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PRIMARY STRUCTURE OF THE GENE CODING FOR THE HAEMAGGLUTININ OF INFLUENZA VIRUS A/LENINGRAD/385/80(H3N2): DETECTION OF A POINT MUTATION RESPONSIBLE FOR THE ANTIGENIC DRIFT

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Summary. — Primary structure of the gene coding for haemagglutinin (HA-gene) of influenza virus A/Leningrad/385/80(H2N2) isolated during the epidemics of influenza in Leningrad in 1980 was determined. The close relationship of HA gene of this virus to the corresponding gene of the virus A/Bangkok/1/79(H3N2) was confirmed. It was shown that a single mutation in an antigenic site (the change from isoleucine to leucine at position 51 of HA1 gene) caused an antigenic drift. One silent mutation was detected (nucleotide 428 of HA1 gene) which points at the relatedness of strains A/Leningrad/385/80 with A/Bangkok/2/79 and with other more recent strains. These data allowed to determine the position of the strain A/Leningrad/385/80 HA gene regarding to the evolutionary relationships of HA genes of influenza A (H3N2 subtype) viruses. The branch leading to the above-mentioned strain is supposed to start from a point common for strains isolated following A/Bangkok/1/79. The mutations of HA genes presented in this subgroup were analysed supporting the notion on limited evolutionary potential of the subtype H3N2 influenza viruses.

Key words: *influenza virus*; *haemagglutinin gene*; *DNA sequencing*; *antigenic drift*; *evolution of influenza virus*

Introduction

The investigation of shift and drift changes of influenza virus remains an urgent problem. The major factor moving the evolution of influenza virus subtypes is the necessity to overcome the natural or vaccine-induced immunity. This can be achieved mainly by the variability of haemagglutinins (HA).

The development of methods of gene cloning and sequencing allows to monitor at molecular level the evolution of genes (especially of HA gene). This is necessary not only for the determination of interdependence between existing virus strains but for the evaluation of the potential of viruses belonging to a concrete subtype without which it is difficult to imagine a well documented prognosis of future changes.

The best studied nucleotide sequences of HA gene are of Hongkong (subtype H3N2) strains (the first of them were isolated in 1968). The evolutionary scheme of interdependence between different strains of this subtype has been created on the basis of the data on the primary structure of HA genes (Both *et al.*, 1983; Petrov *et al.*, 1986). Analysis of these data allowed to reveal the evolution mechanisms and make the conclusion about the limitated evolutionary potential of viruses of this subtype. It is reasonable to suppose that possibilities of genetic drift are restricted, first owing to a relatively small size of antigenic sites and secondly, because not all (in fact, few) amino acid substitutions appear to be neutral (that is not affecting molecular structural and functional stability) and at the same time useful for the virus with regard to epidemiological activity. This is reflected, for example, in the fact that repeated substitutions in the same positions are found during evolution. A part of these substitutions are reversions (Both *et al.*, 1983).

The available information is obviously insufficient for a complete understanding of differences between successful and unsuccessful variants of HA molecular structure and consequently of the mechanisms of influenza virus drift. The data presented in this paper on the primary structure of HA gene of a Bangkok subgroup virus — the A/Leningrad/385/80 — contribute to the understanding of the evolution of influenza A(H3N2) viruses.

Materials and Methods

Viruses. Influenza virus strain A/Leningrad/385/80R(H3N2) (a vaccine strain, Gorev *et al.*, 1983) was obtained by recombination from strain A/Leningrad/385/80(H3N2) isolated during an epidemic in Leningrad in the Virology laboratory of the Institute of experimental Medicine, (U.S.S.R. Academy of Medical Sciences) and strain A/PR8/34(H1N1). According to the authors' data the recombinant inherited the HA nad NA genes from A/Leningrad/385/80.

Haemagglutination-inhibition (HI) test was performed according to a conventional metodh using 4 HAU of the virus; serum was incubated for 60 min at 20 °C and then the mixture reacted with 1 % chicken erythrocytes.

