Prediction of Three-Independent Scales Endowed on Poliovirus Proteinase 2A Sequence

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Mulliken's electronegativity (M) scale was found as a parameterization to predict (elucidate) a virtually specific interaction between Poliovirus proteinase 2A and mitogen-activated protein (MAP) kinase p38 α , as well as that between the 2A and apoptotic protein activating factor 1c (Apaf 1c) (or prion) with intermolecular frequency symmetry (IFS) rule. Also, Lacey's hydropathical (H) scale and Garel's (G) one could be found in the specific relationship between the 2A and the extracellular signal-regulated kinase 2 (ERK2) [or fibroblast growth factor receptor 3 (FGFR3)], and that between the 2A and the c-Jun N-terminal kinase 2 (JNK2) [or forkhead box P2-1 (FOXP2-1)], respectively. Based on these, both the same physicochemical scale and almost the same resonant frequency (f) value would be conserved in the same succession of a signal transduction process in a Poliovirus-infected cell. Furthermore, the 2A could play a trigger role to cause cancer, prion disease, bone disease, or speech and language disorder.

Key words mitogen-activated protein kinase; Poliovirus 2A; apoptotic protein activating factor 1; fibroblast growth factor receptor; forkhead box P2; human DNA replication

We have already developed a novel method by which a specific (simple and concerted) protein–protein (or DNA, RNA) interactions,^{1–6)} including active site of protein,⁷⁾ can be successfully elucidated from both amino acid (aa) and the corresponding RNA (or DNA) (na) sequence²⁾ with the sequence Fourier analysis (SFA). We defined it as the intermolecular frequency symmetry (IFS) rule.²⁾ In the rule, the average nuclear (N) scale,¹⁾ the Garel's (G) relative hydropathy one,²⁾ the Lacey's relative hydropathy (H) one^{3,4)} or the Mulliken's absolute electronegativity (M) one,^{5,6)} is employed as the parameterization. Both the calculation process (see computational method and groups 9–12 described in ref. 6) and the criteria to select the resonant peak between proteins (see a working procedure in ref. 5 and a definition condition described in ref. 9 of ref. 2) had already been reported. Based on the rule, we have hitherto investigated a specific interaction of various proteins to find such a protein as at least three (M, H, G) independent scales are endowed on the same aa (na) sequence. The N scale is regarded as the M one after the G one was found.^{1,2)}

In the course of successive re-examination of a specific interaction between Poliovirus proteinase 2A (149aa; *Picornaviridae*)

naviridae)⁸⁾ and the 3C (183aa; *Picornaviridae*) to binding Poliovirus P1 (881aa; *Picornaviridae*) protein,^{9–13)} we happened to notice that three (M, H, G)-independent scales are endowed on the same aa (na) sequence of the 2A (but not the 3C), when the P1 is appropriately replaced with nine kinds of mitogen-activated protein (MAP) kinases [2 extracellular signal-regulated kinases (ERKs), 4 p38s and 3 c-Jun N-terminal kinases (JNKs)].^{14–16)}

As a typical example, it can be exemplified with the M scale only that one resonant frequency (f) peak (f=0.3848) of the two (f=0.1191, 0.3848) derived from the 2A (Fig. 1) almost overlaps with one resonant peak (f=0.3857) of the desired cross-spectrum from p38 α (360aa; Fig. 2) (but none of ERKs, JNKs and p53). Also, other two (H, G) scales could be found in turn, being arranged in reference and note.^{17,18)} Based on these, it is assumed that the 2A expressed in a Poliovirus-infected cell is activated through MAP kinase before carrying out a specific interaction. Thus, we had a great deal of interest in investigating which kinds of protein can be predicted as a specific binding partner of the 2A among various proteins.

