

PRIMARY STRUCTURE OF SQUID SODIUM CHANNEL DEDUCED FROM THE COMPLEMENTARY DNA SEQUENCE

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SUMMARY: The complete amino acid sequence of a sodium channel from squid *Loligo bleekeri* has been deduced by cloning and sequence analysis of the complementary DNA. The deduced sequence revealed an organization virtually identical to the vertebrate sodium channel proteins; four homologous domains containing all six membrane-spanning structures are repeated in tandem with connecting linkers of various sizes. A unique feature of the squid Na channel is the 1,522 residue sequence, approximately three fourths of those of the rat sodium channels I, II and III. © 1992

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The sodium channel is a voltage-gated ionic channel essential for the generation of action potentials (1, 2). The complete amino acid sequences of the neural sodium channels deduced by cloning and sequence analysis of the cDNAs have all been limited to vertebrates; that is, those from the electric organ of the eel *Electrophorus electricus* (3) and from three distinct sodium channels (designated rat sodium channels I, II and III) of rat brain (4, 5). For invertebrate sodium channels, a partial amino acid sequence deduced from *Drosophila* genomic clones isolated with a vertebrate sodium channel complementary DNA probe has so far been deduced (6). Squid is another important invertebrate for neurophysiology (1, 7). The squid giant axon has long been used for experiments to elucidate molecular mechanisms both of action potential generation and transmission (1, 8), axon-Schwann cell interactions (9) and of axonal transport (10). The present paper reports the isolation of the protein-coding sequence of the squid cDNA and the complete amino acid sequence of this novel sodium channel deduced from the cDNA sequence. Comparisons of the amino acid sequence of the squid sodium channel are made with those of the vertebrate and *Drosophila* sodium channels.

MATERIALS AND METHODS

PCR: We adopted the PCR (polymerase chain reaction) method (11) to isolate part of squid sodium channel gene from genomic DNA of the optic lobe of squid *Loligo bleekeri*. The live squids were captured in the Sagami Gulf of Japan and transported to the Electrotechnical Laboratory (12) where they were sacrificed for the present experiments. Reaction solution for the PCR contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 mM NTP, 200 mM DNA primers and 25 units/ml Taq DNA polymerase (Cetus Co.) (13). For the DNA primers, we synthesized several 20 - 24 mer mixed oligonucleotides based on the

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amino acid sequences commonly conserved among rat I, II & III and eel Na channels, as shown in Fig. 1a. The DNA primers were synthesized with the Cyclone plus DNA synthesizer (Milligen Co.). The PCR was performed in the 100 µl reaction solution with the DNA primers under the conditions of 94°C for 1 min, 54°C for 2 min and 72°C for 3 min in succession, and subjected to 50 cycles of the amplification. DNAs thus amplified were analyzed with 1.5% agarose gel electrophoresis. Major bands excised from the gel were ligated into the M13 mp19 vector, respectively, to be amplified and sequenced with an automatic sequencer (ABI 370A). We also adopted the inverse PCR method (14). For this, 1 mg squid genomic DNA was digested with 1 unit of restriction endonuclease Alu I at 37°C for 30 min, to be ligated with T4 DNA ligase. Circular DNA was used for this template to carry out the inverse PCR with the primer shown in Fig. 1b under the same conditions for the PCR as described above. Amplified DNA was ligated into Puc 119 and M13 mp19 vectors.

cDNA cloning: Total RNA was extracted from the squid optic lobe by the guanidium thiocyanate method (15). Poly (A)⁺ RNA was isolated by repeating oligo (dT)-cellulose chromatography twice (Pharmacia). cDNA was synthesized from 5 mg mRNA of adult squid optic lobe with reverse transcriptase (Amersham, RPN.1256Y). The cDNAs were ligated into λZAP II and transfected into XL-1 Blue cells. The cells were then inoculated into 35 culture dishes (Greiner 94/16) and transferred to the nylon membrane (Amersham, Hybond-N). The filters were treated with alkaline, neutralized and irradiated with UV to crosslink the DNA to the filter. Hybridization with the DNA probe was performed in a hybridization buffer (6X SSC, 5X Dehaldt's solution, 0.5% SDS, 20 mg/ml Sermon sperm DNA) at 65°C over night. After hybridization, filters were washed in 0.2X SSC at 65°C for 20 min.

