Prediction of Endocrine Disruptors Based on a New Structure–Activity Relationship for Sex and Environmental Hormones Using Chemical Hardness Concept

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Classification of the relationship between electronic structures and biological activities of endocrine disruptors (so-called environmental hormones) was attempted using the parameters of absolute hardness (η), absolute electronegativity (χ), and global softness (S), approximately defined as $\eta=1/2(\varepsilon_{LUMO}-\varepsilon_{HOMO})$, $\chi=-1/2(\varepsilon_{HOMO}+\varepsilon_{LUMO})$, and $S=1/\eta$, respectively, based on the hardness concept. The strength of binding affinity and toxicity of the chemicals were approximately proportional to the absolute hardness, and laterally toxic chlorinated PCDDs, PCBs, and DDTs are classified as chemically soft. Here we found that the electronic structures of environmental hormones can be classified into four main groups: 17β-estradiol type (group I), testosterone type (group II), thyroxine type (group III), and HCH (hexachlorocyclohexane) type (group IV). Therefore, if we can predict the coordinate (χ, η) of the electronic structure of one chemical on the η–χ activity diagram, we would be able to predict the receptor with which the chemicals (environmental hormones) interact. For instance, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is classified in group III, therefore, it would bind with the thyroid receptor more than the estrogen receptor (group I). It appears that dibutyl phthalate would not interact with estrogen receptor because it does not belong to group I. In addition, the coordinates of these four groups do not complementarily overlap with the electronic structures of 20 natural amino acid residues. The η–χ activity diagram is a new tool for the prediction of the toxicity and biological activity of environmental hormones.

Key words endocrine disruptor; η–χ activity diagram; hardness concept; estrogen receptor; bisphenol A; antagonist

Environmental hormones such as polychlorinated dibenzo-*p*-dioxins (PCDDs), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-ethane (DDT), and polychlorinated biphenyls (PCBs) are composed of an aromatic system substituted with chlorine at various positions. They are potently toxic in biological systems, and teratogenic to animals.[1)] Moreover, it has been reported that the chemicals 2,2-bis(*p*-hydroxyphenyl)propane (bisphenol A, BPA) and diethyl stilbestrol (DES) can mimic natural hormones, and may disrupt the endocrine systems in animals and humans.[2)] Although the acute toxicities and estrogen activities of these chemicals are dependent on their chlorine substitution pattern, chemical shape, and hydrophobicity, the nature of the structure–activity relationships remains uncertain. Meaningful structure–activity relationships (SARs) between DDTs and DES cannot be explained by the difference in their chemical structures. This important correlation has been stressed by many chemists and biologists. In modern toxicology, it is difficult to predict the degree of toxicity, receptor binding affinity, and chemical structure essential for so-called environmental hormones. It is necessary to establish a methodology to estimate the presence of estrogen activity and chemical structures of endocrine disruptors. Previously, the authors reported a new analysis for the SARs of new quinolones, dioxins and PCBs[3a)] based on the hardness concept[4)] as a biological application of the density functional theory(DFT).[5)] According to this method, it was found that more toxic isomers of dioxins are chemically soft, whereas less toxic isomers are chemically hard. The toxicities and induction abilities of dioxins and PCBs are proportional to the magnitude of value of their absolute hardness (η).[3a,b)] On the other hand, we found that the antibacterial activity of a new quinolone, 1,4-dihydro-4-oxopyridine-3-carboxylic acid derivative such as norfloxacin is completely dependent on the strength of the absolute electronegativity (χ).[3a)]

We report here that inhibition of the binding of such environmental hormones as DDT, DDE(2,2-bis(4-chlorophenyl)-1,1-dichloroethylene), DES, and chlordecone to androgen receptors also correlates well with the value of their absolute hardness. The chemical structures of the main compounds used in this paper are shown in Chart 1. Using another factor, absolute electronegativity (χ), we present an absolute hardness-electronegativity (η–χ) activity diagram as a new measure of predicting the relative toxic potencies and receptor binding affinities of environmental hormones. Based on our results, the electronic structures of environmental hormones can be classified into four groups: 17β-estradiol type (group I), testosterone type (group II), thyroxine type (group III), and HCH (hexachlorocyclohexane) (group IV). That is, coordinates (χ, η) of the electronic structure of environmental hormones are divided into at least four groups, I—IV. If we calculate these coordinates to test to which groups target chemicals belong, we can estimate the receptors with which the chemicals easily interact. In addition, we focus on the relationship between agonist and antagonist for the ligand of estrogens and androgens using the η–χ activity diagram, and present the chemical necessary conditions to be an agonist or antagonist.

Thus, the η–χ activity diagram has become a useful tool for estimating the toxicities and the receptor binding of environmental pollutants as endocrine disruptors. It is generally

