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Comparative Amino Acid Sequences of Dengue Viruses

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Abstract: Amino acid (AA) sequences of 4 serotype of dengue viruses deduced from their nucleotide (nt) sequences of genomic RNA were analyzed for each genome segment and each stretch of 10 AA residues. Precursor of membrane protein (pM), and 4 nonstructural proteins (NS1, NS3, NS4B, NS5) were highly conserved, while another nonstructural protein (NS2A) was least conserved among 5 strains of dengue viruses. When homology was compared among heterotypic viruses, type 1 and type 3 dengue viruses showed close relationships, while type 4 dengue virus was relatively remote from other types. When AA sequences were compared for each 10 AA residues, some highly conserved as well as least conserved domains were identified. Significance of these findings for diagnostic virology and epidemiological survey was discussed.

Key words: Dengue virus, Flavivirus, Amino acid sequence, Homology

Dengue viruses of 4 different serotypes belong to the family Flaviviridae (Westaway *et al.*, 1985), and transmitted among humans by mosquitoes, primarily *Aedes aegypti* (Clarke and Casals, 1965), thus arthropod-borne viruses (or arvoviruses). Disease manifestations among dengue virus-infected humans are various, from mild undifferentiated fever, dengue fever to severe dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). Sequential infection with heterologous type of dengue viruses different from those in the primary infection has been considered to be related with severe manifestation of the disease (Russell, 1971; Halstead, 1966, 1980). The latter investigator postulated that antibody-dependent enhancement of dengue virus growth in human peripheral monocytes was underlying cause of DHF or DSS.

Recently, entire nucleotide (nt) sequences of genomic RNA were analyzed for types 2, 3, and 4 dengue viruses (Deubel *et al.*, 1988; Hahn *et al.*, 1988; Osatomi, 1988; Mackow *et al.*, 1987; Zhao *et al.*, 1986), while type 1 dengue was partially sequenced (Mason *et al.*, 1987). In order to understand intratypic as well as heterotypic homologies of dengue viruses at a molecular level, we compared deduced AA sequences of 5 dengue virus strains.

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Sequences data were referred from following references: type 1 dengue (D1: Mason *et al.*, 1987); type 2 dengue Jamaica genotype (D2J: Deubel *et al.*, 1988); type 2 dengue S1 candidate vaccine strain (D2S: Hahn *et al.*, 1988); type 3 dengue (D3: Osatomi, 1988); type 4 dengue (D4: Mackow *et al.*, 1987; Zhao *et al.*, 1986). Viral genomes were divided into 11 segments [core protein (C), precursor (pM) of membrane protein (M), envelope glycoprotein (E), and nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5)] according to Rice *et al.* (1985) and Speight *et al.* (1988).

Since compared 5 dengue virus strains possessed varying numbers of AA residues for each genome segment, direct sequence comparison was difficult. Sequences were, therefore, compared after alignment by filling up several residues to obtain maximal matching of the corresponding sequence using DNASIS Version 6.0 software (Hitachi, 1989). For fine comparison, each 10 AA residues were compared and numbers of matching AA residies were counted which were divided by the length to obtain scores.

Analysis for each of the 10 combinations of 5 dengue virus strains was summarized in Table 1 and Fig. 1. For all combinatis, pM, NS1, NS3, NS4B and NS5 proteins were highly conserved and NS2A and C proteins were least conserved. Both C and M proteins showed high degree of deviation among 5 dengue virus strains. In NS3 protein, an NTP-motif sequence Gly−X−Gly−Lys (G−X−G−K) of ATP-dependent DNA helicase (Gorbalenya *et al.*, 1988) was found for all dengue virus strains as other flaviviruses (Takegami, personal communication). While NS5 protein was considered as a component of viral RNA-dependent RNA polymerase because of its consensus triplet sequence Gly−Asp−Asp (G−D−D) of viral RNA polymerases (Goelet *et al.*, 1982; Kitamura *et al.*, 1981). Both NS3