First, it was found with the M scale only that a specific interaction between the 2A and apoptotic protein activating factor 1c (Apaf 1c; 1248aa) could be successfully predicted. As shown in Figs. 1 and 3, two resonant peaks (f=0.1270, 0.3828) derived from the 2A (Fig. 1) overlap with two resonant peaks (f=0.1238, 0.3809) of the desired cross-spectrum from the Apaf 1c (Fig. 3).^{19–21)} In addition, a specific interaction between the 2A (f=0.1270, 0.3828) and wild type prion protein (231aa; f=0.1259, 0.3857) [but not mouse prion (232aa; *Mus musculus*)] could be observed with the

Fig. 1. The Desired Cross-Spectrum of the Sense Amino Acid Sequence of Poliovirus 2A with the M scale

The abscissa represents frequencies (f) value 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 stands for the resonant frequency value.

Fig. 2. The Desired Cross-Spectrum of p38 α with the M Scale

See also the caption of Fig. 1.

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Fig. 3. The Desired Cross-Spectrum of Apaf1c with the M Scale
See also the caption of Fig. 1.

Fig. 4. The Desired Cross-Spectrum of the 2A with the H Scale
See also the caption of Fig. 1.

Fig. 5. The Desired Cross-Spectrum of FGFR3 with the H Scale
See also the caption of Fig. 1.

same M scale only (figure not shown). Such specific interaction between the 2A and the mutant prion_{P105L} (but not the prion_{M129V}) could be observed.^{22,23)}

Next, it was found with the H scale only that a specific interaction between the 2A and wild type fibroblast growth factor receptor 3 (FGFR3) (but none of FGFR1, 2 and 4) could be so far found (predicted) with the same H scale only.^{24–27)} As shown in Figs. 4 and 5, two resonant values ($f=0.1191$, 0.3789) derived from the 2A (Fig. 4), overlap with those ($f=0.1248$, 0.3774) of the desired cross-spectrum from the FGFR3 (784aa; Fig. 5). Here, note that the f value of the 2A agrees with that between the 2A and ERK2 (360aa) with the H scale.¹⁷⁾ Therefore, the reverse process (FGFR3→2A→ERK2) may be also possible, because a kinase activity is conserved in the intracellular region of the FGFR3. The same analytical result in the interaction of wild type FGFR3 and of the mutant FGFR3_{G380R} (ex. achondroplasia) to the 2A could be observed.

Finally, it was found with the G scale only that a forkhead box P2-1 (FOXP2-1; 715aa), involved in a severe speech and

language disorder,^{28,29)} can show a specific relationship with the 2A. One resonant peak ($f=0.2920$) (figure not shown) of two characteristic ones ($f=0.2168$, 0.2920) derived from the 2A overlap with one ($f=0.2856$) of the desired cross-spectrum from FOXP2-1 (figure not shown).¹⁸⁾ No specific relations between the 2A and the mutant FOX P2-1_{R553H} could be observed with any scale.

In conclusion, it is predicted that three independent scales are *a priori* conserved in a specific interaction of Poliovirus proteinase 2A, and that its binding partner is possibly ‘wild type’ Apaf1c, prion protein, FGFR3, or FOXP2-1. Based on this, the 2A is involved in cancer, prion disease, bone disease, or speech and language disorder, as far as the method should be correct. Almost the same resonant f value would be conserved in the same succession of a signal transduction pathway in a cell.^{14,30)} Furthermore, referred to the previous studies about the f value,^{1–6)} a pair of the f values can roughly be classified into three groups (*i.e.*, $f=ca.$ $0.20/0.30$, $ca.$ $0.10/0.40$ and $ca.$ $0.15/0.35$), irrespective of the scale. The correlation between their f value and the biological activity has been determined.

