Analysis of the Peptides (Prp106—126, MSI-78A, and Oxaldie 1) with the Same Biological Activity by Discrete Fourier Transform: Toward a Selection Rule in Ligand–Receptor Interaction

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Here, we first report a novel method in which the “desired cross-spectrum” of the peptides Prp106—126, MSI-78A, and oxaldie 1 with the same biological activities is obtained by the multiplication of two cross-spectra derived from the RNA sequence and from the cognate amino acid sequence by discrete Fourier transform (DFT), respectively. Based on a well-known method reported previously, we investigated the cross-spectrum by the multiplication of two of three desired cross-spectra. As a result, we found that one prominent peak occurring in the three cross-spectra showed the same frequency when a binary scale was used as a parameter of nucleotide or amino acid in the analysis. Moreover, we examined the relationship between a binary scale and other physicochemical ones. Almost the same results could be reproduced when the absolute electronegativity scale (or the absolute hardness one) was used, but not in the case of the hydrophobic or electron–ion interacting potential scale reported previously. This indicates that either the absolute electronegativity scale (or the absolute hardness one) or a binary scale, or both is very useful in extracting the information desired for various proteins by the present method from the amino acid and the RNA sequence.

Key words bioinformatic; discrete fourier transform; electronegativity; hardness; binary; prion

We have recently reported1,2) that three synthetic peptides, Prp106—126 (NH2–KTNMKMAGAAAAGAVVGGLG–COOH), MSI-78A (NH2–GIGKFLKKAKFKAFVKILK–K–CONH2), and oxaldie 1 (NH2–LAKLKLALLLK–COOH), and their reversed peptides, namely Prp126—106 (NH2–GLGGVWAAAAAGAMKMKNTK–COOH), A78-ISM (NH2–KKLKVFAKFKKALFKGIG–CONH2) and 1 eidlaxo ((NH2–KKLKLALLLK–CONH2), that are in opposite amino- to carboxy-orientation (vice versa) could exert decarboxylase activity for oxaloacetate in the presence of trifluoroethanol (TFE) in aqueous solution (pH 7.0) to give pyruvate and carbon dioxide. The naturally occurring peptides show generally stronger activity than the corresponding reversed peptides. In this, Prp106—126 corresponds to the amino acid number 106—126 of the prion protein sequence (Homo sapiens), which may be responsible for conversion of cellular prion protein (PrP C) to an infective toxic molecule.3) The secondary structure around the region has been clarified to be the disordered one from the analysis of the three-dimensional structure of recombinant PrPC by NMR measurement.4) While the amino acid sequence,5) the α-helical structure has been postulated by various secondary structure prediction methods.5) MSI-78A6) is a synthetic analogue of naturally occurring magainin 2,7) which is an antibacterial peptide and isolated from the skin of Xenopus laevis. Oxaldie 1 has been rationally designed and synthesized to express decarboxylase activity,5) but the amino acid sequence is homologous to mastparan M (amino acid numbers 1—12: INLKALAA–LKK) (VESPA MANDARIA) or partially to mellitin (amino acid numbers 12—23: GLPALISWIRK) (APIS MELLIFERA). The peptides MSI-78A and oxaldie 1 have been known to form amphipathically α-helical polycationic structures as a common factor necessary for expressing activity. In the field of bioinformatics, the concept of sequence Fourier analysis is not new. Discrete Fourier transform (DFT) analysis has been well known to be able to provide valuable information on the relationship between the characteristic periodicity and bioactivity, higher-ordered dimensional structure, or evolutionary change in the macromolecular sequences of protein, DNA, or RNA. In these studies, the electron–ion interacting potential (EIIP) scale,9,10) or binary scale12,13) has usually been used as a parameter.

The informational system method (ISM)9,10) is a theoretical method using DFT analysis for the informational contents of protein or nucleic acid distribution. It has frequently been emphasized that one characteristic frequency could be extracted from the cross-spectrum of at least two or more protein sequences with the same biological function in this method.10) The analytical results seemed to hint at novel bioinformatics, excluding the stereochemistry of macromolecules. Furthermore, it has been suggested that the ISM result does not depend on the degree of homology between the amino acid sequences,10) indicating that the same periodicity is contained in any amino acid sequences with the same biological activity.