Table 1. Homology % of AA

	D1/D2J	D1/D2S	D1/D3	D1/D4	D2J/D2S	D2J/D3	D2J/D4	D2S/D3	D2S/D4	D3/D4
C	55.4	59.6	66.9	57.7	80.0	48.5	55.4	50.0	56.9	47.7
pM	74.4	75.6	76.7	68.9	88.9	71.1	68.9	71.1	70.0	74.4
M	70.0	68.8	82.5	61.3	96.3	68.8	65.0	67.5	65.0	58.8
E	64.6	65.8	70.4	57.7	92.9	60.2	58.5	61.0	58.7	56.2
average	64.8	65.7	71.7	59.3	90.7	60.4	59.8	61.0	60.2	57.1
NS1	72.6	71.7	78.3	67.7	96.3	72.6	70.6	72.0	70.3	70.9
NS2A					88.3	26.7	25.4	26.3	26.7	30.0
NS2B					93.1	56.2	50.8	53.8	50.8	53.8
NS3					96.2	79.0	75.7	78.4	74.8	76.7
NS4A					98.0	62.7	64.0	60.7	63.3	60.0
NS4B					93.2	71.6	73.2	72.8	74.8	72.0
NS5					95.6	77.6	71.6	78.1	72.5	74.3
				average (nonstructural)	95.3	70.5	67.2	70.3	67.5	68.6
				average (full length)	94.0	67.9	65.3	67.9	65.6	65.7

Abbreviation of virus name and genome segment is shown in text

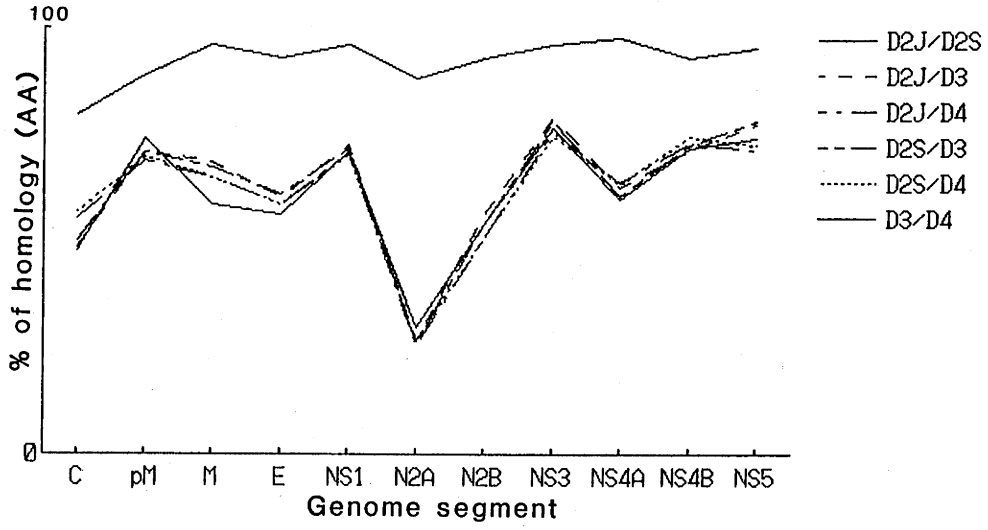

Fig. 1. Graphic presentation of AA homology % in each genome segment. Abbreviation of virus name and genome segment is shown in text.

and NS5 proteins could be important for the survival and replication of the virus and thus highly conserved (Rice *et al.*, 1985, 1986; Strauss and Strauss, 1986).

Intratypic homology between D2J and D2S was higher (91%) than heterotypic homology (57−51 %), although these 2 strains possessed different isolation and passage histories. Among heterotypic combinatis, homology between D1 and D3 (72%) was higher than other combinations, indicating closer relation between D1 and D3, which is compatible with the observation by monoclonal antibodies (Henchal *et al.*, 1982). Homology of D4 to other types of dengue viruses was lower than other combinations (Fig. 2), as illustrated in 3 dimensional scheme of Fig. 3. This scheme was obtained by comparing total score of structural protein region and each distance among 5 viruses was compared with the distance between D2J and D2S which was taken as 1.0.