DNA copies of the viral RNA were synthesized on the virion RNA (vRNA) template using oligonucleotide primers and reverse transcriptase. These and the recombinant plasmid DNAs were used as for the HA gene sequencing. Influenza viruses were grown in chick embryos and purified by differential centrifugation and treatment with Freon-113. The RNA was extracted by the phenol detergent method using pronase. Virus-specific double-stranded DNA for obtaining recombinant DNA was synthesized on vRNA templates in the presence of synthetic oligonucleotide primers and inserted into a *Pst*I site of the plasmid pBR322 using G-C linkers; *E. coli* HB101 strain (Plusnin *et al.*, 1983) was used for transformation.

Clones containing the recombinant DNA were selected by screening of the colonies using labelled vRNA as a probe. For sequencing the virus-specific sequences were recloned in the *Pst*I site of *pUC*18 and *pUC*19 plasmids containing a polylinker. The plasmid DNA was extracted (Birnboim and Dolly, 1979) and purified by gel filtration on Sepharose CL-2B. Terminal labelling of fragments obtained by digestion with restriction endonucleases *Bam*HI, *Hind*III, *Eco*RI,

Table 2. Nucleotide and amino acid changes in the sequences of HA gene cDNAs and in corresponding proteins of the A/Bangkok group viruses

Nucleotide no.,	Amino acid no., antigenic site	Codons with mutations and nonidentified bases and corresponding amino acids of strains		
		A/Leningrad/385/80	A/Bangkok/1/79	A/Bangkok/2/79
59	−7	ATT (Ile)	ATT (Ile)	ATC (Ile)
76	−1	GCC (ala)	GCC (ala)	GTC (val)
128	17	CAT (his)	CAC (his)	CAC (his)
200	41	GAA (glu)	GAG (glu)	GAG (glu)
228	51 site C	CTA (leu)	ATA (ile)	ATA (ile)
234	53 site C	GAC (asp) TAC (tyr)*	GAC (asp)	GAC (asp)
428	117	ACC (tre)	ACT (tre)	ACC (tre)
638	187 site B	ACG (tre)	ACG (tre)	ACA (tre)
639	188 site B	GAC (asp)	GAC (asp)	TAC (tyr)
731	218	GGG (gly)	GGG (gly)	GGA (gly)
910	278 site C	AGT (ser)	AGT (ser)	ATT (ile)
959	294	TTT (phe)	T?T (?)	TTT (phe)
1034	319	GGA (gly)	GGG (gly)	GGG (gly)
HA2 part**				
48	16	GGA (gly)	GG? (gly)	
52	18	GTA (val)	?TA (?)	
56	19	GAC (asp)	G?C (?)	
96	32	ACC (tre)	ACA (tre)	
327	109	GAA (glu)	GAT (asp)	
539	180	TCA (ser)	T?A (?)	
623				
624	208	TGG (try)	T?? (?)	
625	209	GCC (ala)	?CC (?)	

* The mutation was detected by sequencing of the cloned fragment.
** Information on the H2 gene region structure of A/Bangkok/2/79 was not published.

Msp I was carried out using polynucleotide kinases of phage T4 in the presence of γ^{32}P-ATP or the *E. coli* Klenow fragment DNA polymerase I in the presence of α^{32}P-dNTP ("ISOTOP", U.S.S.R.). For sequencing of cDNA copies and of the cloned fragments we used a modified method of Maxam and Gilbert (1977) (Chumpilo and Kravchenko, 1983).