In the preceding study,11) we investigated the ISM in order to elucidate the same biological activity of Prp106—126, MSI-78A, and oxaldie 1 and their reversed peptides (vide supra). In the study, it was accepted that the spectra among amino acid sequences of Prp106—126, MSI-78A, and oxaldie 1 showed the same features as those from the reversed peptides Prp126—106, A78-ISM, and 1 eidlaxo, respectively. This indicates that the naturally occurring amino acid sequence and the reversed one have the same periodicity. However, one major characteristic peak that occurred in

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the cross-spectrum between Prp106—126 and oxaldie 1 (or MSI-78A and oxaldie 1) was not similar or the same as that between Prp106—126 and MSI-78A. Based on this information, there occurred a question as to the appropriateness of the parameters, namely, the EIP scale1,10) assigned to each amino acid which were used in the DFT analysis.

So far, a theoretical manipulation14) and the experimental results15—18) to determine a specific interaction between monomeric ribonucleotide and amino acid have been reported, while the preferentially catalytic activities of RNA at the beginning of evolution was suggested,19) based on the discovery of the enzymic activities in the RNA molecule.20,21) In the theory,19) a basic model of the interaction between monomeric amino acid and monomeric nucleic acid has been proposed to determine the definite-sequence polymers, i.e., in terms of polypeptides or polynucleotides. This model is very attractive, although the molecular structure of a given nucleic acid or amino acid is obscure. In experiments,15) a direct and significant correlation between the amino acid and the anticodon ribonucleotide monophosphate has been examined. The anticodon theory in the origin of coded protein synthesis has been strongly proposed, based on a relationship between the average hydrophobicity rankings of amino acids and that of their dinucleotide monophosphates, while the final conclusion on the protein synthesis seems to have been determined.20,21)

We independently reported that the complementary units (e.g., GA or MXH) of amino acids are evolutionarily conserv as a signal around the active sites of various proteins.23) Such the complementary units are conserved in Prp106—126 (vide supra). The complementary units correspond to something like a restriction site, composed of 6 or 9 nucleotides, that is, one of four nucleic acid or amino acid is obscure. In experiments,15) a direct and significant correlation between the amino acid and the anticodon ribonucleotide monophosphate has been examined. The anticodon theory in the origin of coded protein synthesis has been strongly proposed, based on a relationship between the average hydrophobicity rankings of amino acids and that of their dinucleotide monophosphates, while the final conclusion on the protein synthesis seems to have been determined.20,21)

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terchanged binary scale using group 5 (0 for u and 1 for c, a, and g), group 6 (0 for c and 1 for u, a, and g), group 7 (0 for a and 1 for u, c, and g) and group 8 (0 for g and 1 for u, c, and a). Furthermore, the spectra in Fig. 1 and Fig. 2 are also the same, signifying that the original and its interchanged binary parameters like groups 1 and 5 give the same spectra. Based on the fact that the triplet (i.e., codon) corresponds to a cognate amino acid, the parameter groups 5—8 seem to be more reasonable rather than the groups 1—4.

Next, we determined the parameter group for the amino acids as follows. Based on group 5 (0 for u), we constructed group 9 (1 for N, G, E, P, H, K, A, Q, S, T, R, and D; 0 for other amino acids) by excluding the amino acids having u as the first and the second nucleotide in the codon (or triplet). This concept has come from an indication that the second and third anticodonic bases, namely, the first and second codonic bases, recognize the amino acids. Groups 10—12 were similarly obtained as: group 10 (1 for L, I, N, G, V, E, K, Y, W, M, S, C, F, R, and D; 0 for other amino acids); group 11 (1 for L, G, V, P, A, W, S, C, F, and R; 0 for other amino acids); and group 12 (1 for L, I, N, P, H, K, Y, Q, M, S, T, and F; 0 for other amino acids). Using these parameterization scales, we incrementally calculated \( F_i(m); i = 1a—4a \) for 21 amino acids of Prp106—126 and then obtained the cross-spectrum M2a(m) shown in Fig. 3a. Then the desired cross-spectrum DM(m) of the corresponding RNA sequence in Fig. 2a. The virtual amino acid sequence translated from the antisense mRNA (5’–3’) sequence was also calculated. The obtained cross-spectrum in Fig. 3b is dissimilar to that (Fig. 3a) of naturally occurring amino acid sequence, unlike the case of RNA (or DNA). Such dissimilarity between naturally occurring and virtual amino acid sequence was observed in various other proteins, including MSI-78A and oxalidase 1.