In order to find out the highest and lowest homology regions, more fine analysis of each 10 AA sequences were performed and the result was shown in Fig. 4. Homology tendency among dengue viruses was almost similar to those of other flaviviruses, with highly conserved regions at almost similar regions (Haishi, 1990).

In C protein region a hexapeptide sequence of Asn−Met−Leu−Lys−Arg−Gly was seen in all dengue viruses, similar to other mosquito-borne flaviviruses, except tick-borne encephalitis (TBE) virus (Haishi, 1990). Similar sequence was reported in some other nucleic acid-binding proteins (Heinkoff *et al.*, 1983; Delcuve *et al.*, 1980; Brauer and Roming, 1979). On the other hand the least conserved region was AA No. 2325−2345 in NS4A of dengue and other flaviviruses (Fig 5). Since this region appears to be type (or strain) specific, it would be a suitable sewuence for serotyping of viral isolates or assaying type-specific antibodies.

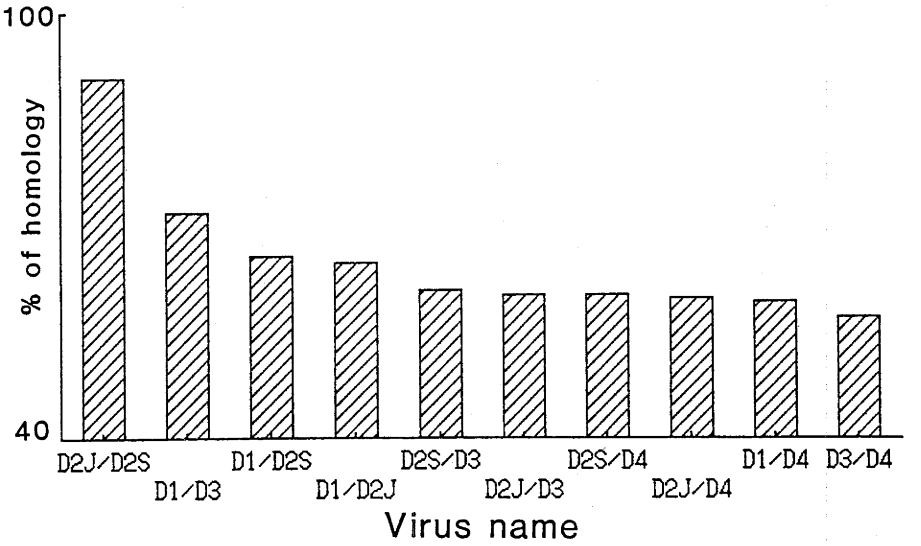

Fig. 2. Graphic presentation of AA homology % of whole virus genome. Abbreviation of virus name is shown in text.

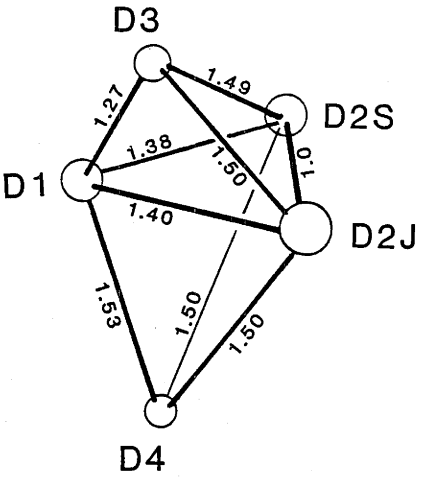

Fig. 3. Three dimensional configuration of dengue virus homology Abbreviation of genome segment is shown in text.

