A Fourth Scale Sensitive to the Magnetic Field; Intermolecular *Frequency* Symmetry in a Specific Interaction between Protein and Low-Molecular Compound

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Received June 25, 2010; accepted September 3, 2010; published online September 17, 2010

We found a new method that a specific interaction between prion, *i.e.*, high-molecular compound, and Cp-60, *i.e.*, low-molecular one, could be successfully elucidated with intermolecular *frequency* symmetry (IFS). To accomplish this, the former sequence is analyzed with a sequence Fourier analysis used average nuclear (N) resonant frequency scale as a fourth one, and the latter structure with a ^{13}C-NMR software. Further, such the symmetry could be observed in a specific interaction between a segment of human immunodeficiency virus (HIV)gag and PA-457 or between 1918 neuraminidase and peramivir. Therefore, the IFS rule seems to be evolutionarily conserved as a necessary condition even in a specific protein-organic compound interaction.

Key words prion; Cp-60; human immunodeficiency virus gag; PA-457; 1918 neuraminidase; Peramivir

We had already reported a method that a specific protein–protein (or DNA, RNA) interaction can be successfully elucidated from both amino acid (aa) sequence and the corresponding RNA (na) one with a sequence Fourier analysis,[1—5] on which some of the Mulliken's absolute electronegativity (M) scale (row 1a in Table 1),[4,5] the Lacey's relative hydropathy (H) one (row 2 in Table 1)[1,3] or the Garel's (G) one (row 3 in Table1),[1] is assigned as a parameterization. Both the calculation process[5] and the criteria (including two kinds of symmetry operation)[1,4] had been already reported. Of other scales, further average nuclear (N) resonant frequency scale, divided into four kinds (N_1, N_2, N_3 and N_4) of types,[6] could be found as a fourth scale,[7—10] which are similar (but not the same) to the M scale more than the H or the G scale (see Table 1). Here, note that 42.57, 10.70, 4.31, 5.77, and 3.27 MHz (T=1), which are the NMR frequency value of ^{1}H, ^{13}C, ^{15}N, ^{17}O and ^{33}S respectively,[11] are used to calculate the N scale by Sanderson's equation.[5]

In the $N_{1—4}$ scale context, first it was noticed that a specific interaction between wild type mature prion protein (231aa),[12] as a biologically active structure, and Cp-60 (Fig. 1)[12,13] might be successfully elucidated under the condition of almost the same criteria as described above. To accomplish this, the desired cross-spectrum (Fig. 2) of the prion is constructed from both the protein (231aa) and the mRNA (693na) sequence using the N_2 scale only[6] as a parameterization. In the Cp-60, the ^{13}C-NMR spectrum was predicted by using a commercially available software.[14] The chemical shift (δ) value predicted is arranged in reference and note,[15,16] including that of PA-457[15,17] or peramivir[15,18] (*vide infra*). At this point, 3 working hypotheses are tentatively endowed on the analytical data of the Cp-60 to investigate a specific interaction with the prion.

1) The Cp-60 is regarded as a virtual mixture (1 : 1) of D and L isomer,[19] which are corresponding to something like a wild type sense and antisense aa sequence, respectively (*vice versa*).
2) The δ value of peak(s) expressed in the spectrum region from 220 to 0 ppm is converted to the frequency (f) scale from 0.5 to 0, referred to Shannon's sampling theorem.[20]

Table 1. Various Scale for Amino Acid (aa) and Nucleic Acids (na)