Finally, the desired cross-spectrum from the mRNA sequence and the naturally occurring amino acid sequence of Prp106—126 was calculated by Eq. 4, which is shown in Fig. 4, in which two characteristic peaks \( f = 0.0313 \) and \( 0.0781 \) occurred in the range of the lower frequency. They differ from those of either mRNA \( f = 0.3125 \) and 0.3438) or amino acid \( f = 0.0469 \) and 0.2344), indicating that the desired cross-spectrum is hybridized.

We applied the series of procedures described above to the amino acid sequence of MSI-78A or oxalidase 1, and their corresponding mRNA to synthesize the desired cross-spectrum. For MSI-78A, the triplet nucleotide with the highest occurring frequency in X. laevis was used, because MSI-78A is a synthetic analogue of magainin 2. The desired cross-spectrum of MSI-78A is shown in Fig. 7, including a cross-spectrum (Fig. 5) from the mRNA sequence and that (Fig. 6) from the amino acid sequence. In this case, one lysine residue at the C-terminal end of the full length of MSI-78A is removed for convenience of computation, because it is fairly similar to that of MSI-78A itself. In Fig. 7, one characteristic peak occurred in the frequency \( f = 0.0938 \). On the
other hand, the peptide oxaldie 1 is completely artificial, and its amino acid sequence is more highly homologous to mast
paran M (amino acid number 2—12) (V. mandarnia) than mellitin (amino acid number 12—23) (A. mellifera). Since, however, the codon usage table of V. mandarnia was not available, the codon preference in A. mellifera had to be adopted. The desired cross-spectrum of oxaldie 1 is shown in Fig. 10, including a cross-spectrum (Fig. 8) derived from the mRNA and that (Fig. 9) from the amino acid sequence. In Fig. 10, one characteristic peak occurred in the frequency $f=0.0938$, while it is accompanied by four other major peaks. Furthermore, based on Fig. 5 and Fig. 8, no sharp peaks around frequency $f=0.3333$ was observed, unlike the case of Prp106—126. This was also indicated in the case of replacing the mRNA sequences of MSI-78A and oxaldie 1 (vide infra) with the lowest occurring codon in X. laevis and A. mellifera, respectively (data not shown). Based on these, a sharp peak at frequency $f=0.3333$ is not always characteris-
tic in the partial gene.

From the spectra in Fig. 4, Fig. 7, and Fig. 10, we noticed that all the desired cross-spectra are nearly similar in the re-

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Fig. 3a. The Cross-Spectrum of the Naturally Occurring Amino Acid Se-
quence of Prp106—126 Using the Binary-Scale Parameterization of Groups 9—12
See also the caption of Fig. 1a.

Fig. 5. The Cross-Spectrum of mRNA Represented with All the Highest
Occurring Codons of MSI-78A Using the Binary-Scale Parameterization of Groups 5—8
See also the caption of Fig. 1a.

Fig. 3b. The Cross-Spectrum of the Virtual Amino Acid Sequence of
Prp106—126 Using the Binary-Scale Parameterization of Groups 9—12
See also the caption of Fig. 1a.

Fig. 6. The Cross-Spectrum of the Amino Acid Sequence of MSI-78A
Using the Binary-Scale Parameterization of Groups 9—12
See also the caption of Fig. 1a.

Fig. 4. The Desired Cross-Spectrum of From mRNA and the Cognate
Amino Acid (Naturally Occurring) Amino Acid Sequence of Prp106—126,
Using the Binary-Scale Parameterization of Groups 5—8 and 9—12, Re-
spectively
See also the caption of Fig. 1a.

Fig. 7. The Desired Cross-Spectrum of mRNA Represented with All the
Highest Occurring Codon and the Cognate Amino Acid Sequence of MSI-
78A Using the Binary-Scale Parameterization of Groups 5—8 and 9—12,
Respectively
See also the caption of Fig. 1a.
region of low frequency. Furthermore, multiplications of these three kinds of desired cross-spectra are shown in Fig. 11 (i.e., Fig. 4*Fig. 7), Fig. 12 (i.e., Fig. 7*Fig. 10), Fig. 13 (i.e., Fig. 10*Fig. 4) and Fig. 14 (i.e., Fig. 4*Fig. 7*Fig. 10). Based on these, the peptides with the same biological activities were clarified to have at least one characteristic peak ($f=0.0938$) in the cross-spectrum, irrespective of the degree of homology between their amino acid sequences. We also investigated the desired cross-spectra for Prp106—126, MSI-78A, and oxaldie 1 after replacing the corresponding nucleotide se-

Fig. 8. The Cross-Spectrum of mRNA Represented with All the Highest Occurring Codons of Oxaldie 1 Using the Binary-Scale Parameterization of Groups 5—8
See also the caption of Fig. 1a.

