potassium channel from a flat worm, behaves as a weak inward rectifier and that the inward current is carried by a naturally occurring ω-current. 90 To date, this is the only potassium channel, apart from Shaker, in which ω-currents have been demonstrated.

The energy change associated with the conformational rearrangement of a VSD is dependent on the total amount of charge that moves during the transition. If this conformational change occurred between 2 discrete states, the total energy change could be readily measured, and equal to the product of the charge and the voltage difference, but VSDs have evolved in such a way that the charge movement is distributed between several smaller transitions that take place before the pore of the channel opens. If the total number of transitions and their voltage dependence were known precisely, it would be possible to obtain the energy change from this information. However, knowing these is always uncertain, even in the best-understood channels such as Shaker. Recently, a method was proposed that makes use of the median value of the charge-voltage relationship, or Q-V curve, and knowledge of the gating charge, to estimate the gating energy change, that is, the energy needed to go from the closed state occupied at negative voltages to the open state. 91 Using this definition, it becomes relevant to have good values for the amount of charge movement involved in channel activation. It was estimated that the energy change involved in opening Shaker channels is −14.19 Kcal/mol. In a recent detailed study of voltage gating in Kv1.2 the energy...
change upon voltage dependent opening was estimated to be $-7.5$ Kcal/mol. Since Q-V curves and good estimates of the gating charge of Shaker, Kv2.1 and Shab channels are available, the total energy change for voltage gating of these channels are calculated and shown in Table 2.

**Conclusions**

Potassium channels and specially the voltage-gated kind (Kv’s) are a diverse family of membrane proteins. This diversity has been widely documented and is reflected in the existence of potassium channels with different activation and deactivation kinetics, channels with slow and fast inactivation and wildly varying ranges of activation by voltage. The mechanism of activation by voltage has been extensively studied in Kv channels. Our current understanding is that the same mechanism is shared by several types of ion channels and even related proteins, such as voltage-gated phosphatases and involves the voltage-driven movement of transmembrane domains associated in a characteristic fold termed the voltage sensing domain or VSD. In spite of the shared mechanism, Kv channels have evolved variations on this theme. The main difference in regards to the VSD functionality seems to be the different sizes if the gating charge, which is the amount of equivalent electronic charges that are moved as the channels open in response to a large variation of voltage. These differences might have a functional consequence, since the gating charge contributes to membrane capacitance, potentially affecting the conduction properties of action potentials.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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**Table 2.** Calculated gating free energy for selected voltage-gated potassium channels.

| Channel type | Charge per channel (e<sub>0</sub>) | Median voltage (mV) | Gating free energy (Kcal/mol) |
|--------------|---------------------------------|--------------------|-------------------------------|
| Shaker       | 13<sup>(a)</sup>                 | -46.8<sup>(a)</sup>| -14.19<sup>(a)</sup>          |
| Kv1.2        | 9.6<sup>(b)</sup>                | -32.7<sup>(b)</sup>| -12.9<sup>(b)</sup>           |
| Kv2.1        | 12.5<sup>(c)</sup>               | -45<sup>(c)</sup>  |                               |
| Shab         | 7.5<sup>(d)</sup>                | -41.4<sup>(d)</sup>| 7.1<sup>(d)</sup>             |

Notes: <sup>(a)</sup>1,9,80; <sup>(b)</sup>47; <sup>(c)</sup>80 ;<sup>(d)</sup>Calculated according to<sup>91</sup> from data in.<sup>80</sup>
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