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References and Notes

- Numao N., Fukui T., Fukazawa Y., *Chem. Pharm. Bull.*, **58**, 1669–1671 (2010).
- Numao N., Kamimoto Y., *Chem. Pharm. Bull.*, **57**, 1305–1307 (2009).
- Numao N., Miyama M., Hanagata N., *Chem. Pharm. Bull.*, **52**, 1026–1028 (2004).
- Numao N., Fujii H., Fukazawa Y., Hanagata N., Tanaka K., *Chem. Pharm. Bull.*, **52**, 377–379 (2004).
- Numao N., Fujii H., Fukazawa Y., Tanaka K., *Chem. Pharm. Bull.*, **51**, 1217–1219 (2003).
- Numao N., Fujii H., Fukazawa Y., Yoshioka K., Okada M., Tanaka K., *Chem. Pharm. Bull.*, **51**, 550–559 (2003).
- Numao N., Kidokoro S., *Biol. Pharm. Bull.*, **16**, 1160–1163 (1993).
- The sequences are of ‘*Homo sapiens*’ used, unless otherwise stated. Various proteins examined in this study are of the following locus number in NCBI database. The length of amino acid (aa) sequence is parenthesized. [Poliovirus VP1/3 (*Picornaviridae*)/CD155], [GAL4 (*S. cerevisiae*)/GAL80 (*S. cerevisiae*)], [E2F1/pRb], [cJun/cFos], [FGFs/FGFRs/Ras], [pkCs/FOXP2-1/P2-2/P2-3], [Poliovirus 2A (NC002058), 3C(NC002058)/Poliovirus P1 (NC002058), ERK1 (HSERK1), ERK2 (HUMERK2A), p38 α (NML001315), p38 β (HSU53442), p38 γ (HSU66243), p38 δ (AF015256), JNK1 (HSU34822), JNK2 (HSU34821), JNK3 (HSU34820)], Apaf1a (NM013229), Apaf1b (NM001160), Apaf1c (AF149794), Prion (AY008282), mouse Prion (NML011170), APP1/2/3 (NM201414), PS1s/2s, BACE1s/2s, FOXP2-1 (NML014491)/P2-2/P2-3], [Poliovirus 2A, 3C, 3D, 3CD/CTRs, BMPRs, FGFR1/2/3 (HUMFGFR3)/4 *etc.*], [Poliovirus 3CD (NC002058), p53 (HSP53), E2F1, SP1, SV40T, dnaA, STATs/Apaf1 promoter DNA sequence (AC013283), Poliovirus RNA sequence [NC002058; (*Picornaviridae*)] *etc.*
- A specific interaction between the 2A ($f=0.1270$, 0.3711) and the P1 ($f=0.3770$) could be observed with the M scale only, similar to that between the 3C ($f=0.0791$, 0.4238) and the latter ($f=0.075$). Moreover, an interaction between Poliovirus capsid protein VP3 (238aa) and the receptor (CD155; 397aa) reported in the previous study⁵⁾ was confirmed with both the M (VP3; $f=0.3643$, CD155; $f=0.1226$, 0.3606) as well as the G (VP3; $f=0.0215$, CD155; $f=0.0122$, 0.4746) scale only.⁵⁾ The result is similar to such a heterodimeric one between GAL4 (881aa; H scale; $f=0.1802$, 0.3213 , G scale; $f=0.1699$, 0.3418) and GAL80 (435aa; H scale; $f=0.1889$, G scale; $f=0.3511$).
- Takahara Y., Ando N., Kohara M., Hagino-Yamagishi K., Nomoto A., Itoh H., Numao N., Kondo K., *Gene*, **79**, 249–258 (1989).