Fig. 9. The Cross-Spectrum of the Amino Acid Sequence of Oxaldie 1 Using the Binary-Scale Parameterization of Groups 9—12
See also the caption of Fig. 1a.

Fig. 10. The Desired Cross-Spectrum of from mRNA Represented with All the Highest Occurring Codons and the Cognate Amino Acid Sequence of Oxaldie 1 Using the Binary-Scale Parameterization of Groups 5—8 and 9—12, Respectively
See also the caption of Fig. 1a.

Fig. 11. Multiplication of the Desired Cross-Spectrum of Prp106—126 and that of MSI-78A.
See also the caption of Fig. 1a.

Fig. 12. Multiplication of the Desired Cross-Spectrum of MSI-78A and that of Oxaldie 1
See also the caption of Fig. 1a.

Fig. 13. Multiplication of the Desired Cross-Spectrum of Oxaldie 1 and that of Prp106—126
See also the caption of Fig. 1a.

Fig. 14. Multiplication of Three Desired Cross-Spectra (Prp106—126, MSI-78A, and Oxaldie 1)
See also the caption of Fig. 1a.
sequence considering the lowest occurring triplet in \( H. \) sapiens, \( X. \) laevis, and \( A. \) mellifera, respectively. The result is shown in Figs. 15—18. This check is of interest since the highest occurring codon is not always used in the naturally occurring mRNA sequence, which encodes polypeptides. As clearly seen in Figs. 15—18, the frequency (\( f = 0.0938 \)) of the prominent peaks are well conserved, though the order of peak height occurring in the multiplied desired cross-spectra is different from those calculated using the highest occurring codon. The comparison of Figs. 11—13 with Figs. 15—17 suggests that the characteristic peak occurring in the present method is fairly susceptible to codon usage.

To verify this, we investigated human calcitonin (NH\(_2\)-CGLNLSTCMLGTYTQDFNKFHTFPQTA1GVGAPCNOH\(_2\)) (hCT)\(^{27}\) and salmon calcitonin (NH\(_2\)-CSNL-STCMLGTYTQDFNKFHTFPQTA1GVGAPCNOH\(_2\)) (sCT),\(^{28}\) in which the region from amino acid number 8 to 22 is the amphipathically \( \alpha \)-helical structure,\(^{29}\) and that from 24 to 32 binds the calcitonin receptor.\(^{30}\) The cross-spectra of hCT and sCT under three kinds of conditions are shown in Fig. 19, Fig. 20, and Fig. 21, in which the mRNA sequences correspond to their naturally occurring, and the highest and lowest frequency codons in \( H. \) sapiens and \( O. \) keta, respectively. Two prominent peaks (\( f = 0.0313 \) and 0.3438) in Fig. 19 were diminished in both Fig. 20 and Fig. 21, indicating that a significant periodicity of the cognate amino acid sequence depends on that of the naturally occurring mRNA sequence. Moreover, the existence of at least two prominent
peaks was indicated in the cross-spectrum using the naturally occurring mRNA sequence of CTs, though it is presently unknown which the prominent peak is. Several such prominent peaks were observed in the cross-spectrum of tumor necrosis factor-α (TNF-α) and TNF-β, but one peak in epidermal growth factor (EGF) and transforming growth factor α (TGF-α), nerve growth factor (NGF), and neurotrophin 3 (NT3) or interleukin-1α (IL-1α) and IL-1β (data not shown). Therefore more detailed investigation will be required to specify (or extract) the prominent peak.

(ii) Electronegativity and Hardness Scale Parameterization

In the course of investigating the relationship between the prominent peaks in DFT analysis and the functional regions on two kinds of CTs using a binary scale, no desired cross-spectrum could be obtained in DFT analysis for the receptor-binding site of sCT, namely, RTNTGSGTP mentioned above, all the amino acids of which belong to group 9. Based on this information, we were interested in replacing a binary scale with any other physicochemical ones such as the EIIP scale, hydrophobic scale, charge scale, or absolute electronegativity and absolute hardness scale as global parameters of nucleotide or amino acid.