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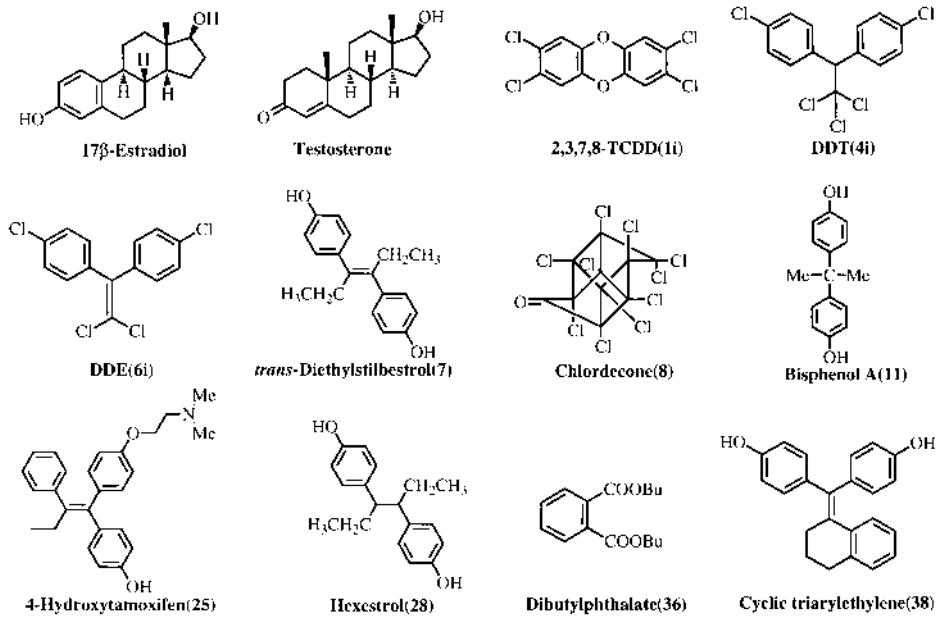

Chart 1. Structures of Several Environmental Hormones

recognized that the binding affinity of a receptor–chemical complex formed at the receptor surfaces depends on the shape of the chemical surface and hydrophobicity of this contact surface. As a consequence of the η–χ activity diagram, however, the electronic structure's coordinates (χ, η) also play a dominant role in the binding force between the receptor and the chemical on their molecular surface. Development of a simple prediction method based on the electron structure of chemicals is urged to elucidate the urgent problems caused by environmental hormones.

Experimental

Molecular Orbital (MO) Calculations To explicate the structure-dependent toxicity of environmental hormones, we employed semiempirical AM1, PM3, Hartree-Fock(HF)/6-31G*, and DFT, gradient-corrected BLYP/6-31G* method in TITAN software[6] to determine the electronic structures of the optimized target chemicals. The values of absolute hardness (η) and absolute electronegativity (χ) were calculated by Eqs. 1 and 2, as defined by Parr and Pearson,[7]

$$\chi=-\mu=-(\partial E/\partial N)_{v(r)}=(Ip+Ea)/2 \quad (1)$$

$$\eta=1/2(\partial\mu/\partial N)_{v(r)}=1/2(\partial^2E/\partial N^2)_{v(r)}=(Ip-Ea)/2 \quad (2)$$

where E is the electronic energy of a molecule, N is the number of electrons, and $v(r)$ is the external electrostatic potential. Ip and Ea are the ionization energy and the electron affinity (eV), respectively, and are used to the approximate η and χ values of environmental hormones in this study, using Eqs. 3 and 4,[7,8]

$$\chi=-1/2(\varepsilon_{HOMO}+\varepsilon_{LUMO}) \quad (3)$$

$$\eta=1/2(\varepsilon_{LUMO}-\varepsilon_{HOMO}) \quad (4)$$

where ε_{HOMO} and ε_{LUMO} are the energy levels for the frontier orbitals. Here, the reciprocal of the hardness is the global softness (S) (Eq. 5),

$$S=1/\eta=(\partial N/\partial\mu)_{v(r)} \quad (5)$$

Receptor-Environmental Hormone Interaction Model Recently, X-ray crystal structures of estrogen receptor's ligand binding domains (hERαLBD) have been reported by Brzozowski *et al.*[9] and Tanenbaum *et al.*[10] As shown in Chart 2, the ligand-binding pocket is composed mainly of ^{353}Glu, ^{394}Arg, and some hydrophobic amino acid residues in hERαLBD. Interestingly, hERαLBD residues are occupied by eighteen residues that contact bound 17β-estradiol. The interaction between hERαLBD and 17β-estradiol is not accomplished by covalent bonding but by hydrogen bonding, charge transfer, and hydrophobic bonding *etc.* As the hERαLBD has a low stereospecificity, the chemicals are similar to the shape of 17β-estradiol work-up as endocrine disruptors (environmental hormones). The interaction model between hERαLBD and BPA is illustrated in Chart 2(b).

The electronic structures of endocrine disruptors become important factors since the structure of hERαLBD is the same. The interactions between such two systems, electron donor (ED) and electron acceptor (EA) can be associated with the following chemical process:

$$ED+EA \leftrightarrows (ED\cdot EA) \quad (6)$$

According to the HSAB principle, with the interaction between ED and EA, the quantity of electron transfer (ΔQ) is determined using Eq. 7[7]

$$\Delta Q=(\chi_{EA}-\chi_{ED})/2(\eta_{EA}+\eta_{ED}) \quad (7)$$

where ΔQ is the charge transferred from electron donor to electron acceptor. Next, the energy's strength (ΔE) of the stabilizing interaction between electron donor and electron acceptor is given approximately by Eq. 8.[8a]

$$\Delta E=-(\chi_{EA}-\chi_{ED})^2/4(\eta_{EA}+\eta_{ED}) \quad (8)$$

Then, the interaction between hERαLBD and environmental hormones is determined by χ and η on the two systems, hERαLBD and environmental hormone. When the difference between χ and η is large, $|\chi_{EA}-\chi_{ED}| \ll \eta_{EA}+\eta_{ED}$, ΔQ value would be small. ΔQ value increases in the case of $|\chi_{EA}-\chi_{ED}| \gg \eta_{EA}+\eta_{ED}$.

