Application of Sequence Fourier Analysis to a Specific Interaction in *Arabidopsis thaliana*

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The sequence Fourier analysis reported previously seems to be applicable to elucidating a simple and concerted interaction in *Arabidopsis thaliana*, similar to that in *Homo sapiens*.

Key words sequence Fourier analysis; MAP kinase cascade; *Homo sapiens*; *Arabidopsis thaliana*; hydrophobic scale

We have recently reported a novel sequence Fourier analysis (SFA) for effective elucidation of a simple and concerted interaction between mainly human proteins using both the mRNA and the cognate amino acid sequence.[1—3] Based on the analytical results, two kinds of physicochemical scales, *i.e.*, the Milliken's absolute electronegativity one (M scale)[2] and the Lacey's hydrophobic one (H scale),[1] seem to be independently conserved in a specific interaction, though their difference has to be determined. Based on this, we had a great interest in examining a similar specific interaction in MAP kinase cascade[4—7] of *Homo sapiens* and *Arabidopsis thaliana*. Because the cascade is evolutionary conserved from unicellular to eukaryote organisms.

First, in *H. sapiens* cascade we examined a specific interaction between one of three kinds of ser/thr protein kinases,[8,9] that is, extracellular signal-regulated kinase 1 (ERK1; 379aa),[10] c-Jun N-terminal kinase 1 (JNK1; 427aa) or p38 MAPKs (α: 360aa, β: 372aa, γ: 367aa, δ: 365aa)], and oncogene ELK1 (428aa) as the binding partner. The former acts downstream of the MAP cascade when the cell is exposed to various external stimuli (or stress). The ELK1 is a transcription factor. In other step of the cascade, a third component such a scaffolding/adapter (*i.e.*, MP1 or JIP1) protein has been involved in a signal transduction.

Under the condition of the H scale[1] and the same criteria,[2] one (f=0.2021) of two characteristic peaks (Fig. 1a; f=0.2021, 0.3081) derived from the desired cross-spectrum of ERK1 almost overlapped with a characteristic peak (Fig. 2: f=0.1963) from ELK1. By the way, we also examined the relationship between ERK2 (360aa) and ELK1. It is because ERK2 is *ca.* 90% identical sequence to ERK1.[11] Differing from ERK1, however, ERK2 expressed a characteristic peak (Fig. 1b; f=0.3296), which is almost overlapped with one (f=0.3267) of two peaks (Fig. 2; f=0.1763, 0.3267) from ELK1. Note the difference between the characteristic peak position (Fig. 1b; f=0.3296) derived from ERK2 and that (Fig. 1a; f=0.2021) from ERK1. It is reason why the two major peaks (Fig. 1b; f=0.2021, 0.3081) expressed in the sense amino acid sequence of ERK2 do not exist in its antisense one (Fig. not shown).

Furthermore, a characteristic peak was observed in between JNK1 (Fig. 1c; f=0.1963, 0.3052) and ELK1 (f=0.1963), and not between any one of p38 analogs (α, β, γ, δ) and ELK1 (data not shown). The homology degree (*ca.* 65%) of amino acid sequence between JNK1 and ERK1 is almost the same as that (*ca.* 68%) between JNK1 and ERK2.[12] On the other hand, no specific interaction between any ser/thr protein kinase and the binding partner ELK1 could be observed with the M scale.

In *A. thaliana*, based on two reports[5,6] we focused on and investigated the specific interaction between MPK3 (370aa) [or MPK6 (395aa)] and WRKY29 (304aa) [or WRKY22 (298aa)], which acts downstream of plant MAP kinase cascade.[6] In this, the former proteins (MPK3, MPK6) belong to ser/thr protein kinase family, and the latter (WRKY29,