- 11) Brenner S., Based on an abstract presented at the Yakult International Symposium, Yakult Hall, Tokyo, on 13 May 1988, pp. 39—46 and references therein.
- 12) Numao N., Takahara Y., Kato S. Japan Tokugansho 61-216689 (WO86/01828) (1986).
- 13) Weidman M. K., Sharma R., Raychaudhuri S., Kundu P., Tsai W., Dasgupta A., *Virus Res.*, **95**, 75—85 (2003).
- 14) In the previous study,²⁾ a specific relationship between Poliovirus 3CD (f=0.3032) and ERK1 (f=0.2021, 0.3086) could be found with the M scale only, similar to that between the 3CD (f=0.3291) and the 5' terminal region (f=0.1582, 0.3262) of Poliovirus RNA sequence with the M one. Such the signal transduction passway could be observed in the relationship between wild type p53 (f=0.2271, 0.2756) and p38 γ (f=0.2944) with the H scale only, similar to that between the p53 (f=0.2227, 0.2856) and Apaf1 promoter DNA sequence (f=0.2207) with the H one.
- 15) Wagner E. F., Nebreda A. R., *Nature Rev. Cancer*, **9**, 537—545 (2009) and reference therein.
- 16) Wu G. S., *Cancer Biol. Ther.*, **3**, 156—161 (2004).
- 17) A specific relationship between the 2A (f=0.0977) and ERK2 (360aa; f=0.1025, 0.4028) (but not ERK1) could be observed with the H scale only.
- 18) A specific relationship between the 2A (f=0.2168, 0.2930) and JNK2 (382aa; f=0.2202) (but not p38s) could be observed with the G scale only.
- 19) Otherwise, such a specific interaction between the 3CD (644aa, *Picornavirida*; f=0.0903) and Apaf1a (1237aa; f=0.0947, 0.3918) [or Apaf1b (1194aa; f=0.0974, 0.3938), but not Apaf1c] could be observed with the M scale only.
- 20) Belov G. A., Romanova L. I., Tolskaya E. A., Kolesnikova M. S., Lazebnik Y. A., Agol V. I., *J. Virol.*, **77**, 45—56 (2003).
- 21) Soengas M. S., Capodiceci P., Polsky D., Mora J., Esteller M., Opitz-Araya X., McCombie R., Herman J. G., Gerald W. L., Lazebnik Y. A., Córdón-Cardó C., Lowe S. W., *Nature* (London), **409**, 207—211 (2001).
- 22) Hegde R. S., Mastrianni J. A., Scott M. R., DeFea K. A., Tremblay P., Torchla M., DeArmond S., Prusiner S. R., Lingappa W. R., *Science*, **179**, 827—834 (1999).
- 23) Furthermore, such a specific interaction between the 2A (f=0.2559) and APP3 (678aa; f=0.2432, 0.2559) (but neither APP1 nor APP2) could be observed with the M scale only, while the same (f) value was not found in the relationship between the 2A and MAP kinase.
- 24) Martinez-Frias M. L., de Frutos C. A., Bermejo E., ECEMC Working Group, Nieto M. A., *Am. J. Med. Genet. A*, **152A**, 245—255 (2010).
- 25) Darnell J. E. Jr., *Science*, **277**, 1630—1635 (1997).
- 26) Rousseau F., Bonaventure J., Legeai-Mallet L., Pelet A., Rozet J.-M., Maroteaux P., Le Merrer M., Munnich A., *Nature* (London), **371**, 252—254 (1994).
- 27) No specific relationship between FGF1 (140aa) and FGFR2 (800aa) previously reported⁴⁾ was confirmed in this study.
- 28) Enard W., Przeworski M., Fisher S. E., Lai C. S. L., Wiebe V., Kitano T., Monaco A. P., Pääbo S., *Nature* (London), **418**, 869—872 (2002).
- 29) Lai C. S. L., Fisher S. E., Hurst J. A., Vargha-Khadem F., Monaco A. P., *Nature* (London), **413**, 519—523 (2001).
- 30) Incidentally, a specific interaction between mutant p53_{R175H} (f=0.3311)^{31,32)} and the Apaf1 DNA promoter sequence (91146—91655) (f=0.1797, 0.3271) could be observed with the M scale only, which differs from a base frame-shifted sequence (91147—91656) previously reported with the H scale.²⁾ In addition, such a specific interaction between the p53_{R175H} (f=0.3311) and JNK3 (422aa; f=0.1768, 0.3306) could be found with the M scale only. The similar result could be also observed in that between Poliovirus 3CD protein and the DNA sequence (data not shown). Based on these, both human DNA replication and the promoter sequence may overlap with each other.²⁾
- 31) Zambeiti G. P., Levine A. J., *FASEB J.*, **7**, 855—865 (1993).
- 32) Bergonetti J., Friedman P. N., Kern S. E., Vogelstein B., Prives C., *Cell*, **65**, 1083—1091 (1991).