Results and Discussion

Electron Structures of Environmental Hormones In the case of BPA, DDE, and 2,3,7,8-TCDD calculated the geometry using the BLYP/6-31G* method, the plots of the value of the total energy (E_{total}; eV) against number (N) of the valence electrons of the two chemicals and its anion, and its cation are shown in Fig. 1. In the Fig. 1, the first derivative $(\partial E/\partial N)_{v(r)}$ is equal to chemical potential (μ) and the second derivative $(\partial^2E/\partial N^2)_{v(r)}$ to hardness. The former changes the electron density around a molecule, and the latter is a measure of the resistance to change in electron density. Furthermore, the first derivative $(-\partial E/\partial N)_{v(r)}$ is approximated as electronegativity (χ) and χ is approximately equal to $|\eta+\varepsilon_{HOMO}|$, by Eqs. 3 and 4. The symbols, S^0, $S^{+\cdot}$, and $S^{-\cdot}$ in the figure express the number of electrons for the neutral, cation radical, and anion radical BPA. In the case of BPA, for instance, the total energies of S^0, $S^{+\cdot}$, and $S^{-\cdot}$ were

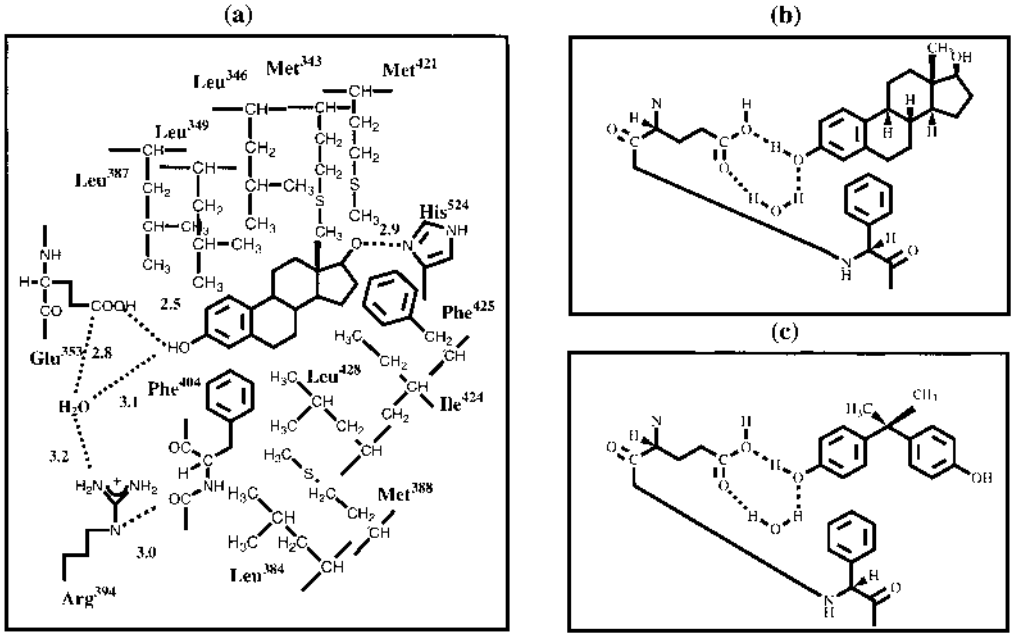

Chart 2. Schematic View of 17β-Estradiol Binding Site (a) and Binding Models of 17β-Estradiol and Bisphenol A[a,b]

a) The hER-17β-estradiol binding pocket shows the structure of side chains of amino acid residues. The data were taken from ref. 15. *b*) The structures show binding model 17β-estradiol (b) and bisphenol A (c) in the hER ligand-binding pocket.

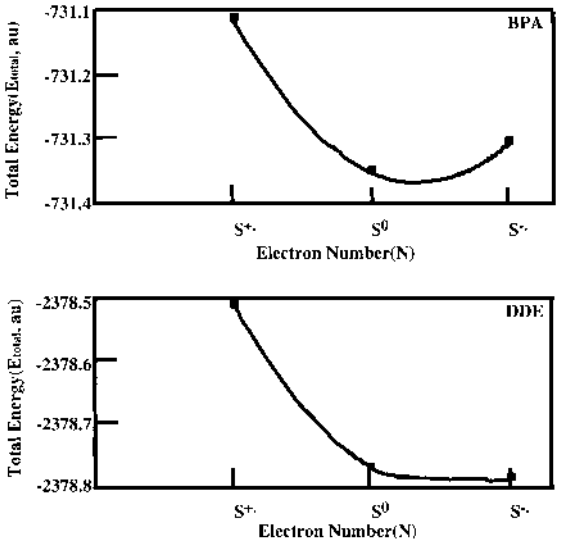

Fig. 1. Plots of Total Energy (au) of BPA and DDE *versus* their Electron Number (*N*)

Calculated using DFT model, BLYP/6-31G* method. $S^{+\cdot}$, S^0, and $S^{-\cdot}$ represent cation radical, neutral, and anion radical, respectively.

−731.5297, −731.789, and −731.987 (au), respectively, using the BLYP/6-31G* method. From these values, the η and χ values were derived as listed in Table 1.