na	1a)	1b)[6]	1c)[6]	2)	3)
u	2.9506	17.3	0.0578	0.69	0.30
c	2.9487	19.3	0.0518	0.62	0.35
a	2.9290	17.5	0.0571	0.26	1.10
g	2.9481	16.7	0.0599	0.44	0.53
t	2.9461	17.4	0.0575	—	0.94
aa	**1a)**	**1b)**	**1c)**	**2)**	**3)**
L	2.9396	32.80	0.0305	3.29	16.71
I	2.9396	32.80	0.0305	3.64	16.36
N	2.9845	25.22	0.0397	16.14	3.86
G	3.0590	42.60	0.0235	14.79	5.21
V	2.9426	33.01	0.0303	7.50	12.5
E	2.9738	25.65	0.0390	14.64	5.36
P	2.9297	31.95	0.0313	7.57	12.43
H	2.9223	24.02	0.0417	12.79	7.21
K	2.9571	31.52	0.0317	16.21	3.79
A	2.9620	34.60	0.0289	12.07	7.93
Y	2.8883	25.24	0.0396	4.57	15.43
W	2.8663	24.51	0.0408	2.57	17.43
Q	2.9695	27.05	0.0370	14.36	5.64
M	2.9161	30.31	0.0330	6.57	13.43
S	3.0126	28.84	0.0347	14.93	5.07
C	2.8998	28.34	0.0353	8.29	11.71
T	2.9815	30.00	0.0333	13.64	6.36
F	2.8650	26.64	0.0375	2.64	17.36
R	2.9719	28.32	0.0353	15.93	4.07
D	2.9927	22.95	0.0436	16.29	3.71

1a): M scale; Mulliken's absolute electronegativity scale. 1b): N scale; Average nuclear resonant frequency scale. 1c): 1/N scale; Inverse N scale. 2): H scale; Lacey's relative hydropathy scale. 3): G scale; Garel's relative hydropathy na scale and the Lacey's reversed aa one.

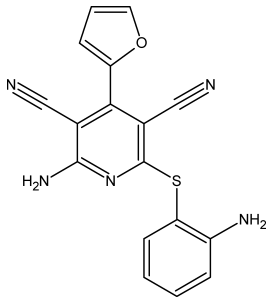

Fig. 1. The Chemical Structure of Cp-60

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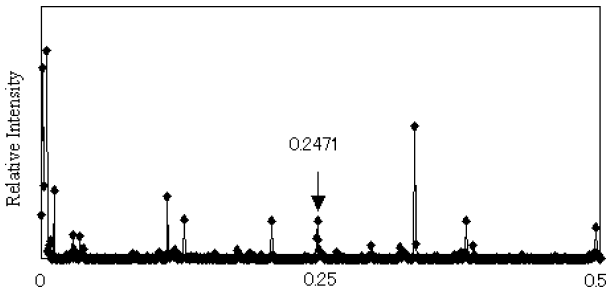

Fig. 2. The Desired Cross-Spectrum of the Sense Amino Acid Sequence of Prion under the N_2 Scale

The abscissa represents frequencies from 0.0000 to 0.5000 and the ordinate relative intensities (amplitudes) in the spectrum throughout all the figure captions in this communication. The number indicated in the figure is the resonant frequency value.

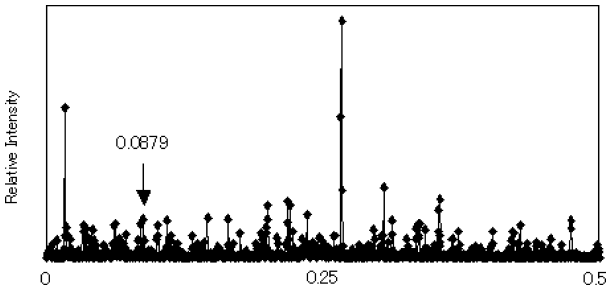

Fig. 3. The Desired Cross-Spectrum of the Sense Amino Acid Sequence of HIV*gag* (149-512) under the N_3 Scale

See also the caption of Fig. 2.

3) The number of peaks selected from the spectrum is 15, and that the confident limit (CL)[14] value predicted is more fixed than that of 16th one.

As a result, one resonant peak[1] (f=0.2471) of the desired cross-spectrum derived from the wild type prion (Fig. 2) could be found to overlap with one (f=0.2380) of two characteristic ones[1] (f=0.2380, 0.2548)[16] from the ^{13}C-NMR spectrum of the Cp-60 under the condition of the same criteria.[1,4] In addition, the same result was observed in the analog A4,[13] while no relationship between mouse prion protein (232aa; *Mus musculus*) and the Cp-60 (or A4) was observed with any scale. No specific interaction of the mutant prion (M129V)[21] with the compounds could be indicated with any scale.

