Binding of Viral Glycoprotein with Trypsin and Its Relation to Virulency. I. Initial Step of Binding

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ABSTRACT. The interaction between surface proteins of some enveloped viruses and trypsin was studied by computer analysis. Prior to the cleavage of the viral protein by trypsin, hydrophobic interaction between them at the vicinity of their active sites may occur. An exposed hydrophobic portion was found there which theoretically could stimulate the interaction. This interaction would be rather non-specific: according to the analysis, trypsin could bind equally well with weakly virulent virus and virulent viruses. Following this interaction, a specific reaction between their active sites would occur. The specificity was found to be related to the virulency of the virus.

The virulency of some viruses depends on the sensitivity of their surface F protein to trypsin. (Proteolytic cleavage at the recognition site of the virus is considered to induce viral infection.) The energy of interaction between these two proteins has been calculated when the active site of trypsin, 170-KDS, was positioned at the cleavage recognition site of the viral F protein. The results showed that mainly two kinds of energy, van der Waals energy and electrostatic energy, are decisive for viral virulency (5).

Previously this problem was approached statistically by deviation analysis, which was originally introduced by Kiho (3). It is based on the following principles. i) The frequency of occurrence of each amino acid in a defined sequence with functional significance more or less deviates from uniform distribution, and the amount of deviation (dev) seems to parallel the function of the sequence. ii) Binding of two peptides results in a decrease of their dev values in accordance with the saturation of their binding abilities. Dev analysis is used to deduce the interacting site in proteins. It was found that the active site of trypsin binds to a viral site (dev site) a few amino acids upstream of the cleavage recognition site.

Abbreviations: SV5, simian virus 5; ReSV, respiratory syncytial virus; VISV, Visna virus; NDV (vir), Newcastle disease virus, virulent strain; NDV (avir), avirulent strain of NDV; RSV, Rous sarcoma virus; MMTV, mouse mammary tumor virus; HIV, human immunodeficiency virus type 1; FPV, fowl plague virus; HTLV-1, human T lymphotropic virus; WSN, influenza virus A/WSN/33; PR8, influenza virus PR8; HVJ (Z), Sendai virus, Z strain. The amino acid abbreviations follow standard conventions.

The results further showed that a neighbor to the active site of trypsin also binds to the dev site first, followed by the interaction with the cleavage recognition site and the cleavage reaction (4). Figure 1 shows the dev sites of NDV (vir) for 165-YLE and 170-KDS of trypsin and the cleavage recognition site for 170-KDS. The details of these sites have already been reported (4, 5). In the present study, the interaction energies between trypsin and the dev site of several viral surface proteins were calculated.

MATERIALS AND METHODS

The interaction energies were calculated as follows. Topological structures of the interacting site of both proteins were constructed using acrophilicity parameters assigned to each amino acid (2). These two structures were then arranged as closely together as possible to visualize the topologically fitted interaction complex. The distances between amino acids in the two structures were determined and three kinds of interaction energy—van der Waals energy $V(V)$, interaction energy between side chain and water $V(S)$, and electrostatic interaction energy $V(E)$ —were estimated using the program PEP-IDENT (5).

Topology in relation to hydrophilicity was investigated using the acrophilicity and hydrophilicity parameter of each amino acid (1, 2). Examples of both profiles are shown in Figure 2. There are four main types of extrema: hydrophilic peak (hydrophilicity and acrophilicity are both positive), hydrophobic peak (negative hydrophilicity and positive acrophilicity), hydrophilic depression (positive hydrophilicity and nega-

Fig. 1. Interaction between trypsin-YLE or -KDS and NDV(vir) at the dev or cleavage site. The acrophilicity profiles of the dev and cleavage sites of NDV(vir) are shown. The circles represent amino acids. The acrophilicity profiles of two trypsin portions (165-YLE and 170-KDS) were positioned as closely as possible to the viral sites. The distances between the amino acids in the respective molecules were determined on pairs less than 50 Å apart, and $V(E)$, $V(V)$, and $V(S)$ were calculated. (A) Trypsin-YLE at its dev site, (B) trypsin-KDS at its dev site, (C) trypsin-KDS at the cleavage site. The values on the abscissa refer to position in the amino acid sequence of NDV(vir) F protein.

tive acrophilicity), and hydrophobic depression (both hydrophilicity and acrophilicity are negative). We paid the most attention to the second kind, hydrophobic peaks. Using the program "PROPART1" (see Appendix), we selected such regions of the amino acid sequence. The size of a hydrophobic peak, HOPK, was taken as the absolute value of the acrophilicity value times the hydrophilicity value.