To analyze the electron structure of environmental hormones, the coordinate of the electron structure was defined as $((-\partial E/\partial N)_{v(r)}, (\partial^2 E/\partial N^2)_{v(r)})=(\chi, \eta)$ as provided by the hardness concept. The geometries of BPA and DDE were calculated using the PM3 and BLYP/6-31G* methods, the dihedral angles (φ) C1′–C2–C1″=C2″ are about 52.07° and −51.39°, and the barrier to the dihedral rotation is about 1.0 and 2.0 kcal/mol, respectively. These low barriers indicate that the chemicals are flexible due to the low steric interaction and have a coplanarity. The φ of BPA obtained by different calculation methods is listed in Table 1, and the result that was obtained using the AM1 (φ=53.96°) is similar to that of BLYP/6-31G*. The numerical data based on AM1 are listed in Table 2. According to the "Receptor-Environmental Hormone Interaction Model" described in the previous section, we calculated the ionization potentials, electron affinities, energy levels and η and χ values of Glu, BPA and 2,3,7,8-TCDD, because the hERαLBD involves glutamic acid (Glu) which forms a hydrogen bond donated by the phenolic hydroxy group of environmental hormones. Using electron energy (E_{ele}; eV) and η–χ scale as the ordinate, the plots of the value of the E_{ele} against the valence electrons of Glu, BPA and 2,3,7,8-TCDD using the AM1 method are shown in Fig. 2.

We discuss the chemical correlation for their electron structures, hardness (η) and electronegativity (χ), of the hERαLBD with environmental hormones. On the basis of two cases of electron donor (the electron structure coordinate (χ_{ED}, η_{ED}) of the environmental hormone), first: $|\chi_{EA}-\chi_{ED}| > 0$ (a), second: $|\chi_{EA}-\chi_{ED}| \sim 0$ (b), in the interaction between EA and ED, we examined the reactivity decided on the sum of η_{EA} and η_{EA}, as illustrated in Fig. 2. Here, the η and χ scale used eV units as the ordinate. In the 2,3,7,8-TCDD (4.954, 4.044)-Glu and BPA (4.596, 4.230)-Glu, the $|\chi_{EA}-\chi_{ED}|$ differences are about 0.005 and 0.729, and the sums, $\eta_{EA}+\eta_{ED}$, are about 9.678 and 10.23, respectively. This indicates that the BPA-Glu is more stable than 2,3,7,8-TCDD-Glu. In the 1,3,6,8-TCDD (4.951, 4.116)-Glu and 2,3,7,8-TCDD-Glu, the 2,3,7,8-TCDD-Glu is more stable than 1,3,6,8-TCDD, because the conditions consist of $|\chi_{EA}-\chi_{ED}| \sim 0$ and $\eta_{2,3,7,8\text{-TCDD}}+\eta_{Glu} < \eta_{1,3,6,8\text{-TCDD}}+\eta_{Glu}$.

In general, the large $|\chi_{EA}-\chi_{ED}|$ differences ((b) in Fig. 3) between EA and ED are apparent that the driving force of the chemical reaction is stronger than that of small $|\chi_{EA}-\chi_{ED}|$

Table 1. Calculated Dihedral Angles and Absolute Hardness and Electronegativity of BPA, DDE, and 2,3,7,8-TCDD

Comp.	Dihedral angles (deg)			Absolute hardness (η)			Absolute electronegativity (χ)		
	AM1	PM3	BLYP/6-31G*	AM1 (eV)	PM3 (eV)	BLYP/6-31G* (au)	AM1 (eV)	PM3 (eV)	BLYP/6-31G* (au)
BPA	53.96	44.49	52.07	4.596	4.624	0.19253	4.230	4.321	0.14376
DDE	−62.90	−63.42	−51.90	4.376	4.300	0.24404	4.900	4.804	0.13888

Table 2. Values of Absolute Hardness, Electronegativity, and Global Softness for Several Environmental Hormones

	Chemical	Absolute hardness (η, eV)[a]	Absolute electronegativity (χ, eV)[a]	Global softness (S, eV)[a]
1i	2,3,7,8-TCDD	4.044	4.954	0.2472
1ii	1,4,6,9-TCDD	4.263	4.949	0.2346
2	2,3,7,8-TCDF	4.060	5.226	0.2463
3i	3,4,5,3′,4′,5′-HCB[b]	4.241	5.319	0.2358
3ii	2,3,6,2′,3′,6′-HCB[c]	4.489	5.124	0.2228
4i	*p*,*p*′-DDT	4.526	5.015	0.2209
4ii	*o*,*p*′-DDT	4.574	5.019	0.2186
5	*p*,*p*′-DDD	4.643	4.912	0.2154
6i	*p*,*p*′-DDE	4.376	4.900	0.2285
6ii	*o*,*p*′-DDE	4.627	4.880	0.2161
7	*trans*-Diethylstilbestrol (DES)	4.582	4.278	0.2182
8	Chlordecone	5.123	5.794	0.1952
9	Mirex	5.461	5.730	0.1831
10	Chlorfenethol	4.674	4.834	0.2139
11	Bisphenol A	4.596	4.230	0.2176
12	4,4′-Sulfobisphenol	4.544	5.186	0.2200
13	Pentachlorophenol (PCP)	4.441	5.425	0.2252
14	α-HCH	5.768	5.806	0.1734
15	Nonylphenol	4.669	4.241	0.2142
16	Dicofol	4.630	5.097	0.2160
17	*trans*-Styrene dimer	4.780	4.409	0.2092
18	*cis*-Styrene dimer	4.825	4.375	0.2073
	Tyroxine	4.233	4.994	0.2362
	17β-estradiol	4.625	4.214	0.2162
	Testosterone	5.014	4.999	0.1994

a) AM1 level. *b*) Dihedral angle (φ)=49°.[3a] *c*) Dihedral angle (φ)=74°.[3a]