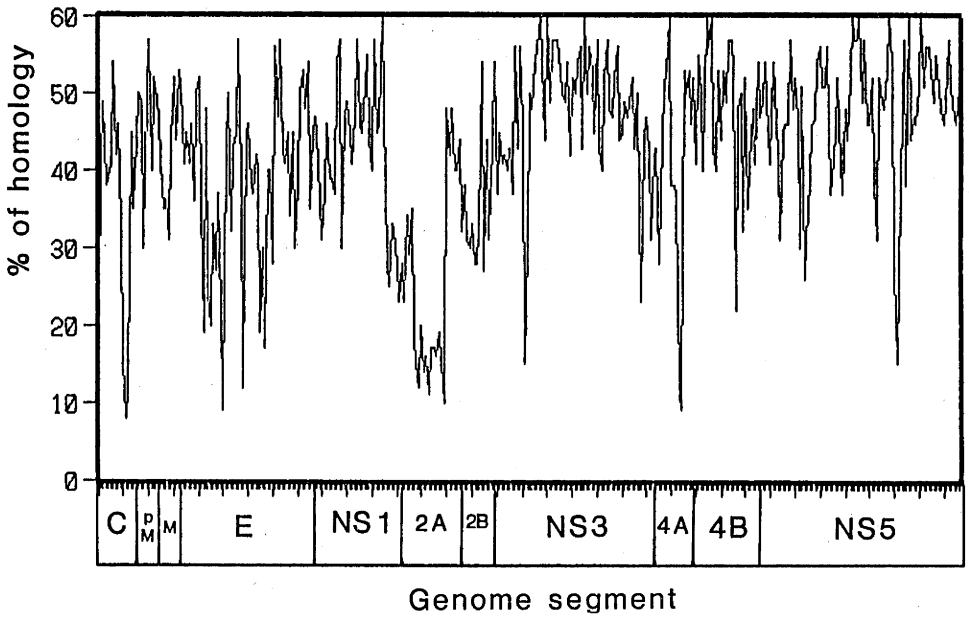

Fig. 4. Graphic presentation of AA average homology % of D2J, D2S, D3, D4 in each 10 AA residues.
Abbreviation of virus name is shown in text.

AA No				
VIRUS	2325	2335	2345	
D2J	TK	KDLGLGNIATQQPESNILDI		DL
D2S	TK	KDLGLGSITTQESESNILDI		DL
D3	TK	RDLGMSKEPGVVSPTSYLDV		DL
D4	TK	TDFGFYQVKTETTILDV		DL
WN	TK	NDIGSLLGHRPEARETTLGVESFLL		DL
JE	TK	ADLKSMFVGKTQASGLTGLPSMAL		DL
KUN	TK	SDISGLFGQRIETKENFSIGEFLL		DL
TBE	TK	ADLSTALWSEREEPRPWSEWTNY		DI
YF	TK	EDLFGKKNLIPSSASPWSWPDL		DL

Fig. 5. The least conserved regions in flaviviruses.
Abbreviation of virus name is shown in text and followings: JE: Japanese encephalitis, KUN: Kunjin, TBE: tick-born encephalitis, WN: West Nile viruses, YF: yellow fever. AA names were shown by single letter codes.

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REFERENCES

1) Brauer, J. F. & Roming, R. (1979): The primary structure of protein S3 from the small ribosomal subunit of *Escherichia coli*. FEBS Letters, 106, 352−357.

2) Clarke, D. H. & Casals, J. (1965): Arboviruses; group B. pp606−658. *In* F. L. Horsfall, Jr., I. Tamm (ed.). Viral and Rickettsial Infections of Man. J. B. Lippincott Co. Philadelphia, Montreal.

3) Delcuve, G., Downing, W., Lewis, H. & Dennis, P. P. (1980): Nucleotide sequence of the proximal protein of the RNA polymerase beta subunit gene of *Escherichia coli*. Gene, 1, 367−373.

4) Deubel, v., Kinney, R. M. & Trent, D. W. (1986): Nucleotide sequence and deduced amino acid sequence of the structural proteins of dengue type 2 virus, Jamaica genotype. Virology, 155, 365−377.

5) Goelet, P., Lomonossoff, G. P., Butler, P. J. G., Akam, M. E., Gait, M. J. & Karn, J. (1982): Nucleotide sequence of tobacco mosaic virus RNA. Proc. Natl. Acad. Sci. U.S.A., 79, 5818−5822.

6) Gorbalenya, A. E., Koonin, E., V., Donchenko, A., P. & Blinov, V. M. (1988): A conserved NTP-motif in putative helicases. Nature, 333, 22.

