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Heptad-Repeat Sequences in the Glycoprotein of Rhabdoviruses

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Abstract. Two or three regions containing three or more successive newly defined heptads of a–d hydrophobic amino acid repeats have been located in the cDNA-derived amino acid sequences of glycoprotein G of all rhabdoviruses examined (rabies, vesicular stomatitis, fish, and plant rhabdoviruses) by computer search. These new heptad-repeats differ from those previously reported in other viruses because of the presence of all the hydrophobic amino acids in positions a or d, and because they are not predicted to form coiled coils by current methods and thus they have not been detected previously in any rhabdoviruses. The two or three heptad-repeat regions were the only parts of the glycoprotein with at least three successive heptad-repeats in all the rhabdoviral sequences studied and had low sequence variability among the members of each of the rhabdoviral genus but show no sequence similarity among the different genus. All these newly detected heptad repeats were in the vicinity of some of the higher hydrophobic regions in each of the rhabdovirus genera studied and were found mostly, but not always, outside the extra amino acid sequences that occur in the longer insect or plant rhabdovirus glycoprotein G. The correspondence of position and structure of these heptad-repeats among all the rhabdoviruses suggests its participation in common function(s), most probably related to viral fusion with cellular membranes.

Key words: heptad-repeats, hydrophobic, rhabdoviruses, glycoprotein

Introduction

Heptad-repeats with a high propensity to form coiled coils were defined as sequences of seven amino acids (aa)—**a** b c **d** e f g—in which the aa in each position were specified by a probability matrix, with the aa usually found in positions a–d, mostly F, Y, I, L, V, N, and A (1). The presence of these repeats has been described in sequences of glycoproteins from many enveloped viruses, such as paramyxovirus, retrovirus, coronavirus, and influenza (2), and is related to regions of high hydrophobicity or to viral fusion (3,4). Even though heptad-repeats with a propensity to form coiled coils had not been found previously in any rhabdoviruses (5), we searched for possible heptad-repeats in glycoprotein G sequences of rhabdoviruses after finding that a phospholipid binding region of the viral hemorrhagic septicemia (VHSV) rhabdovirus, a rhabdovirus affecting salmonid fish, had a heptad-repeat structure that included more hydrophobic aa than those defined earlier by Lupas [6].

Rhabdoviruses possess a glycoprotein (G), which serves as a homotrimeric membrane protein that forms spikes protruding 83 Å from the viral membrane (7,8). Glycoprotein G initiates virus attachment to cellular receptors (9), reacts with neutralizing antibodies (10), and has fusion properties of the rhabdoviruses that are detectable only at low pH (7,11).

The family Rhabdoviridae is mostly made up of the genera Lyssavirus (rabies) and Vesiculovirus (VSV), with two main serotypes—New-Jersey (NJ) and Indiana (Ind) (12,13)—but it also contains fish rhabdoviruses, including the VHSV or the infectious hematopoietic necrosis virus (IHNV) and plant rhabdoviruses, an interesting area of study for comparative virology.

Materials and Methods

Selection of Rhabdoviral Glycoprotein G Sequences

Selection of the sequences for this study was made to obtain representative sequences from as many members as possible of the Rhabdoviridae family. It was limited, first, by the actual number of published cDNA-derived aa sequences of rhabdoviral glycoprotein G and, second, by requiring a low enough number to make the study feasible. The glycoprotein G sequences actually available from rhabdoviruses were divided according to their host as those infecting fish (3 sequences), arthropods (2 sequences), plants (1 sequence), lyssavirus (7 sequences), and vesiculoviruses (3 sequences selected from 34 sequences of VSV-NJ and 3 from 26 VSV-Ind).