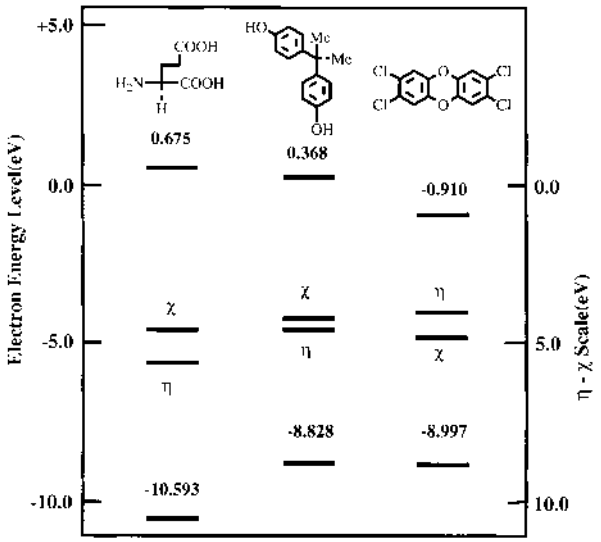

Fig. 2. Calculated HOMO and LUMO Energies, Absolute Hardness, and Absolute Electronegativity for Glu, BPA, and 2,3,7,8-TCDD

differences ((a) in this Fig. 3). The case of (a) means that the interaction between EA and ED is small since the electron transfer is small: $|\chi_{EA}-\chi_{ED}|\sim 0$. On the other hand, the case of (b) means that strong interaction occurs between EA and ED since the electron transfer is large and the stabilization energy (Eq. 8) also is large. As the EA uses same chemicals, for instance, Glu, *etc.* the interaction is classified by two combinations, case (a) $|\chi_{EA}-\chi_{ED}|\sim 0$ and case (b) $|\chi_{EA}-\chi_{ED}|>0$. Finally, by considering both cases, the strengths for the toxicity, enzyme induction, and estrogen activity can be estimated from the numerical data of η.[11]

Structure–Activity Relationship for Androgen Receptor Binding Affinity Kelce *et. al*., reported that environmental hormones, *e.g.*, *p*,*p*′-DDT, chlordecone, DDE, and *trans*-diethylstilbestrol (DES) inhibit androgen binding to the receptor, and are thus potent androgen receptor antagonists.[12] The structure of DDTs is very similar to PCBs substituted with chlorines at various positions. *Para*, *para*′-DDE, a DDT metabolite is an inhibitor of androgen binding to the receptor. These chemicals do not seem to have a characteristic property except that they are hydrophobic, polychlorinated hydrocarbons. Therefore, it is hard to infer a structure–activity relationship based on their chemical structures. However, we found an interesting relationship between electron structure of the environmental hormones and biological activity. According to the method described above, the data of absolute hardness and absolute electronegativity calculated by the AM1 method for several environmental hormones, *e.g.*, chlordecone, *p*,*p*′-DDT, *p*,*p*′-DDE, *o*,*p*′-DDT, *p*,*p*′-DDD, and diethyl stilbestrol is listed in Table 2.

A relationship between the η values of these environmental hormones and their androgen receptor (AR) binding affinity shows a correlation curve obtained from a plot of the logarithmic AR binding affinity against the η value (Fig. 4). The other physical data are not correlated to the AR binding affinity. The potency of this affinity is clearly controlled by the values of absolute hardness, but not absolute electronegativity. In contrast, it is suggested that the activity is also dependent on the global softness (S). The χ values of these chemicals, *p*,*p*′-DDE and *p*,*p*′-DDT, *etc*., are closer to these of progesterone and testosterone than to that of 17β-estradiol. As these chemicals are satisfied by the condition of $|\chi_{testosterone}-\chi_{p,p'\text{-DDE}}|\sim 0$, it is determined that *p*,*p*′-DDE and *p*,*p*′-DDT have no activity as an agonist or antagonist of 17β-estradiol, but do have such activity as an antagonist of testosterone and progesterone.

A η–χ Activity Diagram for Sex Hormones To elucidate the correlation between chemical structure and estrogen activity for sex hormones, we used such physical parameters as absolute hardness, absolute electronegativity, and softness

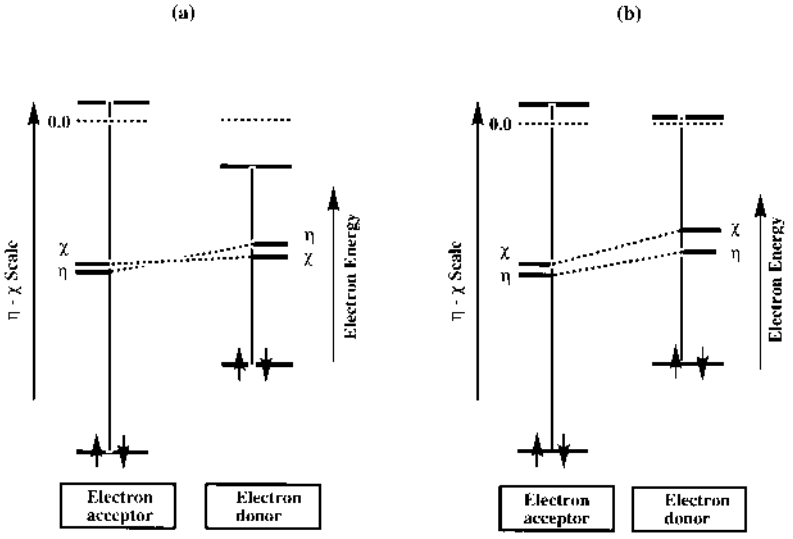

Fig. 3. Correlation of Electronic Energies, Absolute Hardness, and Absolute Electronegativity for Interaction Between Electron Acceptor and Electron Donor

Zero line represents zero energy level. (a) Interaction under the condition, $|\chi_{EA}-\chi_{ED}|\sim 0$ and (b) interaction under the $|\chi_{EA}-\chi_{ED}|>0$.