Sequencing: Insert of each clone was digested with exonuclease III and deletion mutants were sequenced. Both strands of the cDNA have been sequenced by the dideoxy chain termination method with [α-³²P]dCTP (Amersham) and further with dye-labeled oligo primer (ABI 370A).

RESULTS AND DISCUSSION

The conserved sequence of sodium channel genes was amplified with the PCR only for the IV S5-6 primers in the domain IV (Fig. 1a). The synthesized DNA (IV S6A) of about 150 bp (Fig. 1b), encoding upstream region of IV S6 and a part of IV S6 segment of the squid sodium channel, was obtained. Amplified DNA was sequenced, after it was ligated into Puc 119 vector. Following the inverse PCR was applied to the genomic DNA of squid with the primers as shown in Fig. 1b, DNAs were isolated and sequenced, thereby the probe IV I6 of 960 bp was acquired. With this probe, the random primed squid cDNA library in λZAP II was screened. Out of 250,000 clones, we found six positive clones, one of which was SQSR22 of 3.3 kbp. The 800 bp of SQSR22, which was located at the 5' end, was used as the probe IV 7 to obtain the cDNA clone covering further upstream region. This resulted in finding two more clones. One of them was SQSR2-7 of 3.8 kbp (Fig. 2). The restriction endonuclease maps used for the cDNA segments, SQSR2-7 and SQSR22, are shown in Fig. 2.

Fig. 3 shows the nucleotide sequence of the cDNA, together with the amino acid sequence, for squid sodium channel I, determined with the clones of SQSR2-7 and SQSR22. An intron was also observed in IV S3 based on the genomic DNA analysis (Fig. 3). The translational initiation site was assigned to the methionine codon composed of nucleotide residues 583-585 because this is the first ATG triplet that appears downstream of a nonsense codon, TAT (nucleotides 547-549), and is contained in the nucleotide sequence ATTATGG of the residues 580-586, which has a favored sequence of A (or G) XXATGG (X representing any nucleotide) around the initiation codon ATG in the eukaryote. Thus, the open reading frame consists of 4,566 nucleotides, indicating that squid sodium channel I is composed of 1,522 amino acids (Fig. 3) and has the molecular weight of 174,105 daltons estimated from the amino acid sequence. The

a. Amino Acid Sequence

RAT I	KPPDCDPKYNKPGSSVKGDCGNSVGIFFVFSYIIISFLVVMNYIAV
II	GPPDCDEKDHGPGSSVKGDCGNSVGIFFVFSYIIISFLVVMNYIAV
III	APPDCDI ¹ DAIHGPGSSVKGDCGNSVGIFFVFSYIIISFLVVMNYIAV
EEL	GPPDCDPDENPGDVRGNCNPGKGIFFGFSYIIISFLVVMNYIAV

Sense Primer: Pro Pro Asp Cys Asp Pro
5-GGAATT CCN CCN GA_(C) TG_(C) GA_(C) CC-3
42x2³ = 128

Antisense Primer:

	Asn	Met	Tyn	Ile	Ala			
	(AA	T	ATG	TA	T	AT	T	GC)
		C		C		C		
3-TT	(A	TAC	AT	(A	TA	(A	CG	TTCGAAC-5
	G			G		G		
						T		

$2^2 \times 3 = 12$

b. Amplified DNA Sequence

5- CCTCCG GATTGTGACCGA AATTACATCACCACAAGCACCGGAGAAAAATAAAA
(P P N C D P) N Y I T T S T G E K I K
P P D C D P N K V - - N P G S S V K

GTAGTGAACGGTGACTGCCGCATGCCATGGCTTGCTATATCGTATATGGTTTCA
V V N G D C G M P W L A I S Y M V S
- - - G D C G N P S V G I F F F V S

TATATCATCATCGTGTTCATGATTGTCCTCAACATGTACATAGCA-3
Y I I I V F M I V F (N M Y I A)
Y I I I S F L V V V (N M Y I A)