Analysis of Rhabdovirus Glycoprotein G

The hydrophilicity profile, signal peptide, and predicted transmembrane regions of the cDNA-derived aa glycoprotein G sequence of VHSV (Table 1) and of other rhabdoviruses were obtained by using the SOAP, ANTIGEN, and the PSIGNAL programs from the PCGene package (Intelligenetics, Geneve, Switzerland) using an average group length of 9 aa. The newly defined

Table 1. Rhabdoviral sequences of the glycoprotein G selected for this study

Host	Virus	Description	# aa	Reference	AC[a]
Fish	VHSGER	VHSV 07.71	507	24	X 59148
	VHSDK	VHSV DK	507	23	X 66134
	IHNGP	IHNV RB-1	508	25	M 16023
Mammal	RABMOK	rab Mokola	524	16	S 59447
	RABPV	rab PV	524	26	M 32751
	RHRBGD	rab ERA	524	27	J 02293
	RABSAD	rab SADB19	524	28	M 31046
	RABMEP	rab MEP	524	29	M 32751
	RABLEP	rab LEP	524	30	X 69122
	RABCVS	rab CVS	524	31	—
Cattle	VSVGPNO8	VSV N.J.-I	517	13	M 21424
Horse	VSVGPNJA	VSV N.J.-II	517	13	M 21416
Swine	VSVGPN29	VSV N.J.-III	517	13	M 21565
	RHGPORS	VSV Orsay	511	32	M 11048
	RHGM	VSV San Juan	511	33	J 02430
	RHVSVGR	VSV Indiana	511	34	X 03633
Insect	SIGMA	Drosophila	526	35	X 06171
	BEFV	Bov.eph.fev.	623	36	M 94266
Plant	SYNV	ATCC-PV263	628	37	M 73626

VHSV = viral hemorrhagic septicemia; IHNV = infectious hematopoietic necrosis virus; RAB = rabies; VSV = vesicular stomatitis virus; BEFV = bovine ephemeral fever virus; SYNV = sonchus yellow net virus.
The 26 sequences of cDNA of VSV-Ind have been assigned GenBank accession nos. M 35207–M 35232 (15). The 34 sequences of cDNA of VSV-NJ have been assigned GenBank accession no. J 04326 (13). — not found.
[a]Accession numbers of GenBank data libraries.

hydrophobic heptad-repeat sequences were searched with the program PSEARCH. Amino acids with $-\Delta G$ values ≥ 0.4 kcal/mol for transfer of the aa side chain from water to ethanol were used as hydrophobic aa (single-letter code aa, W, F, Y, I, L, L, V, M, A, H, T) (14). The subsequence used to search was (hydrophobic aa) XX (hydrophobic aa) XXX, repeated two times (with X indicating any aa). Then the positions at which more than two heptad-repeats were found successively arranged in the sequence of the glycoprotein G were selected (Table 2). Heptad-repeats with a high propensity to form coiled-coil regions were searched for with the program COIL (1) from the same package.

Results

The total number of aa in glycoprotein G varied from 507 to 526 aa for most of the rhabdoviruses, except for BEFV and SYNV, which had 623 and 628 aa, respectively (Table 1). At least two regions in which hydrophobic aa were found in the a–d positions of heptads successively arranged (repeated) three or more times were located in all the rhabdoviral glycoprotein G sequences selected by computer search (Fig. 1). No attention was paid to the aa compositions of the rest of the positions (b, c, e, f, or g) within the heptads, contrary to the prediction method by using the program COIL (1), which associates a probability with each aa in every heptad position (being the aa in a–d, for example, mainly = F, Y, I, L, V, M, and A, because those aa were the ones with the highest probability of being found in known coiled coils in protein structures).

Therefore, the occurrence of hydrophobic a–d heptad-repeats of the kind defined in this work (aa in the a–d positions of the heptad-repeats = F, Y, I, L, V, M, A, + W, H, T, aa in the single-letter code) is not by itself an indicator of a coiled-coil structure. We thought to include aa W, H, and T as new candidates for the a or d positions because of the appearance of these aa in the a or d positions of the heptads in a phospholipid binding domain in VHSV (6). Prior reported analysis of the VSV glycoprotein G sequence using the method of Lupas, while looking for the presence of at least four successive heptad repeats (with the shortest peptides still exhibiting a stable coiled-coil structure in solution) failed to identify any predicted coiled-coil domains (1,2,5). Even on shortening the length of the aa window to three heptad-repeats in the program COIL, there were no coiled-coil regions predicted with a high enough probability in any of the rhabdoviral sequences shown in Table 1.