The calculation method of the EIIP value$^{9,10}$ is pursued to give the mean quasivalence number $Z^*$ for each nucleotide and amino acid side chain.

$$Z^* = \frac{\sum_{i=1}^{\text{atom}} N_i Z_i}{\sum_{i=1}^{\text{atom}} N_i}$$

where $N_i$ and $Z_i$ denote the number of the i-th atom and its valence number, respectively. The $Z^*$ scale was first invented to approximate the pseudopotential felt by a conduction electron in bulk metal,$^{32}$ while some found that the relation between the $Z^*$ parameterization scale and biological activities was questionable.$^{33-35}$ Our separate check also concluded that none of the EIIP, $Z^*$ (parameters not shown), hydrophobic$^{15}$ and charge scale (parameters not shown)$^{36}$ was appropriate as a parameterization for this purpose in the sense of the similarity of various spectra derived from a binary scale (data not shown).

We considered that the original EIIP scale can be substituted with the absolute electronegativity $\chi$ and/or hardness $\eta$ developed in theoretical chemistry. To do this, the atomic $\chi$ values of Pauling,$^{37}$ Mulliken,$^{37}$ and Pearson$^{38}$ and the atomic $\eta$ values of Pearson$^{38}$ were employed. These values were plugged into Eq. 5 as a substitute for $Z_i$ and used as the absolute electronegativity scale or hardness scale. Note that the average operation adopted in Eq. 5 is based on the postulate of geometric mean introduced originally by Sander$^{39,40}$ and has the same ability of the virtual crystal approximation to represent the average potential in the random-potential medium.$^{42}$

In addition, we directly obtained molecular $\chi$ and $\eta$ of four bases and 20 amino acids in their neutral forms under the aqueous condition (dielectric constant, 78.5) by the molecular orbital (MO) calculation using the relationships,$^{43}$

$$\chi = \frac{-E_{\text{HOMO}} + E_{\text{LUMO}}}{2}$$

$$\eta = \frac{E_{\text{LUMO}} - E_{\text{HOMO}}}{2}$$

where $E_{\text{HOMO}}$ and $E_{\text{LUMO}}$ denote the orbital energies of the highest occupied MO (HOMO) and the lowest unoccupied MO (LUMO), respectively. The semiempirical MO calculation at the PM3 level$^{44,45}$ was performed for this purpose using the MOPAC package.$^{46}$ All of the parameters obtained in these scales are listed in Table 1.

The desired cross-spectra based on Mulliken’s and Pearson’s $\chi$ were fairly similar, but not the same as, to that from the binary scale. Note that these two kinds of $\chi$ values for atoms are essentially the same, because the ratio of Mulliken’s $\chi$ for atom to Pearson’s $\chi$ is almost constant$^{7,38}$ (or see (d) and (e) in Table 1), while their energy units are different. In addition, both Pearson’s $\chi$ and $\eta$ gave the same desired cross-spectra. Although Pauling’s $\chi$ values for atoms are widely accepted, they showed lesser similarity to the binary scale than Mulliken’s $\chi$, based on the relative intensity

| Table 1. Various Scales for Nucleic Acids (na) and Amino Acids (aa)$^{(a)}$ |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| (a) | (b) | (c) | (d) | (e) | (f) | (g) |
| na | aa |
| u | 0.0561 | 0.3 | 2.7055 | 2.9506 | 6.9364 | 5.9918 | 4.7941 |
| c | 0.134 | 0.35 | 2.63 | 2.9487 | 6.9367 | 6.1242 | 4.8176 |
| a | 0.126 | 0.53 | 2.625 | 2.929 | 6.8979 | 6.205 | 4.4626 |
| g | 0.806 | 1.1 | 2.6793 | 2.948 | 6.9407 | 6.1967 | 4.6002 |
| t | 0.1335 | — | 2.6221 | 2.9461 | 6.9236 | 5.9836 | 4.6732 |

Note: (a) EIIP scale from ref. 10. (b) Hydrophobic scale from ref. 15. (c) Pauling electronegativity scale from ref. 37. (d) Mulliken electronegativity scale from ref. 37. (e) Pearson electronegativity scale from ref. 38. (f) Pearson hardness scale from ref. 38. (g) The Absolute electronegativity in aqueous conditions.
of various peaks in the spectrum.