Fig.1. The experimental procedure of PCR used for squid cDNA cloning. *a*: The sense and antisense primers for the RCR extracted from the amino acid sequences of rat I, II & III and eel sodium channels. The sense primer of the amino acid sequence PPDCDP was used, corresponding to the one 1298-1302 between IV S5 and IV S6 (Fig.3). The inclusion of the next two nucleotides from the amino acid¹³⁰³ is indicated by (**). The antisense primer of the amino acid NMYIA was used, corresponding to the sequence 1344-1348 in IV S6 (Fig.3). For the both primers, the degenerated combinations of codons were all synthesized for the PCR. *b*: The sequence of DNA (SQSO3) cloned after the PCR. The nucleotide and amino acid sequences are represented at *upper* and *middle* columns, respectively, with the comparison to the amino acid sequence of rat sodium channel I. The positions of inverse PCR primers are shown with the arrows.

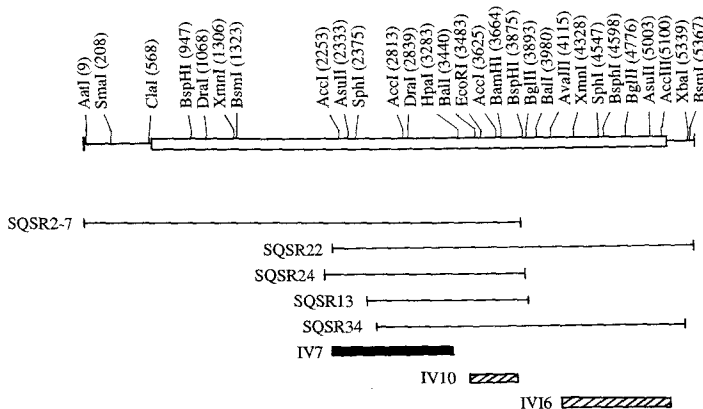


Fig.2. Restriction endonuclease map of squid cDNAs of SQSR2-7 and SQSR22. The map stands for the cleavage sites at the 5' terminal nucleotide by respective restriction endonucleases. Protein-coding regions are indicated by open boxes; the probe IV 7 to screen upstream is indicated by a closed box while probes, IV 10 and IV I6, are both represented by hatched boxes.