Results

Analysis of the antigenic properties of the original strain A/Leningrad/385/80 and of its relationship with other viruses of the Bangkok subgroup was performed by a standard HI test using polyclonal antisera (Table 1). It showed that this strain was a drift variant of virus A/Bangkok/1/79. Drift

changes are connected specifically with the HA. Rabbit serum against the recombinant R16 possessing Neq neuraminidase allowed to exclude the steric hindrance, that might be caused by antibodies against the homologous neuraminidase (NA). A close resemblance between strains A/Leningrad and A/Bangkok/ 2 was detected: these strains and sera against them crossreacted in a homologous titre. However, they were not identical, since different patterns

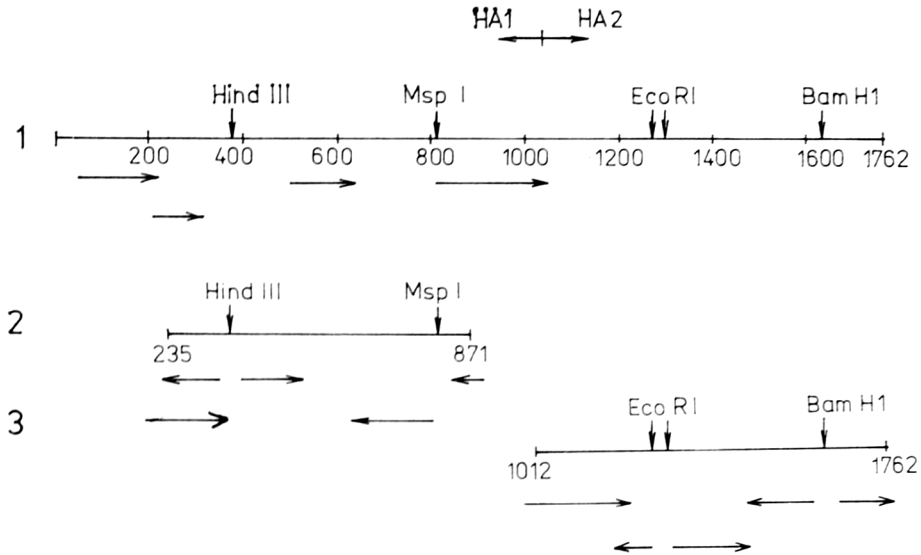

Fig. 1

The strategy of sequencing influenza virus strain A/Leningrad/386/80 HA gene and of cloned DNA-copies of its fragment. 1. Restriction map and schematic representation of the sequencing HA-gene from vRNA; 2. and 3. the same for the 2 cloned fragments.

Table 1. The characterization of influenza viruses A (H3N2) of 1979-1980 (titres in HI test)

Viruses	Sera against strains*			
	A/Bangkok/1/79	A/Bangkok/2/79	A/Leningrad 385/80	R16**
A/Bangkok/1/79	320	80	80	80
A/Bangkok/2/79	80	640	160	160
A/Leningrad/385/80	80	320	160	160
A/Leningrad/385/80R	80	320	160	320

* R16 — rabbit antiserum, the rest — rat antisera

** the recombinant strain R16, inheriting HA gene from strain A/Leningrad/385/80 and NA gene from A/Equine/Prague/1/56 was kindly provided by Gorev, N.F., Influenza Research Institute, U.S.S.R. Ministry of Health, Leningrad.

Fig. 2

The nucleotide sequence of influenza virus A/Leningrad/386/80 (H3N2) HA gene cDNA and a corresponding amino acid sequence.

*Controversal results in the sequencing vRNA and cDNA; **in parenthesis the sequences of strain A/Bangkok/1/79 (H3N2) are presented, since corresponding sequences of strain A/Leningrad/386/80 were not determined; ***the deletion of 3 nucleotides is observed following the triplet 225 of HA2.