To confirm such the analytical method, next we investigated a specific interaction between wild type human immunodeficiency virus (HIV)gag protein (512aa; *Retroviridae*),[22] and PA-457.[23,24] Of various segments of the gag protein, it could be found with the N_3 scale[6] only that one resonant peak (f=0.0879) of the desired cross-spectrum (Fig. 3) derived from the HIV*gag* (364aa), composed of aa number 149 (N terminus) to 512 (C terminus)[22] on the gag protein, is overlapped with one (f=0.0843) of two characteristic ones (f=0.0843, 0.4132)[17] from the ^{13}C-NMR spectrum of the PA-457. No specific interaction between the former mutant (A364V)[24] and the PA-457 could be observed with any N scale, as might have been expected.

Based on these[12] and our previous study,[25—27] finally we investigated a specific interaction between 1918 neuraminidase (1918 NA),[28] that is, the variant 256F of pandemic influenza A virus [A/New York/1/18] (*Orthomyxoviridae*), and a clinical neuraminidase inhibitor such as

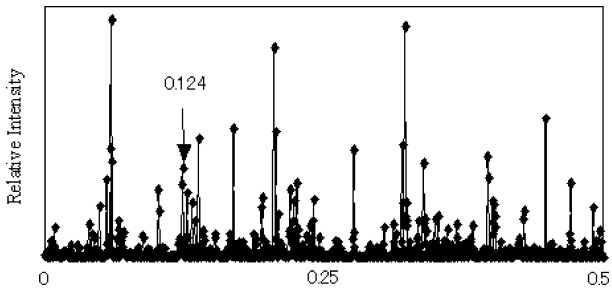

Fig. 4. The Desired Cross-Spectrum of the Sense Amino Acid Sequence of Neauramidase (256F) of Influenza A Virus [A/South Carolina/1/1918(H1N1)] under the N_3 Scale

See also the caption of Fig. 2.

zanamivir, oseltamivir carboxylate or peramivir[29—31] to know whether such the frequency symmetry can be observed in their intermolecular interaction. Because all three drugs are reported to be powerful inhibitors of influenza A virus.[29] As a result, it could be formally indicated with the N_3 scale[6] only that one resonant peak (f=0.1240) of the desired cross-spectrum derived from the mature 1918 NA (434aa; 256F) (Fig. 4) could be overlapped with one (f=0.1244) of two characteristic ones (f=0.1244, 0.3610)[18] of the peramivir only, while no specific interaction could be observed in zanamivir with any scale. In the oseltamivir carboxylate, a typical heterodimeric interaction could be indicated (data not shown). The same analytical result was observed in another variant 1918 NA (256L) isolated from A/Brevig Mission/18 (data not shown).[32]

Although test samples exemplified here may not be sufficient to discuss the generality, the intermolecular *frequency* symmetry (IFS) rule is evolutionarily conserved as a necessary condition even in a specific interaction between protein and low-molecular compound, while the correlation of the f value with the biological activity has to be determined. Based on this, NMR chemical shift is an important factor to elucidate a relationship between the genetic code and the physicochemical properties of amino acid.[33] A desired lead-compound against disease due to a protein could be effectively found from a user database. In addition, the rule is sensitive to a substitution on the sequence or the chemical structure.

Acknowledgements We (N.N.) appreciate very much Dr. T. Hamada for ref. 17, Prof. M. Shibasaki for ^{13}C-NMR information of oseltamivir in D_2O, and F. Arai for long supporting this research.