5'--GAGAGAGGCGCTGACACAGCTCACTAACTTG	
CCGACCGGATCTCTGGACTTTTACCTGAACAGCAGCCATCTTTTCGACAATCAGCAACGACATTTGGAGAAATACCAACACAGCTACTACGCGACACGCCGTTTGAA	
ACAACTTTTCGTTTGTCAACATTTGTCGATACACAGGATTTGTTTATAGTTTGTGTCGCCCGGATCGGCCCAATTTTCGATAAGACACATTTGTTCAACCAACC	
TGGTGCTACACAGCAGTTACCATTTTACGAAATGACGACTCTGTCGAGAACCATATGCTGTTCTCAAGTACACCGCTCCACACAGCAGTCTGACCCGCAACTCAGCG	
CAAAACACATCCGAAATTTTACATGCTGTCGCCAATCCGCTTGACATTAGCAACATGATGCTGCACATCGGCGCAGCAAGAAAGAGGACATCATGTTCACTGCTGA	
GCACCGATCCATCTCTCGCAACAGGAAAGTTTACCTGAATACCTGCGGCCCTCTCCAAAGGATTTATATGGGATACCTCTGGAAGAGATCGATAAGCAATATTATGGAT	
	M D 588 2
GAA AAA TAT ACT GCA AAG AAT AGA GAT AAG ACA TTT GTG CTC ATA GAG AAG AGA TTT AAG AAG AAT ATC ATT CAT CGG TTT TCA	672 30
E K Y T A K N R D K T F V V I E K R F K K N I I H R F S	
CCT AAG AGG TCA CTT TTC TTA TTC ACA CCA CCG AAT CCC ATC CGG CGC CTA GCT GTT TGC ATT GCC ACA AAT GTA TGC TTT GAC	756 58
A K R S L F L F P R N P L A V C I A T N V C F E D	
TAC CTC TTG ATG TTC ACC ATA ATG ATA AAC TGT GTC TTC CTT GCT ATG CCA GAT ATA TCA GAA TTT GCT GAA TAC ATA TTC CTT	840 86
Y F L M F T I M I N C V F L A M P D I S E F A E Y I F L	
GGT ATC TAC ACA ATG GAA ATG GCT ATA AAG TTA GTT GCA GGG GGA TTT TTT ATT GAC AAA TAT ACA TAT CCA CGG CAC GAA TGG	924 94
G I Y T M E M A I K L V A G G F F I D K Y T V L R D A W	114
AAC TGC TTG GAT TTC ACA GTA ATC ATG ATT TCA TAT ATC ACC CTG CTC TTA CAG ACA ATT AAT GAC AAA GTT ATC AGC GAC ATC	1008 142
N C L D F T V I M I S Y I T L L L Q T I N D K V I S D I	
ACG GGC CTG CGG ACC TTT CGT GTG CCA TGG AGG ACT CTT TCT ATC ATC CCT GGT TTA CCA ACC ATG ATC AAT GCC CTT	1092 170
T G L R T F R V L R A L R T L S I J P G L K T M V Y N A L	
TTA CGT GCG CTT CGT ATG CTC ATT TCT GTA CTC ATT CTC ATC CTC TTC TGT TTG TGG ATC TTC TCC CAA GCT GGT GTT CAG CTT	1176 198
L R A L R M L I S V L I L I L F C L W I F S A G V I	
TTT GGA GGA GCT TTG CGC CAC AAA TGT GTC TTA CAA ATC CAC GGT AGT CCA GCC TTT GGA AAA ACC TAC GAT GAG TTC TAT GCT	1260 226
F G G A L R H K C V L Q I H G S P A F G K T Y D E F Y A	
GAA CAC ATT GAA AAT AGT GAT AAC TGG TTA GCA AAA GGT AAT GGA GAA TAC GTT CTC TGC GGG AAT GCG ACC GGG GCA GGA CCC	1344 254
E H I S F N S D N W L A K G N G E Y V L C G N G A P	
TGT CCT ACC AAC TAT ACC TGC CTA CCT GAC ATC GCG GAA AAC CCT AAC TAC GGA TAT ACG AAC TTC GAC AGC ATT GGC TGG AGT	1428 282
C P T N Y T C L P D I G E N P N Y G Y T N F D S I G W S	
ATG CTT ATA TCA TTT CAG TTA CTA ACT CAG GAT TAT TGG GAA GAT GTA TAT AAC AAG GTT ATT GCG CCT CAT AGT CCA TGG ACT	1512 310
M L I S F T V I W E D V Y N K V I R W T	
GTG ATC TAT TTC ATC GTC ATT AAC TTC TTC GGA TCA TTG TAC CTT ATG AAT CTA ATG TTG GCA GTT GTT GCA ACT GCC TAT GAA	1596 338