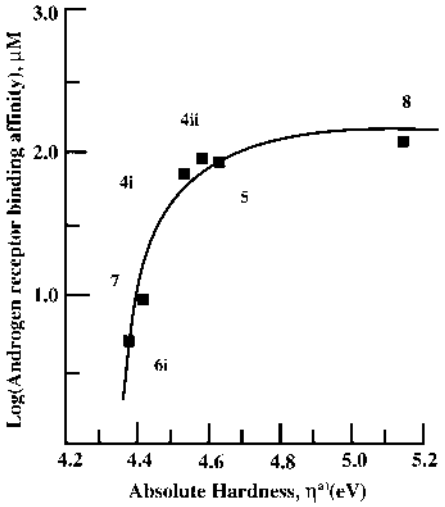

Fig. 4. Plots of Absolute Hardness against Androgen Receptor Binding Affinity (IC_{50}) of Environmental Hormones[a,b]

a) AM1 level. *b*) **6i**; *p,p′*-DDE, **7**; diethylstilbestrol, **4i**; *p,p′*-DDT, **4ii**; *o,p′*-DDT, **5**; *p,p′*-DDD, **8**; chlordecone.

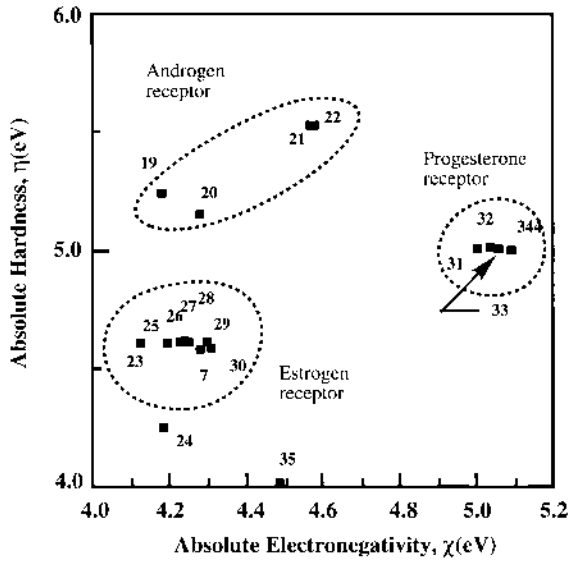

Fig. 5. Plot of η–χ Activity Diagram for Sex Hormones and their Derivatives[a—c]

a) **19**, androstenediol; **20**, pregneolone; **21**, epiandrosterone; **22**, androsterone; **23**, moxestrol; **24**, 4-hydroxytamoxifen; **25**, 17β-estradiol; **26**, estriol; **27**, hexestrol; **28**, 17α-estradiol; **7**, *trans*-diethylstilbestrol; **29**, estrone; **30**, equilin; **31**, testosterone; **32**, ethisterone; **33**, progesterone; **34**, 4-androstene-3,17-dione; **35**, equilenin. *b*) Symbol ■ represents the coordinate (χ, η) of electronic structures of hormones and their derivatives. *c*) AM1 level.

in our studies. With regards to sex hormones' SARs, Fig. 5 shows a 2-D (two dimensional) plot of the electron structures of sex hormones of global minimized energy to the absolute hardness–electronegativity ($\eta-\chi$) activity diagram. We represented for the first time a 2-D plot of female and male hormones clustered in two electronic structures using absolute electronegativity as the abscissa and absolute hardness as the ordinate. The electronic structures of female hormones are controlled by the property of chemically soft and bases, while the male hormones are controlled by the property of chemically hard and acids. For instance, η- and χ- values of 17β-estradiol (η=4.614 and χ=4.194) are smaller values than testosterone (η=5.014 and χ=5.037), therefore, 17β-estradiol is a softer hormone (S=0.217) than testosterone (S=0.199). The gap of χ value of sex hormones expresses the orientation and strength of polarizability because the electron donation of chemicals is determined by magnitude and sign of χ value. Large χ values are characterized as acids, and small χ values as bases. According to our results, we can predict which chemicals bind with estrogen or progesterone receptors, based on the electronic structural pattern of chemicals in the η–χ activity diagram.

In this study we showed that the electronic structures of sex hormones can be classed to three types: (i) hard acidic male hormones, (ii) hard basic androgen hormones, and (iii) soft basic female hormones, using an η–χ activity diagram. In the diagram the distribution area of sex hormones corresponds to the chemical property of the receptor ligand binding cavity. Therefore, the binding center in the ligand binding