RESULTS AND DISCUSSION

Figure 3A shows the energies $V(E)$ and $V(S)$ when the active site of trypsin, 170-D, is positioned at the cleavage site of the surface proteins of various viruses (for example, 89-QRRF in the case of NDV(vir) (Fig. 1C)). The values of $V(E)$ have a positive correlation with the viral virulency (5), which suggests that the electrostatic interaction between the trypsin active site and the cleavage site of the virus is decisive for virulent infection. In contrast, $V(S)$ is inversely correlated with virulency.

Figure 3B shows the energies $V(E)$, $V(S)$ and $V(V)$ when trypsin 170-KDS is positioned at a dev site of these proteins (for example, 87-RRQR in the case of NDV(vir) (Fig. 1B)). In contrast to the case of the cleavage site, the $V(E)$ values are variable and have no relation with the virulency. In some cases, no electrostatic interaction is operative, i.e., $V(E)=0$. $V(S)$ also has little correlation with virulency. However, the values are

rather uniform among these viruses, which suggests that 170-KDS binds to the dev sites of all viruses rather equally. The pattern for $V(V)$ was intermediate between those of $V(E)$ and $V(S)$.

Figure 3C shows that when trypsin 165-YLE binds to its corresponding dev site of a viral surface protein (for example, 83-TSGGR in the case of NDV(vir) (Fig. 1A)), the values of $V(S)$ and $V(V)$ are uniformly distributed among the viruses considered, and the $V(E)$ values are variable. The above results suggest that trypsin can bind fairly equally to the dev sites of all the viruses studied in this report. $V(S)$ is the controlling factor here, indicating that this process mainly operates by hydrophobic association. The binding is followed by the interaction with the cleavage site, in which the electrostatic force is the main factor on which viral virulency depends. Thus the first step is rather non-specific, but the second step is specific. These features coincide with the natures of $V(S)$ and $V(E)$, which control these processes. By the first hydrophobic interaction, the dielectric constant of the surrounding medium will decrease, which in turn stimulates the ionic interaction, resulting in the transfer of trypsin from the dev site to the cleavage site.

The first step can occur easily if the hydrophobic portion of the virus concerned is exposed on its surface (hydrophobic peak). Energetically, exposure of a hydro-

A Hydrophilicity and Acrophilicity

B Hydrophobic Peak

Fig. 2. (A) Hydrophilicity (shown by the curve) and acrophilicity (shown by the vertical bars) profiles of F protein of NDV(vir). The hydrophilicity or acrophilicity values (ordinate) are averaged for six amino acids consecutively along the sequence (abscissa). The cleavage site and dev sites are in the region between residues 80 and 90. (B) The hydrophobic peaks found in the same region as above are shown. The ordinate indicates the HOPK values.

phobic portion in aqueous medium is rather rare. Although rare, hydrophobic sites on the surface could easily bind with another hydrophobic sites. Figure 2A shows the hydrophilicity and acrophilicity profiles of the portion of F-protein of NDV(vir) in which two dev sites and the cleavage site occur: in the region between

residues 80 and 90. The hydrophobic peaks are shown in Figure 2B. The most notable HOPKs were found at 82-TT-83, whose values were 0.2692 and 0.3833 at 82-T and 83-T, respectively. These residues are exposed around the dev sites for trypsin binding. Two other important HOPKs were found at 18-VV-19 and at 100-

Fig. 3. Interaction energies of enveloped viruses. (A) Trypsin 170-KDS interacting with the cleavage site. (B) Trypsin 170-KDS interacting with the corresponding dev site. (C) Trypsin 165-YLE interacting with the corresponding dev site.

VA-101. The other peaks were small and scattered. Furthermore, a hydrophobic peak was also found in trypsin close to the active site, at 165-YL-166. Its HOPK values were 0.0667 and 0.1528 at 165-Y and 166-L, respectively. The interaction of these hydrophobic peaks would facilitate the binding of trypsin 165-YLE to the viral dev site, and this step is most affected by V(S).

Hydrophobic peaks near the binding site (dev site) were also found in other cases, including various F proteins of paramyxoviruses, as shown in Table I. This raises the possibility that the above discussion can be generalized. In the binding reaction of biological macromolecules, it is frequently said that the first interaction is ionic. For example, at the initial stage of viral infec-

tion, the adsorbed virus can be released by salt at high concentration. (After adsorption, the virus penetrates the membrane and enters the cell, where the hydrophobic interaction is operative and the infected virus cannot be released by high salt.) In view of the large value of the dielectric constant of water, it is difficult to consider simple ionic interaction as the first step of viral adsorption. Binding other than or in addition to ionic interaction, such as the binding of trypsin to the viruses described above, should be considered.