V I Y F I V I N F F G S L V L M N L M L A V V A T A Y E	
CTT GAA GTC AAG AAC ACC GGA AAA AAA CTG CAA CAG ACA GCA GCT ACA GCC CGC GAA CAA AGC STA AAG GAA CAA GAA AGA CGT	1680 366
L E T V K N T G K L T A A T A R E Q S C A G A A A R Q	
AAT ACT CTA ACC GTA TCT GAA GCA GAT TCC CAT GTT GAC GAT CCA AAC TGT ACT TGT TGC GAA CAG TGC TGT GGA TGC TGC TAC	1764 394
N T L T V S E A D S H V D D R N C T C C E Q C C G C C Y	
AAC CCT TGG TTA AGG GTG CAG TCC TTT GCC CAT TGC ATC ATT ACA GAT TCA TTC ACT GAG GTT TTC ATT ATC TTC ATC ATT GTA	1848 422
N P I S R V S L T D S F T E V F I I H	
TTG AAC ACA GTC TTT CTT CCA ATG GAG CAC CAT GCA ATG TCC ATG GAG CTG AAG AAT CTA CTT AAA GTC GCC AAT TAT GTA TTT	1932 450
L N T V F L A M E H H G M S M E L K N V L K V A N Y V F	
ACC ACT CTA TTC ATG CTT GAA GCT ATA CTA AAA CTT GCA TTC AAT AAA CAA TAC TTC AAG AGT GCG TGG AAT ATC TGG GAT	2016 478
T T V F V L E A I L K L L A F N K Q Y F K S G W N I C D	
CTT GTC CTC GTT GTC GCC AGC CTA ATT GAT TTG GGA GTG GAA GGG CTC AAA GGT GTA TCT GTG TCT CCG TCT TTT CGA CTG TTA	2100 506
L V V V V A S L I D L G V E G L K G V S V F R S F R L L	
GGA GTT TTC CAC TTG GCT CAA TCT TGG ACA ACA ATG GCA CTG CTT CTG TGT ATA ATC CTA AAT GAT CTT GCG TCG TTA GGT TAC	2184 534
R V F H L A Q S W T T M R L L L C I I L N T L G S L G Y	
TTG ACG ATA ATA CTC ATC ATT GTC ATC TAC ATT TTT GCT GTC ACT GGA CTG CAA CTT TTC CAC ACA GAG TAT ACA CCG GAC AAA	2268 562
L T I I L I I V I F A V T G L Q L F H T V F G K	
TTT ACG GGC GAG CCC GTT CCC AGG TGG AAC TTT AAC GAC TTT CTC CAC TCT TTT ATG ATG GTA TTT CCA ATC CTA TGT GGA GAA	2352 590
F R G E P V P R W N F N D F L H S F M M V F R I L C G E	
TGG ATT GAA CCG ATG TAC GAT TGC ATG CGT GGC TCT AAT GGC CTT TGC TTT CTC ATA TTC ATT CCA GTA ACT GTG TTT GGA AAA	2436 618
W I E P M Y D C M R A C N G L C F L I F I P V T V F G K	
ACA CTG TTC TTC CTG TTC ATC GGT CTG GTC CTG GGT GCG TTT GGT TCT GAT ACG GTG GAA CAA GAA GTG TCC AGC TTT	2520 646
T L F F L F I G L V L G A F G S D T V E Q E V E V S S F	
GCC TTG CCT GGG CCA GAA TCC AAG CCA TGC TGC TGC AGA GAA AGG GGA ATA TCT GCT ACG GAT GAC AAT GTT AAG GAT GAC GGT	2604 674
A L P G P C S V R E R G I S A T D N V K D C	
CAA GAT GAA GTC CAA CAA AAC TCT GAA GAA ACT AAA ATT GAC TTA CCG AAT AAT GAC AAA CAA TCC AAA GAT GGA ATG ATT CTG	2688 702
Q D E V Q Q N S E E T K I D L R N N D K Q S K A D G M I L	
GAA AAC AAT TGC AAC AAT GAC AGT TTG GCA ATG GTC GGT TCA CTA GGG AGC ATC CCA GAC ATA GAT GGC TCC AGC GTG GAA	2772 730
E N N C S L N A S L G S I P D I M D G S S V E	
GAT GAT ATT TCT TCA TGC CAG CAA AAA GAC ATC CCA CCA TGT CTA CCA CTT TTT ATC AGC TCA GAA TTT AAA TGT CTT CCA GAA	2856 758
D D I S S C Q Q K D I Q P C L P L F I S S R F R C L R E	