The two regions in at least three successive heptads found in most of the rhabdoviral sequences studied were situated around aa 100–150 (amino terminal) and around aa 350 (carboxy terminal). In contrast, the location of the heptad-repeats in the proteins of 20 envelope viruses reported previously was found in only one region situated either around aa 150 (near the amino-terminal aa) or around aa 400–500 (near the carboxy-terminal aa), but not in both positions simultaneously for each individual viral protein (■). VHSV and Sigma rhabdoviruses showed still another carboxy-terminal heptad-repeat region situated between aa 377–400 and 416–443, respectively (Fig. 1). The carboxy-terminal heptad-repeats of rabies and vesiculoviruses, and the second carboxy-terminal heptad-repeat of VHSV and Sigma rhabdoviruses, contained one of the putative glycosylation positions. All of the heptad-repeats lay outside and around an internal core of glycoprotein G where seven cysteines (at around aa 170–300) were highly conserved (maximal alignment with a minority of gaps) in all the rhabdoviruses (Fig. 1).

The heptad-repeat sequences show no aa sequence homologies among different rhabdovirus genera but were highly conserved in both aa sequence and relative position in glycoprotein G among members of the same genus, including VSV-NJ and VSV-Ind (Table 2). For simplicity, the heptad-repeat sequences that are displayed in Table 2 extend only from the first hydrophobic aa in positions a or d to the first nonhydrophobic aa in position a or d.

The aa variability of the heptad-repeats was further studied in cases in which a large number of published aa sequences were available, rabies (7 sequences), VSV-NJ (34 sequences), and VSV-Ind (26 sequences). The maximum variation in the number of different aa that appeared at any given position in VSV-NJ (13), VSV-Ind (15), and rabies (16) were obtained at the car-

Table 2. Heptads of hydrophobic repeats of rhabdovirus glycoprotein G