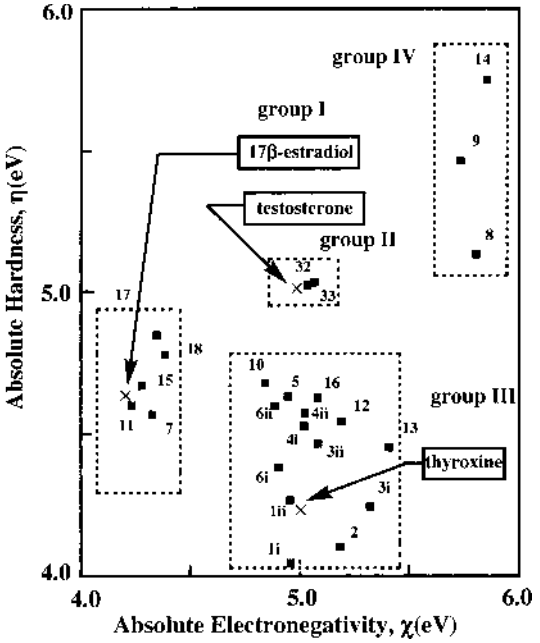

Fig. 6. Plot of η–χ Activity Diagram for Environmental Hormones[a—c]

a) AM1 level. *b*) **1i**, 2,3,7,8-TCDD; **1ii**, 1,4,6,9-TCDD; **2**, 2,3,7,8-TCDF; **3i**, 3,4,5,3′,4′,5′-HCB; **3ii**, 2,3,6,2′,3′,6′-HCB; **4i**, *p*,*p*′-DDT; **4ii**, *o*,*p*′-DDT; **5**, *p*,*p*′-DDD; **6i**, *p*,*p*′-DDE; **6ii**, *o*,*p*′-DDE; **7**, DES; **8**, chlordecone; **9**, mirex; **10**, chlorfenethol; **11**, bisphenol A; **12**, 4,4′-sulfobisphenol; **13**, PCP; **14**, α-HCH; **15**, nonylphenol; **16**, dicofol; **17**, *cis*-styrene dimer; **18**, *trans*-styrene dimer; **32**, ethisterone; **33**, progesterone. *c*) Symbols ■ and × represent the coordinate (χ, η) of electronic structures of environmental hormones, 17β-estradiol, testosterone, and thyroxine, respectively.

cavity of 17β-estradiol must be soft chemically. In fact, the hydrophobic aromatic ring of 17β-estradiol is fixed by the side chain of ^{404}Phe. If the electronic structure of environmental and sex hormones can be computed, we are able to predict the receptors to which the chemicals bind. For instance, ethinyl estradiol (4.192, 4.613) and estrone (4.299, 4.615) have the estrogen activity since the coordinates (χ, η) of the electron structure of these chemicals are distributed in the area of 17β-estradiol in the η–χ activity diagram and satisfy the condition $|\chi_{\text{ethinyl estradiol}}-\chi_{17\beta\text{-estradiol}}|\sim 0$. In the case of BPA (4.230, 4.596), it is suggested that BPA has higher affinity than the styrene dimer for the estrogen receptor, as described in the discussion about Fig. 3. As expected, 2,3,7,8-TCDD (4.954, 4.040) has lower affinity for estrogen receptor than BPA, because 2,3,7,8-TCDD is located in a different area from the coordinate of the electron structure of 17β-estradiol in the η–χ activity diagram. Preferably, we assume that 2,3,7,8-TCDD has no agonist or antagonist as estrogen-like activity, but does have androgen-like activity.

An η–χ Activity Diagram for Environmental Hormones, Agonist and Antagonist Concerning structure–activity relationships of the chemicals suspected of being environmental hormones, we provide a η–χ activity diagram as a coordinate of electronic structures for such hormones based on the results described above. A plot of η *vs.* χ is shown in Fig. 6 using χ as the abscissa and η as the ordinate. The authors are able to classify environmental hormones into four groups: group I, DES and bisphenol A, *etc.* have soft bases, and endocrine disruptor effects are clearly confirmed; group II, androgen such as testosterone, *etc*., and their analogs are classified as hard acids; group III, aromatic polychlorinated hydrocarbons like dioxins, dibenzofurans, DDTs, and their analogs have potent toxicity, and these chemicals are classified as soft acids; group IV, aliphatic polychlorinated hydrocarbons such as mirex, chlordecone, and HCH are less toxic, and are classified as hard acids. From the diagram, more toxic 2,3,7,8-TCDD, 2,3,7,8-TCDF, and 3,4,5,3′,4′,5′-HCB (=coplanar PCB), *etc.* have smaller η values than less toxic chlorfenethol, *p*,*p*′-DDT, and HCH, *etc.* According to the chemical hardness concept, as soft molecules have small η values, and hard molecules large η values, toxic 2,3,7,8-TCDD, 2,3,7,8-TCDF, and 3,4,5,3′,4′,5′-HCB and chlorfenethol, *p*,*p*′-DDT, and HCH, *etc.* are classified as soft and hard molecules, respectively.

On the other hand, the χ values of BPA and DES are lower than those of dioxins, DDTs, chlordecone, and HCH. Obviously, the distribution of chemicals in group I is different from the chemicals of groups II and III in the diagram. The differences are related to the reactivity of environmental hormones. The target receptor of BPA and DES is an estrogen receptor (ER) which belongs to the superfamily of nuclear receptors,[13,14] but dioxins, DDTs, and PCBs preferably bind to the thyroid receptor (T4R)[15,16] and Ah receptor.[17,18] An interesting fact was learned: DDE is also classified in group III as well as dioxins. Therefore, the η–χ activity diagram in Fig. 6 also can suggest whether the chemicals are environmental hormones or not. The strength of affinity for hER is the following order: group III<group II<group I. For instance, dioxins have an electronic structure similar to thyroxine (×), and are included in group III. The η–χ activity diagram indicates that dioxins would bind to the thyroid receptor and androgen receptor rather than to the estrogen receptor, whereas DES interestingly belongs to group I.