The desired cross-spectrum of Prp106—126 under the condition of Mulliken’s $\chi$ is shown in Fig. 24. The cross-spectra of the nucleotide sequence and of the amino acid sequence are shown in Fig. 22 and Fig. 23, respectively. All the spectra (Figs. 22—24) were similar to those (Figs. 2, 4, and 7) derived from the binary scale, respectively. In addition, a similar tendency was supported by the semiempirical MO calculation for four bases and 20 amino acids (see Figs. 25—27), while the relative intensity of various peaks was not strictly in accord with the binary, Pauling’s, Mulliken’s, or Pearson’s, parameterization. Based on these, the absolute electronegativity scale is a plausible substitute for the binary scale, although more detailed investigation is required to determine finally which scale is superior for elucidating the same biological activities of proteins.

On the other hand, under the condition of Mulliken’s $\chi$ the desired cross-spectrum of the receptor-binding region from 24 to 32 (i.e. RTNTGSGTP) of sCT could be successfully featured (Fig. 28), unlike the case of the binary scale. The cross-spectrum of the receptor-binding region (i.e., QTAIGVGAP) of hCT and that (i.e., RTNTGSGTP) of sCT is shown in Fig. 29, indicating that one ($f=0.3438$) of two prominent peaks observed in Fig. 19 is a candidate for the receptor binding region. However, the amino acid sequences

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Fig. 22. The Cross-Spectrum of mRNA of Prp106—126 Using Mulliken’s Electronegativity
See also the caption of Fig. 1a.

Fig. 23. The Cross-Spectrum of the Amino Acid Sequence of Prp106—126 Using Mulliken’s Electronegativity
See also the caption of Fig. 1a.

Fig. 24. The Desired Cross-Spectrum from mRNA and the Amino Acid Sequence of Prp106—126 Using Mulliken’s Electronegativity, Respectively
See also the caption of Fig. 1a.

Fig. 25. The Cross-Spectrum of the mRNA Sequence of Prp106—126 Using the Electronegativity Value for a Molecule
See also the caption of Fig. 1a.

Fig. 26. The Cross-Spectrum of the Amino Acid Sequence of Prp106—126 Using the Electronegativity Value
See also the caption of Fig. 1a.

Fig. 27. The Desired Cross-Spectrum from mRNA and the Amino Acid Sequence of Prp106—126 Using the Electronegativity Value for a Molecule, Respectively
See also the caption of Fig. 1a.
from 8 to 32 of the two CTs were involved in another prominent peak \((f=0.0313)\) (Fig. 30). Especially, the C-terminal region of hCT was necessary for its peak (data not shown). In addition, the peak was fairly prominent in hCT (active form) composed of 32aa as well as in procalcitonin (inactive form) composed of 116aa (data not shown). Thus one prominent peak relevant to the receptor binding could not be discriminated.

If a specific interaction between ligand and receptor can be regarded as something like a complex with the same biological activity (or function) in a broad sense, the prominent peak (or peaks) involved in the specific intermolecular interaction may be extracted. Based on this working hypothesis, we are now in the process of constructing a selection rule that can elucidate the ligand–protein interaction, such as prion and 37-kD laminin receptor, hCT and hCT receptor, TNF-\(\alpha\) and 55-kD TNF receptor, EGF and EGF receptor, IL-2 and IL-2\(\alpha\) receptor, various viruses and their receptors, etc. Such a selection rule will be discussed elsewhere, although its theoretical base is as yet unsolved.

Conclusions

We demonstrated DFT analysis to elucidate the same biological activity of Prp106—126, MSL-78A and oxalidie 1 using the mRNA and amino acid sequences. As a result, the prominent peak occurring in the cross-spectrum derived from two of three desired cross-spectra was the same when a binary scale was used as a parameter of nucleotide or amino acid. However, the prominent peak was not always single, like the case of CTs or TNFs. Otherwise, almost the same result could be reproduced when the average absolute electronegativity scale (or the absolute hardness scale) was used instead of a binary scale. Based on these, various proteins with the same biological activities may be rationally elucidated from both the naturally occurring mRNA and the cognate amino acid sequence, using either a binary scale or the mean absolute electronegativity scale (or the mean hardness scale) or both, although more detailed investigation will be required to specify the prominent peak.

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References and Notes