	ATC	ATG	AAG	ACT	ATC	ATT	GCT	TTG	AGC	TAC	ATT	TTC	TGC
		met	lys	tre	ile	ile	ala	leu	ser	tyr	ile	phe	cys
				1									10
CTG	GTT	TTC	GCC	CAA	AAC	CTT	CCC	GGA	AAT	GAC	AAC	AGC	ACA
leu	val	phe	ala	gln	asn	leu	pro	gly	asn	acp	asn	ser	tre
									20				
GCA	ACG	CTG	TGC	CTG	GGA	CAT	CAT	GCA	GTG	CCA	AAC	GGA	ACG
ala	tre	leu	cys	leu	gly	his	his	ala	val	pro	asn	gly	tre
					30								
CTA	GTG	AAA	ACA	ATC	ACG	AAT	GAT	CAG	ATT	GAA	GTG	ACT	AAT
leu	val	lys	tre	ile	tre	asn	asp	gln	ile	gly	val	tre	asn
	40										50		
GCT	ACT	GAA	CTG	GTT	CAG	AGT	TCC	TCA	ACA	GGT	AGA	CTA	TGC
ala	tre	gly	leu	val	gln	ser	ser	ser	tre	gly	arg	leu	cys
G*							60						
TAC	AGT	CCT	CAC	CGA	ATC	CTT	GAT	GGG	AAA	AAC	TGC	ACA	CTG
asp	ser	pro	his	arg	ile	leu	asp	gly	lys	asn	cys	tre	leu
tyr													
			70										80
ATA	GAT	GCT	CTA	TTG	GGA	GAC	CCT	CAT	TGT	GAT	GGC	TTT	CAA
ile	asp	ala	leu	leu	gly	asp	pro	his	cys	asp	gly	phe	gln
									90				
AAT	GAG	AAA	TGG	GAC	CTT	TTT	GTT	GAA	CGC	AGC	AAA	GCT	TTC
asn	gly	lys	try	asp	leu	phe	val	gly	arg	ser	lys	ala	phe
					100								
AGC	AAC	TGT	TAC	CCT	TAT	GAT	GTG	CCA	GAT	TAT	GCC	TCC	CTT
ser	asn	cys	tyr	pro	tyr	asp	val	pro	asp	tyr	ala	ser	leu
	110										120		
AGG	TCA	CTA	GTT	GCC	TCG	TCA	GGC	ACC	CTG	GAG	TTT	ATC	AAT
arg	ser	leu	val	ala	ser	ser	gly	tre	leu	glu	phe	ile	asn
							130						
GAA	GGC	TTC	AAT	TGG	ACT	GGA	GTC	ACT	CAG	AGT	GGG	GGA	AGC
glu	glu	phe	asn	try	tre	gly	val	tre	gln	ser	gly	gly	ser
			140										150
TAT	GCT	TGC	AAA	AGG	GGA	TCT	GAT	AAC	AGT	TTC	TTC	AGT	AGA
tyr	ala	cys	lys	arg	gly	ser	asp	asn	ser	phe	phe	ser	arg
									160				
CTG	AAT	TGG	TTG	TAC	GAA	TCA	GAA	AGC	AAA	TAT	CCA	GTG	CTG
leu	asn	try	leu	tyr	glu	ser	glu	ser	lys	tyr	pro	val	leu
170													
AAC	GTG	ACT	ATG	GCA	AAC	AAT	GGC	AAT	TTT	GAC	AAA	CTG	TAC
asn	val	tre	met	pro	asn	asn	gly	asn	phe	ASP	lys	leu	tyr
	180										190		
ATT	TGG	GGG	GTT	CAC	CAC	CCG	AGC	ACG	GAC	AAA	GAA	CAA	ACC
ile	try	gly	val	his	his	pro	ser	tre	asp	lys	glu	gln	tre
							200						
AAC	CTA	TAT	GTT	CGA	GCA	TCA	GGG	AGA	GTC	ACA	GTC	TCT	ACC
asn	leu	tyr	val	arg	ala	ser	gly	arg	val	tre	val	ser	tre
AAG	AGA	AGC	CAG	CAA	ACT	ATA	ATC	CCG	AAT	ATC	GGG	TCT	AGA
lys	arg	ser	gln	gln	tre	ile	ile	pro	asn	ile	gly	ser	arg