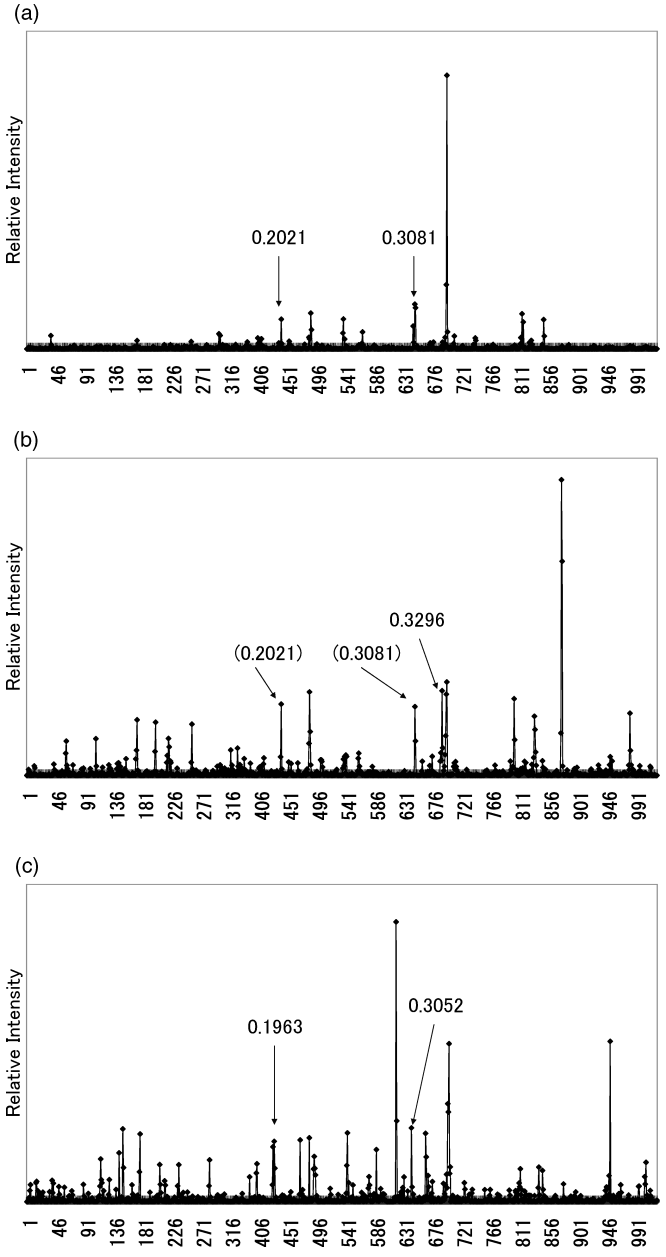

Fig. 1. The Desired Cross-Spectrum of the Sense Amino Acid Sequence of ERK1 (a), ERK2 (b) and JNK1 (c)

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 frequency value.

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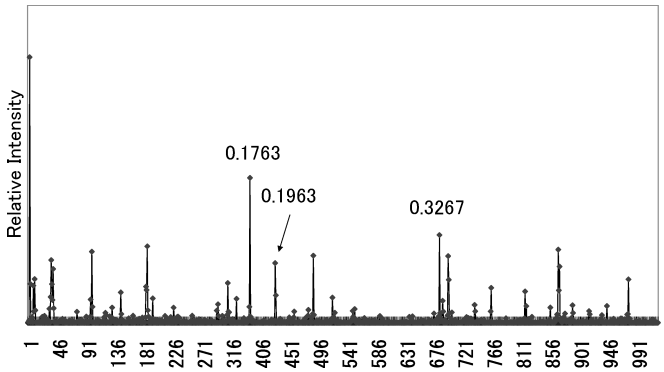

Fig. 2. The Desired Cross-Spectrum of the Sense Amino Acid Sequence of ELK1

See also the caption of Fig. 1.

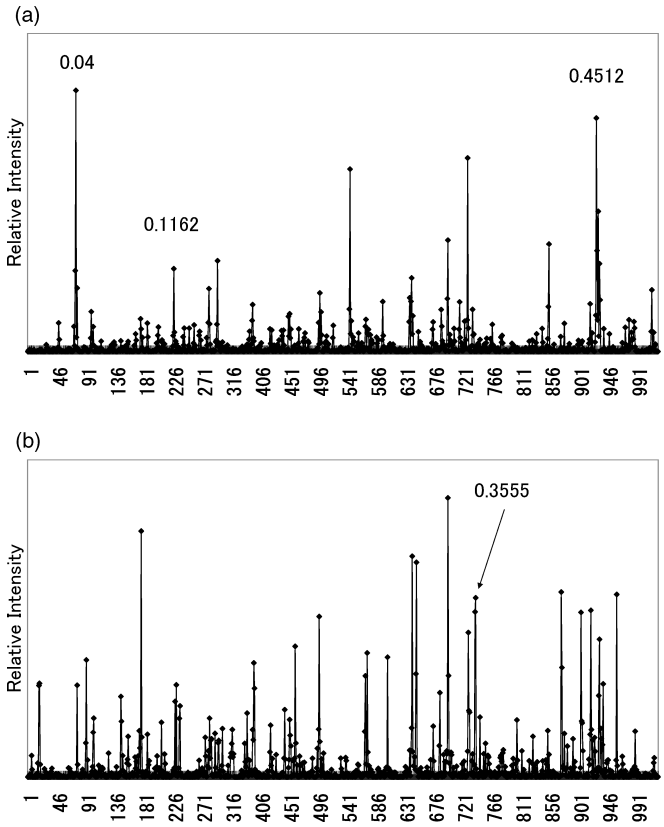

Fig. 3. The Desired Cross-Spectrum of the Sense Amino Acid Sequence of MPK3 (a) and MPK6 (b)

See also the caption of Fig. 1.