Fig.3. Nucleotide sequence of the cDNA encoding squid sodium channel (*upper*) and its deduced amino acid sequence (*lower*). The sequence was determined with the clones of SQSR2-7 and SQSR22 (Fig.2). Both strands of the cDNA were sequenced with either Sequenase ver.2.0 (USB) or klenow large fragment (Takara Co.). The nucleotide sequence is numbered, starting from the first residue of SQSR2-7. The position where an intron is inserted is indicated by an arrow (↗). This was determined by the genomic DNA analysis on the repeat IV.

[illegible]

Fig. 3 - Continued

1,522 amino acid sequence is far shorter than those of vertebrate sodium channels; 2,009, 2,005, 1,951 and 1,820 for rat I, II, III and eel (3, 4, 5). This is one of the features of the squid sodium channel, which is mainly because the sequences exposed to the cytoplasmic side are shorter (Fig.4). The squid cDNA is AT rich since the content of AT and GC were 58 and 42%, respectively (Fig.3.).

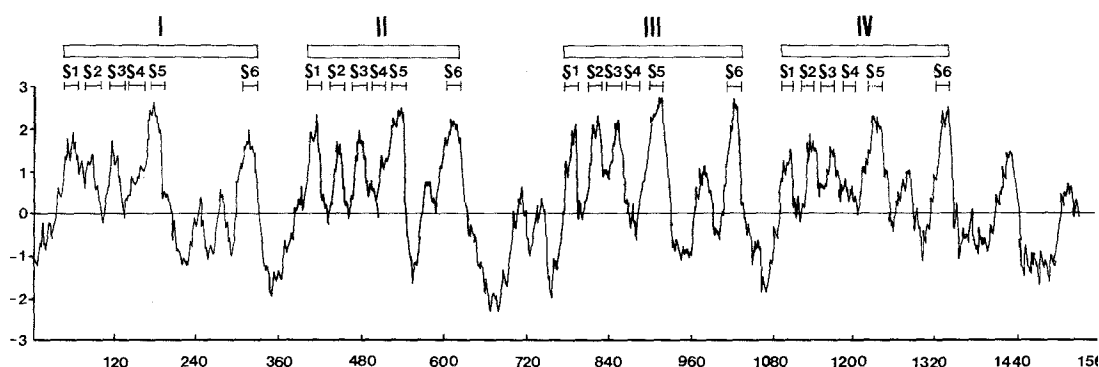


Fig.4. Hydropathy profile of squid sodium channel. The profile was obtained with use of the Kyte and Doolittle program (Kyte, J. and Doolittle, R.F. (1982) *J. Mol. Biol.* **157**, 105) where we run it with a window of 19 residues. Locations of homologous repeats I, II, III and IV are indicated by open boxes. Altogether 24 hydrophobic peaks are found and indicated by their corresponding segments S1-S6.

The alignment of the amino acid sequences of squid sodium channel can be composed with rat sodium channels (4, 5) and the *Electrophorus* sodium channel (3). The degree of the amino acid sequence homology is 48, 47, 46 and 42% for rat I/squid, rat II/squid, rat III/squid and eel/squid, respectively; the degree is rather low as compared with that of 87 and 61% for rat I/rat II and rat I/eel, respectively. For evaluation of amino acid sequence homology, a gap was counted as one substitution. The degree of the nucleotide sequence homology between rat (or eel) and squid sodium channels is even lower. This could be one of the reasons why the cloning of squid sodium channel cDNA has long been unsuccessful with the rat cDNAs. In fact we had tried to screen cDNA library of squid optic lobe with the rat sodium channel cDNA which was kindly offered by Professor M. Noda, but we failed at both high and low stringencies.

The squid sodium channel is assumed to have the same transmembrane topology as proposed for other sodium channels (4, 5) in which the four repeated domains (I, II, III and IV) of homology, each containing the six membrane-spanning segments (S1, S2, S3, S4, S5 and S6), are oriented in a pseudosymmetric fashion across the membrane, and in which the amino- and carboxy-terminal residue on the cytoplasmic side of the membrane. These were predicted by analysis for local hydropathicity on the deduced squid amino-acid sequence (Fig.4). Segments S1, S2, S3, S5 and S6 are apolar, while segment S4 and a linker between S5 and S6 are slightly apolar and partly even hydrophilic (Fig.4). These are quite similar to those of vertebrate sodium channels (3 - 5). For these characteristics of the segments, Guy *et al.* (16) proposed a model with four homologous domains but each containing eight homologous transmembrane segments, S1 through S8, where three segments (S1, S2 and S3) are relatively apolar and two segments (S5 and S8) are quite apolar. S8 is the nomenclature that Noda *et al.* (4) called S6. The close resemblance of sodium channel topology between vertebrate (rat and eel) and invertebrate (squid and fly) is consistent with the idea that sodium channel evolution took place before the separation of vertebrate and invertebrate species (2, 6).