Fig. 2 continued

									230				
CCC	TGG	GTA	AGG	GGT	CTG	TCT	AGT	AGA	ATA	AGT	ATC	TAT	TGG
pro	try	val	arg	gly	leu	ser	ser	arg	ile	ser	ile	tyr	try
					240								
ACA	ATA	GTA	AAA	CCG	GGA	GAC	ATA	CTG	TTA	ATT	AAT	AGT	AAT
tre	ile	val	lys	pro	gly	asp	ile	leu	leu	ile	asn	ser	asn
	250										260		
GGG	AAC	CTA	ATT	GCT	CCT	CGG	GGT	TAC	TTC	AAA	ATA	CGC	ACT
gly	asn	leu	ile	ala	pro	arg	gly	tyr	phe	lys	ile	arg	tre
							270						
GGG	AAA	AGC	TCA	ATA	ATG	AGG	TCA	GAT	GCA	CCT	ATT	GGC	ACC
gly	lys	ser	ser	ile	met	arg	ser	asp	ala	pro	ile	gly	tre
			280										290
TGC	AGT	TCT	GAA	TGC	ATC	ACT	CCA	AAT	GGA	AGC	ATT	CCC	AAT
cys	ser	ser	glu	cys	ile	tre	pro	asn	gly	ser	ile	pro	asn
									300				
GAC	AAG	CCC	TTT	CAA	AAC	GTA	AAC	AAG	ATC	ACA	TAT	GGG	GCA
asp	lys	pro	phe	gln	asn	val	asn	lys	ile	tre	tyr	gly	ala
					310								
TGT	CCC	AAG	TAT	GTT	AAG	CAA	AAC	ACT	CTG	AAG	TTG	GCA	ACA
lys	pro	lys	tyr	val	lys	gln	asp	tre	leu	lys	leu	ala	tre
	320								HA 1				
GGA	ATG	CGG	AAT	GTA	CCA	GAG	AAA	CAA	ACT				
gly	met	arg	asn	val	pro	glu	lys	gln	tre				
						1	HA 2						
						AGA	GGC	ATA	TTC	GGC	GCA	ATA	GCA
						arg	gly	ile	phe	gly	ala	ile	ala
		10										20	
GGT	TTC	ATA	GAA	AAT	GGT	TGG	GAG	GGA	ATG	GTA	GAC	GGT	TGG
gly	phe	ile	glu	asn	gly	try	glu	gly	met	val	asp	gly	try
								30					
TAC	GGT	TTC	AGG	CAT	CAA	AAT	TCT	GAG	GGC	ACC	GGA	CAA	GCA
tyr	gly	phe	arg	his	gln	asn	ser	glu	gly	tre	gly	gln	ala
				40									
GCA	GAT	CTT	AAA	AGC	ACT	CAA	GCA	GCA	ATC	GAC	CAA	ATC	AAT
ala	asp	leu	lys	ser	tre	gln	ala	ala	ile	asp	gln	ile	asn
50										60			
GGG	AAA	CTG	AAT	AGG	GTA	ATC	GAG	AAA	ACG	AAC	GAG	AAA	TTC
gly	lys	leu	asn	arg	val	ile	glu	lys	tre	asn	glu	lys	phe
						70							
CAT	CAA	ATC	GAA	AAG	GAA	TTC	TCA	GAA	GTA	GAA	GGG	AGA	ATT
his	gln	ile	glu	lys	glu	phe	ser	glu	val	glu	gly	arg	ile
		80										90	
CAG	GAC	CTC	GAG	AAA	TAC	GTT	GAA	GAC	ACT	AAA	ATA	GAT	CTC
gln	asp	leu	glu	lys	tyr	val	glu	asp	tre	lys	ile	asp	leu
								100					
TGG	TCT	TAC	AAC	GCG	GAG	CTT	CTT	GTC	GCT	CTG	GAC	AAC	CAA
try	ser	tyr	asn	ala	glu	leu	leu	val	ala	leu	glu	asn	gln
				110									
CAT	ACA	ATT	GAA	CTG	ACT	GAC	TCG	GAA	ATG	AAC	AAA	CTG	TTT
his	tre	ile	glu	leu	tre	asp	ser	glu	met	asn	lys	leu	phe
120										130			
GAA	AAA	ACA	AGG	AGG	CAA	CTG	AGG	GAA	AAT	GCT	GAG	GAC	ATG
glu	lys	tre	arg	arg	gln	leu	arg	glu	asn	ala	glu	asp	met
						140							
GGC	AAT	GGT	TGC	TTC	AAA	ATA	TAC	CAC	AAA	TGT	GAC	AAT	GCT
gly	asn	gly	cys	phe	lys	ile	tyr	his	lys	cys	asp	asn	ala
		150										160	