Table I. DISTANCES (IN TERMS OF AMINO ACIDS) BETWEEN THE BINDING SITE OF TRYPSIN-YLE (A DEV SITE), THE HYDROPHOBIC PEAK, AND THE CLEAVAGE SITE (2) IN VARIOUS VIRAL PROTEINS.

virus	hydrophobic peak	dev site for trypsin-YLE	cleavage site

virus	c	a	
SV5	8-11	2-5	
ReSV	7	4	
NDV (vir)	1-2	8	
RSV	-1	7	
VISV	16	5 (3)	
MMTV	16	2	
FPV	13	3	
HTLV-1	5	2	
HIV	5-8	7-10	
NDV (avir)	-2	9	
HVJ (Z)	-4	8 (4)	
PR8	-1	5	

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APPENDIX

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10 '      Protein Topology Analysis (PROPART1)
20 '
30 DIM SEQ$(1800),P$(1800)
40 CLS 3:CONSOLE 0,25,0,1:SCREEN 3,0,0,1
50 '
60 ' menu
70 '
80 PRINT "**** Selectable Menu ****":PRINT:PRINT
90 PRINT "[ 1 ] : Hydrophilicity Profile ":PRINT
100 PRINT "[ 2 ] : Acrophilicity Profile":PRINT
110 PRINT "[ 3 ] : Hydrophilicity and Acrophilicity Profile":PRINT
120 PRINT "[ 4 ] : Quit":PRINT:PRINT:PRINT
130 PRINT:INPUT "Select the number (1 - 4)..... ";ZZ$
140 IF ZZ$="4" THEN END ELSE GOTO 150
150 '
160 CLS 3:FILES 2:PRINT:PRINT
170 INPUT "File Name .....";SN$
180 OPEN "2:"+SN$ FOR INPUT AS #1
190   INPUT #1,F$,CMT$
200   NSEQ=0
210   WHILE NOT EOF(1)
220     INPUT #1,A$
230     NSEQ=NSEQ+1:SEQ$(NSEQ)=A$
240   WEND
250   CLOSE #1
260 '
270 INPUT "START----",A
280 INPUT "NUMBER OF AA,TEST----",B
290 J=1
300 FOR I=A TO A+B
310 P$(J)=SEQ$(I)
320 J=J+1
330 NEXT I:NSEQ=J-1
340 PRINT:PRINT
350 INPUT "The residue number for averaging (1-10).....";NA:PRINT:PRINT
360 INPUT "If you know the active site, key-in the residue number.....";ACTN
370 '
380 '      Acrophilicity and Hydrophilicity
390 '
400 '      T.P.Hopp
410 '      Synthetic Peptides in Biology and Medicine 1985,p3-12
420 '
430 DATA Arg,Asp,Glu,Lys,Ser,Asn,Gln,Gly,Pro,Thr
440 DATA Ala,His,Cys,Met,Val,Ile,Leu,Tyr,Phe,Trp
450 '
460 DATA 3,3,3,3,0.3,0.2,0.2,0,0,-0.4
470 DATA -0.5,-0.5,-1,-1.3,-1.5,-1.8,-1.8,-2.3,-2.5,-3.4
480 '
490 DATA 0.3,2.1,0.5,1.4,1.8,2.3,-0.2,3,2.6,-0.1
500 DATA -0.5,-0.4,-2.6,-1.8,-0.7,-2.5,-2.5,-2,-2.7,-3
510 '
520 DATA R,D,E,K,S,N,Q,G,P,T
530 DATA A,H,C,M,V,I,L,Y,F,W
540 '
550 DATA 1,0,1,1,2,1,2,2,3,2,3,3,4,3,4,4,5,4,5,5
560 '
570 DIM NAM$(20),IUP$(20)
580 DIM NS(10),NF(10),HYP(20),ACR(20),AVR(800),PAVR(800),PSE(800),PPSE(800)
590 '
600 FOR I=1 TO 20:READ NAM$(I):NEXT
610 FOR I=1 TO 20:READ HYP(I):NEXT
620 FOR I=1 TO 20:READ ACR(I):NEXT
630 FOR I=1 TO 20:READ IUP$(I):NEXT
640 FOR I=1 TO 10:READ NS(I),NF(I):NEXT
650 '
660 FOR I=1 TO NSEQ
670   FOR J=1 TO 20