7) Hahn, Y. S., Galler, R., Hunkapiller, T., Dalrymple, J. M., Strass, J. H. & Strauss, E. G. (1988): Nucleotide sequence of dengue 2 RNA and comparison of the encoded proteins with those of other flaviviruses. Virology, 162, 167−180.

8) Haishi, S. (1990): Comparative nucleotide sequences of flavivirus genomic RNA and deduced amino acid sequences of viral proteins. Trop. Med., 32 (1), 17−31.

9) Halstead, S. B. (1966): Mosquito-borne haemorrhagic fevers of South and Southeast Asia. Bull. WHO., 35, 3−15.

10) Halstead, S. B. (1980): Dengue haemorrhagic fever: a public health problem and a field for research, Bull. WHO., 58, 1−21.

11) Heinkoff, S., Keene, M. A., Fechtel, K. & Fristrom, J. (1986): Gene withn a gene: nested *Drosophila* genes encode unrelated proteins on opposite DNA strands. Cell, 44, 33−42.

12) Kitamura, N., Semler, B. L., Rothberg, P. G., Larsen, G. R., Asler, C. J., Dorner, A. J., Emini, E. A., hamecak, R., Lee, J. J. van der Werf, S., Anderson, C. W. & Wimmer, E. (1981): Primary structure, gene origanization and polypeptide expression of poliovirus RNA. Nature, 291, 547−553.

13) Mackow, E., Makino, Y., Zhao, B., Zhang, Y. M., Markoff, L., Buckler-White, A., Guiler, M., Chanock, R. & Lai, C. (1987): The necleotide sequence of dengue type 4 virus: analysis of genes coding for nonstructural proteins (1987). Virology, 159, 217−228.

14) Mason, P. W., McAda, P. C., Mason, T. L. & Fournier, M. J. (1987): Sequence of the dengue−1 virus genome in the region encoding the three structural proteins and the major nonstructural protein NS1. Virology, 161, 262−267.

15) Osatomi, K. (1988): Complete nucleotide sequence of type 3 dengue virus genomic RNA and analysis of structural proteins. PhD Thesis for the Faculty of Pharmaceutical Sciences, Nagasaki University.

16) Rice, C. M., Lenches, E. M., Eddy, S. E., Shin, S. J., Sheets, R., L. & Strauss, J. H., (1985): Nucleotide sequence of yellow fover virus: implications for flavivirus gene expressioon and evoluti. Science, 229, 726−733.

17) Rice, C.M., Strauss, E.G. & Strauss, J. H.(1986): Structure of the flavivirus genome. pp276−326. *In* S. Schlesinger & M. J. Schlesinger (ed.). Togaviridae and Flaviviradae. H. Fraenkel-Conrat & R. R. Wagner (Ser. ed.) The Viruses. Plenum Press, New Youk.

18) Russell, P. K. (1971): Immunopathological mechanisms in the dengue shock syndrome. pp831−838. *In* D. B. Amos (ed.). Progress in Immunology. Academic Press, New York.

19) Speight, G., Coia, G., Parker, M. D. & Westaway, E. G. (1988): Gene mapping and positive identification of the nonstructural proteins NS2A, NS2B, NS3, NS4B and NS5 of the flavivirus Kunjin and their cleavage sites. J. Gen. Virol., 69, 23−34.

20) Strauss, J. H. & Strauss, E. G. (1986): Replication of the RNAs of alphaviruses and flavivirus. pp 71−90. *In* J. J. Holland, P. Ahlquist, & E. Domingo (ed.). RNA Genetics, Vol. 1. CRC Press, Florida.

21) Westaway, E. G., Brinton, M. A., Gaidamovitch, S. Ya., Horzinek, M. C., Igarashi, A., Kaariainen, L., Lvov, D. K., Porterfield, J. S., Russell, P. K., & Trent, D. W. (1985): Flaviviridae. Intervirology, 24, 183−192.

22) Zhao, B. Mackow, E., Buckler-White, A., Markoff, L., Chanock, R. M., Lai, C. J. & Makino, Y. (1986): Cloning full−length dengue type 4 viral DNA sequences: analysis of genes coding for structural proteins. Virology, 155, 77−88.