Fig. 2 continued

TGC	ATA	GGG	TCA	ATC	AGA	AAT	GGA	ACT	TAT	GAC	CAT	GAT	GTA
cys	ile	gly	ser	ile	arg	asp	gly	tre	tyr	asp	his	asp	val
								170					
TAC	AGA	GAC	GAA	GCA	TTA	AAC	AAC	CGG	TTT	CAG	ATC	AAA	GGT
tyr	arg	asp	glu	ala	leu	asn	asn	arg	phe	gln	ile	lys	gly
				180									
GTT	GAG	CTG	AAG	TCA	GGA	TAC	AAA	GAC	TGG	ATC	CTG	TGG	ATT
val	glu	leu	lys	ser	gly	tyr	lys	asp	try	ile	leu	try	ile
190										200			
TCC	TTT	GCC	ATA	TCA	TGC	TTT	TTG	CTT	TGT	GTT	GTT	TTG	CTG
ser	phe	ala	ile	ser	cys	phe	leu	leu	cys	val	val	leu	leu
						210							
GGG	TTC	ATC	ATG	TGG	GCC	TGC	CAA	AAA	GGC	AAC	ATT	AGG	TGC
gly	phe	ile	met	try	ala	cys	gln	lys	gly	asn	ile	arg	cys
		220											
AAC	ATT	TGC	ATT	TGA	GTG	TAT	TAG	***	TTA	AAA	ACA	CCC	TTG
asn	ile	cys	ile										
TTT	CTA	CT											

of the interaction of sera against these strains with virus A/Bangkok/1 were observed (1/2 to 1/8 of the homologous titre, respectively).

The strategy of sequencing is shown in Fig. 1. The information was obtained mainly by sequencing the cloned DNA copies (80%). In those regions, where the sequence was determined by both methods, complete coincidence of the data was observed with the exception of one case (nucleotide 234 of HA1 region) which will be discussed below. Nucleotide sequence of HA gene was established (beginning with 27th nucleotide) on the 3′-terminus of vRNA except of 100 nucleotides flanking the restriction endonuclease recognition sites (which were labelled).

The cDNA nucleotide sequence of the HA gene and the corresponding amino acid sequence are presented in Fig. 2. Amino acids are numbered

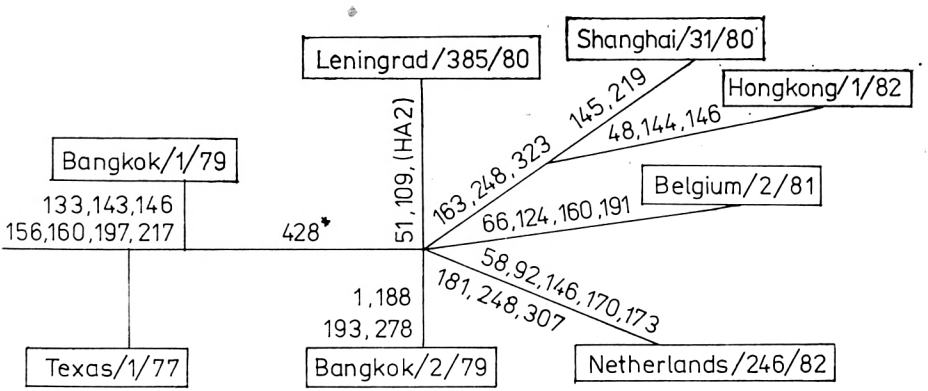

Fig. 3

Evolutionary interrelationships of human influenza virus A (H3N2) HA genes(Petrov *et al.* 1986). Figure also shows the localization of the amino acid exchanges. *The localization of silent nucleodite substitution.