Amino-terminal hydrophobic repeat									
VHSGER	68		**F**EDINKG	LVSVPTR	**I**IHLPLS	VTSVSAV	ASGHYLH		102
VHSDK	68		**F**EDINKG	LVSVPTK	**I**IHLPLS	VTSVSAV	ASGHYLH		102
IHNGP	99	IHKV	LYRTICS	TGFFGGQ	TIE				119
SIGMA	151		VKDHPVM	LDPYTNN	YVDAIFP				171
BEFV	149		LIQHKPF	LNPYDNI	IYD				165
SYNVG	90		ITGARWN	YVGISIP	VFKIVTN				110
RABMOK	140	WLRT	VTTTKES	LLIISPS	IVEMDIY				164
RABPV	140	WLRT	VKTTKES	LVIISPS	VADLDPY				164
RHRBGD	140	WLRT	VKTTKES	LVIISPS	VADLDPY				164
RABSAD	140	WLRT	VKTTKES	LVIISPS	VADLDPY				164
RABHEP	140	WLRT	VKTTKES	LVIISPS	VADLDPY				164
RABLEP	140	WLRT	VKTTKES	LVIISPS	VTDLDPY				164
RABCVS	140	WLRT	VRTTKES	LIIISPS	VTDLDPY				164
VSVGPN08	134	TVTD	AEAHIVT	VTPHSVK	VDEYTGE	WID			161
VSVGPNJA	134	TVTD	AEAHIVT	VTPHSVK	VDEYTGE	WID			161
VSVGPN29	138		AEAHIIT	VTPHSVK	VDEYTGE	WID			161
RHGPORS	134	TVTD	AEAAIVQ	VTPHHVL	VDEYTGE	WVD			161
RHGM	134	TVTD	AEAVIVQ	VTPHHVL	VDEYTGE	WVD			161
RHVSVGR	134	TVTD	AEAVIVQ	VTPHHVL	VDEYTGE	WVD			161
Carboxy-terminal hydrophobic repeat									
VHSGER	288	TDIQ	MRGATDD	FSYLNHL	ITNMAQR	TECLDAH			319
VHSDK	288	ADVQ	MRGATDD	FSYLNHL	ITNMAQR	TECLDAH			319
IHNGP	327	TPYL	LSKFRSP	HPGINDV	YAMHKGS	IYH			354
SIGMA	316	ISKM	VSGLPTS	VFDLSYL	IQV				336
BEFV	385		IGSYKRA	WCEYRPF	VDK				401
SYNVG	320		IEGVNRA	FEDLELT	YCSATCD	LFA			343
RABMOK	345		TNVYYKR	VDKWADI	LPS				361
RABPV	330		FGKAYTI	FNKTLME	ADAHYKS	VRTWNEI	IPS		360
RHRBGD	330		FGKAYTI	FNKTLME	ADAHYKS	VRTWNEI	IPS		360
RABSAD	330		FGKAYTI	FNKTLME	ADAHYKS	VRTWNEI	IPS		360
RABHEP	330		FGKAYTI	FNKTLME	ADAHYKS	VQTWNEI	IPS		360
RABLEP	330		FGKAYTI	FNKTLME	ADAHYKS	VRTWNEI	IPS		360
RABCVS	330		FGKAYTI	FNKTLME	ADAHYKS	VRTWNEI	IPS		360
VSVGPN08	332	VGPV	FTIINGS	LHYFTSK	YLRVELE				356
VSVGPNJA	332	VGPV	FTIINGS	LHYFTSK	YLRVELE				356
VSVGPN29	332	VGPV	FTIINGS	LHYFTSK	YLRVELE				356
RHGPORS	328	TGPV	FTIINGT	LKYFETR	YIRVDIA	APILSRM	VGMISGT	TTE	369
RHGM	328	TGPA	FTIINGT	LKYFETR	YIRVDIA	APILSRM	VGMISGT	TTE	369
RHVSVGR	328	TGPA	FTIINGT	LKYFETR	YIRVDIA	APILSRM	VGMISGT	TTE	369