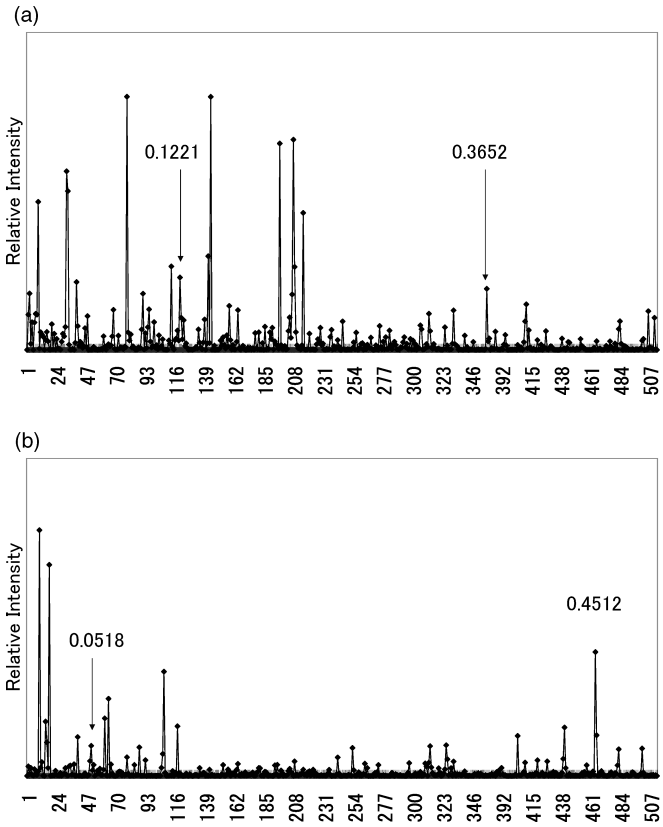

Fig. 4. The Desired Cross-Spectrum of the Sense Amino Acid Sequence of WRKY29 (a) and WRKY22 (b)

See also the caption of Fig. 1.

WRKY22) are transcription factors involved in the expression of defense genes in *A. thaliana* against bacterial flagellin. As a negative sample, furthermore, we adopted WRKY42 (528aa).[6]

Under the same analytical conditions of *H. sapiens* described above, a characteristic peak derived from MPK3 (Fig. 3a; f=0.1162) or MPK6 (Fig. 3b; f=0.3555) overlapped with either of two peaks (Fig. 4a; f=0.1221, 0.3652) of WRKY29. None of MPKs showed such the characteristic peak in the relationship with WRKY42.[13,14] In addition, the degree of homology sequence between MPK3 and MPK6 is reported *ca.* 75%.[15] The analytical result was similar to that between of FGF1 (f=0.3027) and of FGF2 (f=0.2129) to FGFR2 (f=0.2224, 0.2942) reported previously,[1] and that the degree of homology sequence between FGF1 and FGF2 has been reported *ca.* 55% only.[16] Based on these analytical results and those (ERK1/ERK2) described above, the same or similar periodicity existing in the naturally occurring amino acid sequence of MPK3/MPK6 (or FGF1/FGF2) is more conserved in the corresponding antisense (or virtual) sequences, rather than the case ERK1/ERK2, irrespective of their homology degree. Even more apparent but almost the same periodicity is suggested in the analytical results from ERK1 and JNK1 (see Figs. 1a, c).

Based on this information, furthermore we examined the relationship between MPK3 (or MPK6) and WRKY22,[6] which belongs to the same group to WRKY29. The degree of homology sequence in the DNA-binding domain[13] between WRKY29 and WRKY22 is *ca.* 75%, while that between their full sequences is more or less 30%. As a result, two characteristic peaks (f=0.4512, *ca.* 0.05) derived from MPK3 (Fig. 3a) or WRKY22 (Fig. 4b) could be simultaneously observed from two different directions, that is, from the former to the latter protein and *vice versa*. On other hand, WRKY22 showed no characteristic peak in the relation with MPK6 (data not shown). Based on these, WRKY22 may not have the same bioactivity as WRKY29. Additionally, we investigated MPK4 (376aa), which is involved in distinct signal transduction process responding to various environmental tresses.[17] It could not show a characteristic peak in the relationship with any WRKY protein described here, as expectedly. No relationship between MPKs and WRKYs could be

observed with the M scale.

Although two test samples, that is, *A. thaliana* and *H. sapiens*, investigated here are not still sufficient to discuss the selection of the preferential parameterization scale, this analytical method itself seems to be applicable to a simple and concerted interaction in various plants, likely *H. sapiens*.

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