from the first residue of the mature protein; the nucleotide 78 from the 3′-terminus of vRNA is the first nucleotide of the triplet coding for the first amino acid. The mature HA molecule contains 329 amino acid residues in the HA1 region and 221 amino acid residues in the HA2 region. Comparing the HA gene nucleotide sequence of virus A/Leningrad/385/80 and of the most closely related strains A/Bangkok/1/79 and A/Bangkok/2/79 (Both and Sleigh, 1981; Both *et al.*, 1983) a number of differences was found; these are shown and summarized in Table 2.

The missense mutation in codon 51, leading to the substitution of isoleucine to leucine residue is the most important difference of virus A/Leningrad/385/80 HA gene sequence from that of strains A/Bangkok/1/79 and A/Bangkok/2/79. This amino acid is a part of the antigenic site C. This change apparently stimulated the virus drift. No other differences in the HA amino acid sequences which would occur in their antigenic sites were detected in the HA of strains A/Leningrad/385 and A/Bangkok/1/79. On the other hand, two differences were observed with virus A/Bangkok/2/79. The first two strains have aspartic acid residue at position 188 (site B); the third strain has tyrosine in this position. The first two strains have serine residue at position 278 (site C) and the third strain has isoleucine in this position.

One amino acid change was detected in the conservative HA2 region of the HA of virus A/Leningrad/385/80 in comparison with virus A/Bangkok/1/79 (this is a change at pos. 109 of HA2 from aspartic acid to glutamine acid). The mutation at residue 327, leading in this case to a meaningful change, was noticed not only among viruses of influenza A/Hongkong subtype but also earlier. Valine was located at pos. 18 of HA2 of the strain A/Leningrad/385/80; the authors could not identify the first nucleotide of corresponding codon in the gene of virus A/Bangkok/1/79, however, the strain A/NT/60/68 has isoleucine at residue 18. Amino acid changes in HA2 are detected rather infrequently because this very conservative region has a structural function. So, viruses A/NT/60/68 and A/Bangkok/1/79 differ only by 3 amino acids in this region. Probably, the mutation detected by us at residue 18 is also typical for strain A/Bangkok/1/79. In latter case this mutation can be considered the fourth one.

A single silent mutation makes strains A/Leningrad/385/80 and A/Bangkok/2/79 interrelated by the presence of C in the position 428 of the HA1 region of HA-gene. Thymidine is located in this position in strain A/Bangkok/1/79 and in earlier strains. The mutations at position 428 of HA1 region and at position 96 of HA2 region of virus A/Leningrad/385/80 (in comparison with virus A/Bangkok/1/79) were confirmed by restrictase mapping, since the change in the first position mentioned causes appearance of a new site for *Mva*I; the change in the second position causes appearance of a new site for *Msp*I. We detected a deletion of 3 nucleotides (676-678 in HA2 region) in the nonstructural region of HA gene of virus A/Leningrad/385/80; thus, this region of strain A/Leningrad/385/80 contains 32 nucleotides instead of usual 35 nucleotides typical for other isolates of subtype A/Hongkong. The strain A/USSR/2/85 (H3N2) has an analogous mutation (Petrov *et al.*, in press).

Discussion

Summarizing all the data obtained one can see that a close relationship was established between HA genes of viruses A/Leningrad/385/80 and A/Bangkok/1/79, although a single silent mutation has been found pointing at relatedness of A/Leningrad/385/80 and A/Bangkok/2/79. So, there are only 2 meaningful differences between strains A/Leningrad/385/80 and A/Bangkok/1/79: at the residue 51 of HA1 (antigenic site C) and at the residue 109 of HA2. There are also 5 silent mutations, none of them is located in the region coding for the antigenic site. Five meaningful differences were detected between strains A/Leningrad/385/80 and A/Bangkok/2/79 (triplets 1, 51, 188, 193, and 278) from them 3 mutations were located in antigenic sites B (188) and C (51 and 278); 7 silent changes of nucleotides were also detected in this case.