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680     IF   P$(I)=IUP$(J) THEN PSE(I)=HYP(J)
690     NEXT J
700     NEXT I
710 '
720     FOR I=1 TO NSEQ
730       FOR J=1 TO 20
740         IF   P$(I)=IUP$(J) THEN PPSE(I)=ACR(J)
750       NEXT J
760     NEXT I
770 '
780 ' averaging
790 '
800     N0=NS(NA):N1=NF(NA)
810     FOR I=N0 TO NSEQ-N1:SUM=0
820       FOR K=I+1-N0 TO I+N1:SUM=SUM+PSE(K):NEXT K:AVR(I)=SUM/NA:NEXT I
830     FOR I=N0 TO NSEQ-N1:PSUM=0
840       FOR K=I+1-N0 TO I+N1:PSUM=PSUM+PPSE(K):NEXT K:PAVR(I)=PSUM/NA:NEXT I
850     MX=1:MA=AVR(1):PMX=1:MP=PAVR(1)
860     FOR I=2 TO NSEQ:IF AVR(I)>MA THEN MX=I:MA=AVR(I):NEXT I
870     FOR I=2 TO NSEQ:IF PAVR(I)>MP THEN PMX=I:MP=PAVR(I):NEXT I
880 '
890     PRINT:PRINT
900     INPUT "Do you want to print-out the calculated data (y/n) ";YY$
910     IF YY$="N" OR YY$="n" THEN GOTO 930
920     IF YY$="Y" OR YY$="y" THEN GOTO 1340
930 '
940     WIDTH 80,25:WINDOW(-20,-3)-(205,3):COLOR 7:CLS
950     LINE(-1,-2.5)-(200,2),7,B
960     LINE(-1,0)-(200,0),7:LINE(-1,1)-(.5,1):LINE(-1,2)-(.5,2)
970     LINE(-1,-1)-(.5,-1):LINE(-1,-2)-(.5,-2):LOCATE 10,0
980     CIRCLE (5,-2.9),1,1,0,3.14, ,F,1:CIRCLE (50,-2.9),1,3,3.14,6.28, ,F,3:CIRC
990     LOCATE 10,0:PRINT "R and K":LOCATE 26,0:PRINT "D and E"
1000    LOCATE 42,0:PRINT "H"
1010    LINE (140,-2.9)-(145,-2.9),2:LOCATE 60,0:PRINT "Active Site"
1020    LOCATE 30,22:PRINT "Sequence      Number"
1030    FOR I=1 TO 190 STEP 10:LINE(I,2)-(I,1.9):NEXT J=13
1040    FOR I=20 TO 190 STEP 20
1050      LOCATE J,21:PRINT USING "###";I:J=J+7
1060    NEXT:LOCATE 5,12
1070    PRINT "0":LOCATE 4,16:PRINT "-1":LOCATE 4,20:PRINT "-2"
1080    LOCATE 5,8:PRINT "1":LOCATE 5,4:PRINT "2"
1090 '
1100    IF ZZ$="2" THEN GOTO 1190 ELSE GOTO 1110
1110 '
1120 ' hydrophilicity profile
1130 '
1140    FOR I=1 TO NSEQ
1150      LINE(I,-AVR(I))-((I+1),-AVR(I+1))
1160    NEXT I
1170    LINE (ACTN,0)-(ACTN,-AVR(ACTN)),2
1180    IF ZZ$="1" THEN END ELSE GOTO 1190
1190 '
1200 ' acrophilicity profile
1210 '
1220    FOR I=1 TO NSEQ
1230      LINE (I,0)-(I,-PAVR(I))
1240    NEXT I
1250 '
1260    LINE (ACTN,0)-(ACTN,-PAVR(ACTN)),2
1270 ' hydrophilic residues
1280    FOR I=1 TO NSEQ
1290      IF   P$(I)="R" OR   P$(I)="K" THEN CIRCLE (I,-PAVR(I)),1,1,0,3.14, ,F,1
1300      IF   P$(I)="D" OR   P$(I)="E" THEN CIRCLE (I,-PAVR(I)),1,3,3.14,6.28, ,
1310      IF   P$(I)="H" THEN CIRCLE (I,-PAVR(I)),1,6
1320    NEXT I
1330    END

```

```

1340 ' print-out data
1350 '
1360 LPRINT CHR$(27);"!";"< ";CMT$;" >":LPRINT:LPRINT
1370 LPRINT CHR$(27);CHR$(&H22);
1380 LPRINT "Averaging group length =";NA:LPRINT
1390 LPRINT "      [Sequence]      Hyd      Acr      Avr(H)      Avr(A)"
1400 LPRINT
1410 FOR I=1 TO NSEQ
1420   FOR K=1 TO 20
1430     IF P$(I)=IUP$(K) THEN AA$=NAM$(K)
1440   NEXT K
1450   LPRINT USING "### && & & ";I, P$(I),AA$;
1460   LPRINT USING "##.## ##.##";PSE(I),PPSE(I);
1470   IF I<NO THEN LPRINT:GOTO 1500
1480   IF I>NSEQ-N1 THEN LPRINT:GOTO 1500
1490   LPRINT SPC(10);USING "##.#### ##.#### < ###";AVR(I),PAVR(I),I
1500 NEXT
1510 GOTO 930

```