Hydrophobic amino acids (W, F, Y, I, L, V, M, A, H, T) in heptad positions a and d are in bold. Each heptad is separated by a space. The numbers to the left and right of the sequences are the amino-terminal positions of the first and last amino acids of the heptads, respectively. VHSGER, VHSDK, and SIGMA contain another set of heptad-repeats between amino acids 377–400, 377–400, and 416–443, respectively.

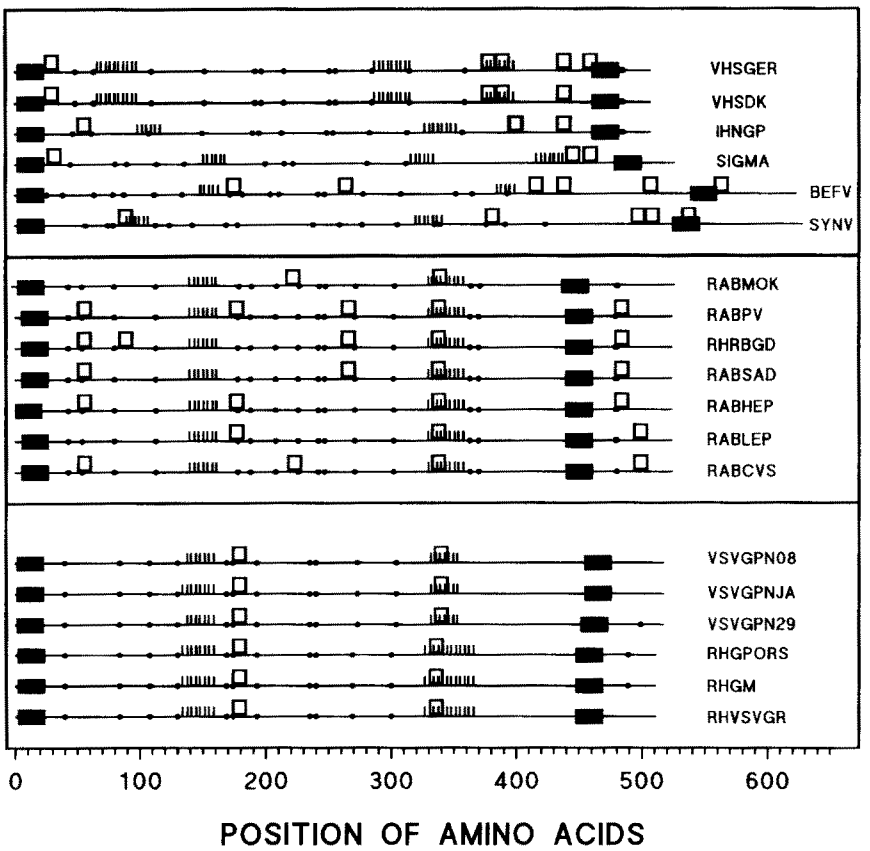

Fig. 1. Sequences of amino-terminal and carboxy-terminal a–d heptad-repeats in rhabdovirus glycoprotein G. The heptad-repeats (| | | | |) were located by using the program PSEARCH (PCGene package, Intelligenetics). $-\Delta G$ values ≥ 0.4 kcal/mol for transfer of the amino acid side chain from water to ethanol were used to define the hydrophobic amino acids (single-letter code aa, W, F, Y, I, L, L, V, M, A, H, T) (14). The subsequence used for the search was (hydrophobic aa) XX (hydrophobic aa) XXX, repeated two times (X for any aa). Then the positions where more than 2 heptad-repeats were successively found in the glycoprotein G sequence were selected. The highly hydrophobic and continuous hydrophobic aa sequences of the predicted transmembrane and signal peptide regions were not considered. Symbols indicate cysteine (●), putative carbohydrate (□), and predicted transmembrane and signal peptide (■) relative positions in rhabdoviral glycoprotein G. The names of the rhabdoviruses are explained in Table 1.

boxy-terminal positions (from aa 400 to 500), including the regions close to the predicted transmembrane hydrophobic stretch, the cytoplasmic tail, and the amino-terminal signal peptide. Thus, there were no variations in aa in the a–d positions in the aa 68–102 heptad-repeat and only one (aa 288, T or A) in aa 288–319 heptad-repeat of the two available VHSV sequences. Similarly, no variations in aa in the a–d positions in the aa 140–164 or in the aa 330–360 heptad-repeats, except those for RABMOK (aa 158, 161, 345, 348, and 359) were found among the rabies strains and all the aa that changed were also hydrophobic (Table 2). Few aa variations (aa 134, T or S; aa 141, H or Q; and aa 332, V or A) were found in the aa 134–161 or the aa 332–356 heptad-repeats of the 34 VSV-NJ isolates studied (13). Finally, only one aa variation (aa 141, V or A; or aa 335, I or V) was found in each of the aa 134–161 or the aa 328–369 heptad-repeats of the 26 isolates of VSV-Ind studied (15).

To investigate the possible relationships between the heptad-repeats and the positions of the extra numbers of aa in BEFV and SYNV, a multiple alignment of sequences was performed with RABMOK, RABPV, VHSV-07.71, and VSVGPN08 by using the CLUSTAL program (PCGene package, Intelligenetics). As Figure 2