Thus, the sequencing results (Table 2) and antigenic analysis (Table 1) of strains A/Leningrad/385/80 and A/Bangkok/1/79 coincided. It follows that the molecular basis for the drift of the first strain from the second one is the change of a single amino acid, located in the antigenic site C (Ile $_{51}$ — Leu). At the same time upon comparison of strains A/Leningrad/385/80 and A/Bangkok/2/79 certain contradictions are revealed, i.e. more pronounced structural differences between haemagglutinins of these strains are not reflected in HI test. In our opinion this is explained not only by disadvantages of the use of polyclonal antisera (one can succed thereby in revealing drift differences of strains A/Leningrad/385/80 and A/Bangkok/1/79) but also by the fact that two changes (Ile $_{51}$—Leu and Ile $_{278}$—Ser) are located in the antigenic site C and can compensate each other due to their close location. Obviously to clarify the situation it is necessary to use a sufficiently representative set of monoclonal antibodies to the HA of indicated strains.

The mutation at residue 53 of strain A/Leningrad (from aspartic acid to tyrosine) detected upon sequencing of cloned DNA copy HA-gene, needs a special discussion. When determining the primary structure in this region using vRNA no mutation was detected. The change in the cloned DNA can be explained by two ways: 1. an error of reverse transcriptase occurred during cDNA synthesis on the vRNA template. (It is important that the modified nucleotide is the first in the cloned fragment and follows immediately the connector sequence). 2. at random selection of a mutant gene during the cloning. The second suggestion seems to be more likely, since first, the presence of minor components in RNA, differing from the main bulk of corresponding genes is characteristic for the population of influenza virus (Petrov *et al.*, 1986) and second, because mutations in amino acid 53 (always and/or in the first base of the triplet) already have been observed in a number of Hongkong subtype strains and their variants selected by monoclonal antibodies (Webster *et al.*, 1983).

Thus, in evolutionary scheme of Hongkong subtype HA genes, the branch, leading to strain A/Leningrad/385/80 should be started from the point common for all strains, following A/Bangkok/1/79 (Petrov *et al.*, 1986). The presence of mutation at position 428, common for strains A/Leningrad/385/80

and A/Bangkok/2/79, detectable in none of the earlier representatives of this subtype, including A/Bangkok/1/79 witness in favour of this scheme; a part of the scheme of HA gene evolution of H3N2 subtype is presented in Fig. 3. The scheme includes strains isolated after 1977.

According to the scheme, changes of 3 amino acid residues in antigenic site A (133, 143, and 146) and 3 amino acid — in antigenic site B (156, 160, and 197) are mutations, common for the group of strains, starting from A/Bangkok/1/79; these mutations distinguish them from the group of viruses, related to strain A/Texas/1/77. The reversion of amino acid in position 217 is also common for these viruses. The following changes of amino acids among some strains in this group have emerged in the antigenic sites: 144 (Hongkong/1/82), 145(Shanghai/31/80),146(Hongkong/1/82) and Netherlands/246/82 — site A, 160(Belgium/2/81) and 188(A/Bangkok/2/79) and Hongkong/1382 — site B, 51(A/Leningrad/385/80) and 278(A/Bangkok/2/79) — site C.

Three of these mutations (at positions 51, 145, and 160) induce the appearance of such amino acids, which were absent earlier in these positions, and the mutation at residue 51 is unique because the mutations were not detected in this point neither before nor after strain A/Leningrad/385/80. The mutation at position 188 in the strain A/Bangkok/2/79 HA gene is a repeated one and the changes at position 188 of strain Hongkong/1/82 as well as at positions 144, 146, and 278 of strain Hongkong/1/82 and of other strains are reversions. The presence of repeated mutations and, especially, of reversions corresponds to the suggestion that the number of different variants of amino acid composition of antigenic sites meeting the requirements of retaining structural and functional stability of the protein molecule is severely restricted.

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