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6) The N_1, N_2, N_3 and N_4 types are composed of (Naa, Nna), (Naa, 1/Nna), (1/Naa, Nna) and (1/Naa, 1/Nna), respectively.
7) The N_4 scale[6] was newly found in the interaction between prion (231aa; f=0.1279, 0.3857) and proteinase K (279aa; f=0.1377), while the relationship between the former (f=0.1279, 0.3799) and the latter (f=0.1377) could be already elucidated with the M scale.[1,8] In addi-

tion, the N_1 scale only[6] was found in the specific relationship of wild type p53 (393aa; f=0.2227) (but not the mutant p53V173L) with c-Jun N-terminal kinase 2 (382aa; f=0.2173, 0.2935).[1] Further, the N_4 scale only[6] was indicated in the relationship of tumor necrosis factor α (TNF-α; 157aa; f=0.2246, 0.2812) with the 55kDaTNF receptor[4,9,32] (426aa; f=0.2739). The M (H or G) scale could not be found in latter two samples.

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12) The sequences are of *Homo sapiens* used, unless otherwise stated. Various proteins, nucleic acid sequences or organic compounds examined in this study are of the following locus number in NCBI database or CAS number.
[Prion (AY008282), mouse Prion (NM011170)/Proteinase K (TAPKD), ADAM10, E2F1, Cp-60 (or A3, A4, A5), Quinacrine], [CT, salmon CT/CTR], [Poliovirus VP1/CD155], [TNFα (HSTNFR), β/TNFRI (HUMTNFRC), II], [P53 (HSP53)/JNK1, 2(HSU34821), 3, CP31398, PFTα, PFTμ, MIRA1, PRIMA1, Iso-showdmycin], [cFos/cJun], [HIVgag (HIVBH102)/HIVprotease1, PA-457, Betulinic acid], [ISNR, IGFRI, EGFR/L-783281, L-767827], [A/Aichi/2/68 (H3N2)(FLAHAL), A/New York/18(EU199384)(H1N1), A/Brevig Mission/18(H1N1) (AF250356), A/South Carolina/18(H1N1) (AF117241)/Sialic acid, Sialyllactose, Zanamivir (139110-80-8), Oseltamivir carboxylate (*rf.* GS4071 for Oseltamivir), Peramivir (RWJ-270201)], *etc.*
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14) C-NMR predictor, version 4.07, Advanced Chemistry Development Inc., Toronto ON, Canada, 1999.
15) The chemical shift (δ) values of Cp-60,[16] PA-457[17] or Peramivir[18] predicted with ACD/C-NMR software are as follows. The content of each parenthesis means confident limit (CL) value estimated by the predictor C-NMR and frequency (f) value converted to investigate the IFS rule.
16) (CL/f) δ: 173.77 (10.8/0.3949), 164.71 (5.6/0.3743), 148.25 (9.1/0.3369), 141.23 (4.6/0.3210), 141.17 (0.6/0.3208), 134.15 (4.7/0.3049), 123.87 (0.8/0.2815), 117.75 (1.9/0.2676), 116.13 (1.2/0.2639), 114.54 (2.6/0.2603), 112.09 (1.3/0.2548), 104.71 (4.3/0.2380), 100.87 (4.5/0.2293), 89.04 (10.3/0.2024), 82.91 (11.2/0.1884).
17) (CL/f) δ: 181.8 (0.2/0.4132), 109.7 (0.7/0.2493), 80.85 (0.7/0.1838), 56.25 (0.5/0.1278), 50.28 (0.2/0.1143), 49.1 (0.7/0.1116), 46.8 (0.8/0.1064), 40.5 (0.6/0.0920), 37.79 (0.8/0.0859), 37.08 (0.7/0.0843), 34.22 (0.1/0.0778), 29.6 (0.7/0.0673), 25.23 (0.3/0.0573), 25.23 (0.3/0.0573), 19.3 (0.3/0.0439).
18) (CL/f) δ: 174.28 (3.3/0.3961), 168.81 (1.8/0.3837), 158.82 (0.7/0.3610), 73.06 (1.2/0.1660), 54.75 (4.6/0.1244), 54.2 (5.5/0.1232), 50.8 (7.4/0.1155), 46.49 (1.6/0.1057), 45.5 (3.7/0.1034), 29.78 (1.7/0.0677), 25.19 (2.8/0.0573), 23.35 (0.6/0.0531), 23.19 (0.6/0.0527), 11.98 (1/0.0272), 11.98 (1/0.0272).
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