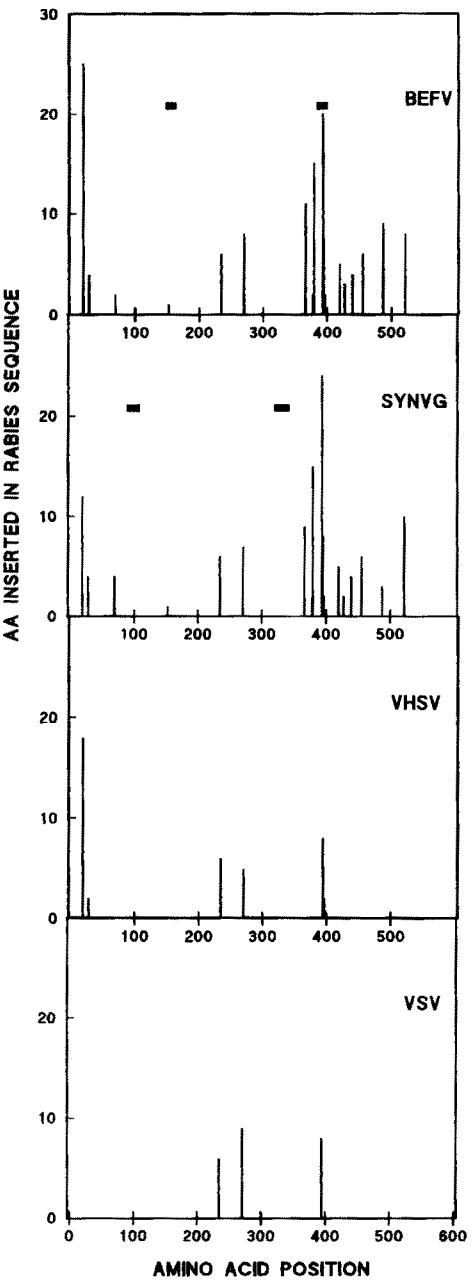

Fig. 2. Number of amino acids inserted in the sequence of rabies glycoprotein G for maximal sequences alignment. The sequences shown in the figure were aligned with glycoprotein G sequences from RABMOK and RABPV by the CLUSTAL program from PCGene (Intelligenetics). (| | | | |) = position of heptad-repeats. BEFV bovine ephemeral fever; SYNVG = sonchus yellow net virus; VHSV = viral haemorrhagic septicemia virus 07.71; VSV = vesicular stomatitis virus (VSVGPNO8). The position number of the amino acids in glycoprotein G corresponds to RABMOK and RABPV.

shows, the relative excess of aa BEFV and SYNV was distributed in short stretches from aa 10 to 20 placed around positions belonging to the carboxy-terminal portion of the molecule (around aa 400) and to the signal peptide, but generally did not coincide with the positions of the heptad-repeats (Fig. 1), except for the BEFV carboxy-terminal heptad-repeat. VHSV07.71 and VSVGPN08 were included as controls and did not show any large insertions of aa ($\geq$10 aa) at positions around aa 400. In most cases the rhabdovirus heptad-repeats in the glycoprotein G were either followed or preceded by short regions (10–15 aa) of high hydrophobicity (Fig. 3), as has been reported in other envelope viruses (2).

Discussion

The function(s) of the newly defined heptad-repeats in glycoprotein G of rhabdovirus are not known at present. The search procedure allows any of the 10 hydrophobic aa (14) in each specified position a or d, and might detect chance runs of aa unrelated to coiled-coil structures, particularly if these include a genuine alpha helix. The presence of numerous helix-breaking proline residues scattered through the newly found heptad-repeats (Table 2) is a major factor arguing against the existence of these structures as alpha helixes.

Several lines of indirect evidence suggest that the new heptad-repeats situated in the amino-terminal region of glycoprotein G or in the regions situated around them could somehow be related to rhabdoviral membrane fusion with host membranes, neutralization, and/or phospholipid binding. For instance, the presence of a fusion-defective mutant of VSV in aa 117 first indicated that the adjacent region (aa 118–136) could be involved in the membrane fusion activity of VSV glycoprotein G. Site-directed mutagenesis finally identified the sequence of aa 123–137 of VSV glycoprotein G as a putative fusogenic peptide involved in low-pH–induced membrane fusion (5,17). These VSV fusion-defective mutants map immediately before the amino-terminal new heptad-repeat (aa 134–161 for most VSV studied) of VSV (Table 2). Of the rest of the identified VSV fusion-defective mutants at aa positions 120–150, 190–210, 300–360,