Based on the sodium channel model by Noda *et al.* (3, 4) where sodium channel has four homologous domains, each having six homologous transmembrane segments, S1 - S6, we will compare amino-acid sequence of squid sodium channel with those of vertebrate sodium channels

in more detail. For S4 segments which have widely been recognized to serve as voltage sensors (3, 5, 17, 18), squid S4s contain 4, 3, 5 and 8 basic residues (arginine R or lysine K) at every third position in the domains I, II, III and IV (Fig.3), respectively, while those of rat or eel contain 4, 5, 5 and 8 basic residues correspondingly (3 - 5). The difference is on II-S4 where two lysine residues of vertebrate are substituted for histidine⁵¹⁰ and glutamine⁵¹³ residues of squid (Fig.3). As for S2, it is noted that glutamic acid (negatively charged) and lysine (positively charged), which are located in the 15th and 19th positions for each domain, are all conserved for vertebrate and squid sodium channels in their identical positions. In addition, segment S2 in repeats I and III contains a conserved glutamic acid or aspartic acid residue both for vertebrate and squid, which locates 10 residues upstream from the above-mentioned glutamic acid. Note that the aspartic acid residue in III-S2 is replaced by an asparagine residue in *Drosophila*. However, lysine (a positively charged residue) conserved in III-S2 for every vertebrate, which locates one residue downstream from the just above-mentioned aspartic acid residue, is substituted for isoleucine⁸²¹ in squid. Segment S3 in every repeat contains a conserved aspartic acid residue (the 6th position in each domain) for both vertebrate and squid at an equivalent position. In II-S3, a negatively charged residue, 10 residues downstream from the above-mentioned aspartic acid, is conserved for both vertebrate (all glutamic acid) and squid (aspartic acid). In IV-S3, a negative charged residue, 15 residues downstream from the aspartic acid in the 6th position, is conserved both for vertebrate and squid. But it is noted that, in I-S3, a conserved glutamic acid residue for vertebrate, located at the position 11 residue downstream from the aspartic acid (the 6th position in I-S3), is substituted for a leucine residue in squid.

A cytoplasmic linker of squid between domains III and IV is quite similar, in its amino-acid sequence, to those of vertebrate (17, 19, 20), particularly for their positively charged positions; three successively aligned lysine¹⁰⁴⁸⁻¹⁰⁵⁰ residues, two successively aligned lysine^{1072,73} & ^{1077,78} residues, a lysine¹⁰⁸¹ and arginine¹⁰⁸⁵ residue are all conserved for squid and vertebrate, except that lysine residues^{1065,66}, conserved lysine residues for vertebrate, are replaced by non-charged ones, glutamine and asparagine in squid. This exception is, however, consistent with the site directed mutagenesis experiment (20) showing that inactivation is not altered in rat III sodium channels expressed in *Xenopus* oocytes where lysine residues mentioned above are substituted by non-charged ones. A serine residue, which can be phosphorylated in a PKC (protein kinase C) dependent manner to cause both inactivation slow and reduce peak sodium current in rat II sodium channel (21), is replaced by threonine¹⁰⁷⁶ at the equivalent position for squid. This suggests that potentiality of phosphorylation at the position is conserved. Two potentially glycosylated sites of asparagine²³¹ and asparagine²⁴⁸ residues are conserved between I-S5 and I-S6 for squid and vertebrate (4, 5, 22). The asparagine²⁴⁸ residue could be glycosylated while the asparagine²³¹ residue could not be glycosylated because of the presence of an aspartic acid²³³ residue in its next neighbour (23). In squid one more potential site of glycosylation between I-S5 and I-S6 is on an asparagine²⁵⁸ residue.

In conclusion, the structural features characteristic of squid sodium channel are shared by vertebrate sodium channels. We should here point out that the deduced amino acid sequence of squid is different from that of *Drosophila* at some critical points; in the squid sodium channel the II-S4 segment contains 3 positively charged residues at every third position, while the *Drosophila*

sodium channel contains 5, the same as in vertebrate, in its equivalent segment. In a III-IV linker, the phosphorylated site (serine and threonine¹⁰⁷⁶ residues for vertebrate and squid, respectively) is replaced by an arginine residue in *Drosophila*, suggesting that it is non-phosphorylated.

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