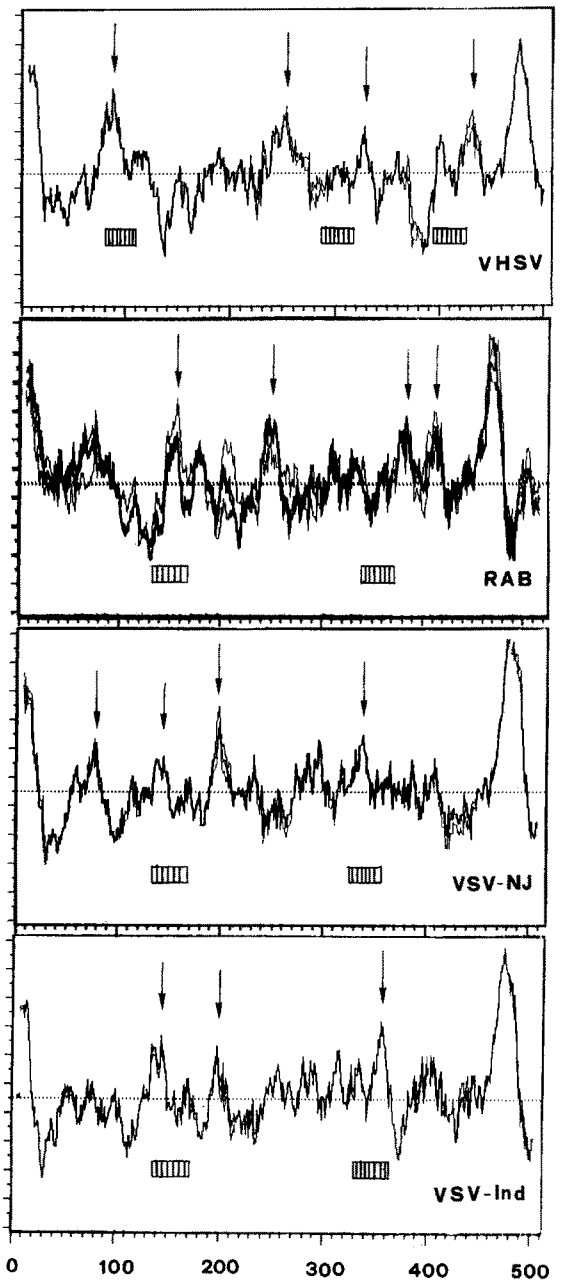

Fig. 3. Hydrophobic profiles and location of the heptad-repeats. The hydrophobic profiles from most of the sequences in Table 1 were obtained with the program SOAP from PCGene (Intelligenetics). The profiles from the different isolates or serotypes from VHSV, rabies, VSV-NJ, and VSV-Indiana were superimposed and the figures obtained are shown. The vertical arrows indicate the position of the highest hydrophobic peaks, whereas horizontal bars show the position of the heptad-repeats. The y axis shows the hydrophobic scales, and the X axis shows the amino-terminal position of the amino acids of rhabdovirus glycoprotein G.

and 409–419, only the positions 300–360 also maps nearby another of the new heptad-repeat–rich region aa 332–356 in VSV-NJ or aa 328–369 in VSV-Ind. Analogous to influenza hemagglutinin, in which pH-dependent coiled coiling of heptad-repeats exposes the upstream fusion peptide (4) and because fusion of rhabdoviruses also has a low-pH dependence, causing exposure of unidentified hydrophobic aa region(s) (7,8,12), the new rhabdovirus heptad-repeats identified in this work could also be related to rhabdoviral fusion. On the other hand, some rabies monoclonal antibody–resistant (MAR) mutants have been mapped in aa 330–338 and aa 342–343, both inside the aa 330–360 new rabies heptad-repeats (10,18–20).

Finally, a phospholipid binding domain of glycoprotein G of VHSV has been recently identified by using pepscan, synthetic peptides, and purified or recombinant glycoprotein G (21) and purified VHSV, solid-phase phospholipid-binding assays (6). That work extended the well-known observation of phospholipid interactions of mammalian rhabdoviruses (VSV and rabies) to fish rhabdoviruses. Study of the 15-mer phospholipid-binding peptide sequence (p106, aa 99–113) identified by pepscan showed that p106 was at the carboxy-terminal part of an a–d hydrophobic new heptad repeat with a predicted α-helix structure (1,2,4) and led to the design of a synthetic peptide (p2) that, by containing P106 and the heptad repeat, increased the specific activity of phospholipids binding about 10-fold (6). The sequence of p2 in VHSV 07.71 (22) was totally conserved in the sequence of glycoprotein G of the other VHSV sequence reported to date (23) (Table 2). P2 (aa 82–109) was inside a region of 5 a–d hydrophobic new heptad repeats (aa 68–102). Whether or not this highly conserved domain and/or its nearby regions may be related to membrane fusion (2) induced by glycoprotein G of VHSV is not known at present. However, there is a similar pH dependence of VHSV in phospholipid (6) binding and membrane fusion (11).

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