The diagram is able to classify the agonists and antagonists into the chemicals, that is, 4-hydroxytamoxifen is an antagonist for 17β-estradiol which is an agonist for estrogen receptor. From the chemical hardness concept, the χ value of antagonist is nearly equal to the χ value of agonist, but a large gap is observed in the η values between them. For instance, electronic structure differences of the potent antagonists, tamoxifen, 4-hydroxytamoxifen, and ICI are $\Delta\chi=|\chi_{\text{e.h}}-\chi_{17\beta\text{-estradiol}}|=+0.0095$—$+0.0135$ and $\Delta\eta=|\eta_{\text{e.h}}-\eta_{17\beta\text{-estradiol}}|=+0.317$—$+0.414$ values against the agonist (17β-estradiol), whereas poor active agonists correspond to $\Delta\chi=\sim\pm 0.0$ and $\Delta\eta=-$sign values. These findings are similarly observed in electronic structures between progesterone, and DDE[18] which is an antagonist for androgen. Generally, it appears that the chemical evidence for antagonists and agonists can be demonstrated by the coordinates in the η–χ activity diagram, as shown in Fig. 7. The electronic structure (χ_1^{a}, η_1^{a}) of the agonist 1(●) is similar to that of the ligand 1 (■) (χ_1^0, η_1^0), whereas the difference $\Delta\eta$ ($=\eta_1^{\text{t}}-\eta_1^0>0$) for antagonist 1 is higher than the agonist 1 in the difference ($\Delta\eta=\eta_1^{\text{t}}-\eta_1^0$, $\Delta\chi=\chi_1^{\text{t}}-\chi_1^0$) between the values of the antagonist 1 (△) (χ_1^{t}, η_1^{t}) and ligand 1. The chemical 2 (χ_2^{a}, η_2^{a}) (△) is an agonist of ligand 2, however, this does not make an agonist of ligand 1 since the difference $\Delta\chi_{22}^{\text{a0}}$ ($=\chi_2^{\text{a}}-\chi_2^0$) of agonist 2 is higher than the difference $\Delta\chi_{12}^{\text{a0}}$ ($=\chi_1^{\text{a}}-\chi_2^0$) for agonist 1. Thus, we can draw the new rules for agonist/antagonist of environmental hormones:

(i) An agonist has an electronic structure similar to a ligand.

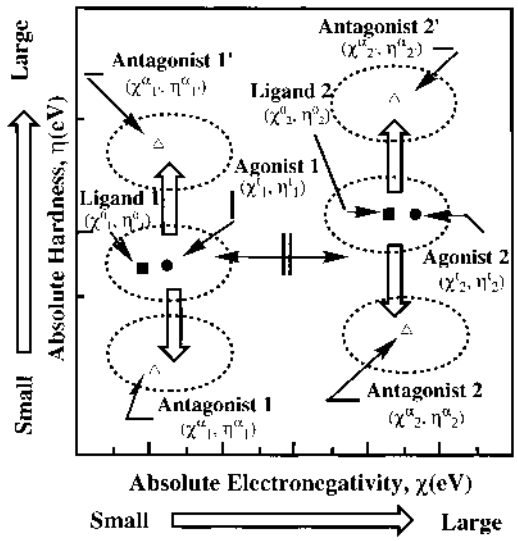

Fig. 7. An η–χ Activity Diagram for Relation of the Electronic Structures among Ligand, Agonist, and Antagonist of Environmental Hormones

Arrow indicates that an electronic structure of the antagonist of ligand 2 (■) is coordinate at symbol 2 (△). The sign ≠ indicates that agonist 1 (or 2) is not an agonist of ligand 2 (or 1).

$$\Delta\eta=\eta_1^0-\eta_1^a=\sim\pm0.0,\quad \Delta\chi=|\chi_1^0-\chi_1^a|=\sim\pm0.0$$

(ii) A condition for a pure antagonist of the ligand is

$$\Delta\eta=\eta_1^0-\eta_1^t=>0.0,\quad \Delta\chi=|\chi_1^0-\chi_1^t|=\sim\pm0.0$$

(iii) A condition is neither agonist nor antagonist.

$$\Delta\eta=\eta_1^a-\eta_1^t=\pm>0.0,\quad \Delta\chi=|\chi_1^a-\chi_1^t|=>0.0$$

Phthalic acid esters (PAE) commonly used as plasticizers also are known as an endocrine disruptors.[19] To predict the bioactivity and receptor binding of PAE, we examined the coordinate of electronic structure of PAE in the η–χ activity diagram (Fig. 6). The coordinate of dibutyl phthalate (DBP: **36**) was (5.431, 4.884), and the calculated difference ($\Delta\chi$, $\Delta\eta$) of the coordinate between **36** and 17β-estradiol was (−1.217, −0.259). As DBP does not belong to group I, this indicates that DBP has no estrogen activity. Preferably, PAE should interact with androgen and thyroid receptors more than estrogen receptor. Our method is available for the prediction of estrogen activity of cyclofenil (**37**) and cyclic triarylethylene (**38**), *etc.*[20,21] These chemicals belong to group I and are soft.

An η–χ Activity Diagram for Electronic Structures between Amino Acid Residues in the Estrogen Receptor Ligand Binding Site and Environmental Hormones To elucidate the relationship between natural amino acids, components of the receptor, and environmental hormones, we calculated the value of absolute hardness (η) and electronegativity (χ) for amino acids with neutral structure, H_2N–CH(R)–COOH (R=side chain). All values of η and χ for 20 natural amino acid residues calculated by Eqs. 1 and 2 are plotted in the η–χ activity diagram shown in Fig. 8. It is evident that the natural amino acids are classified into two groups: type (a) and type (b). Each group is divided into soft bases (type (a)) and hard bases (type (b)), for instance, Phe and Tyr are classified as type (a), however, Gln and Ile are classified as type (b). Interestingly, a blank area for electronic

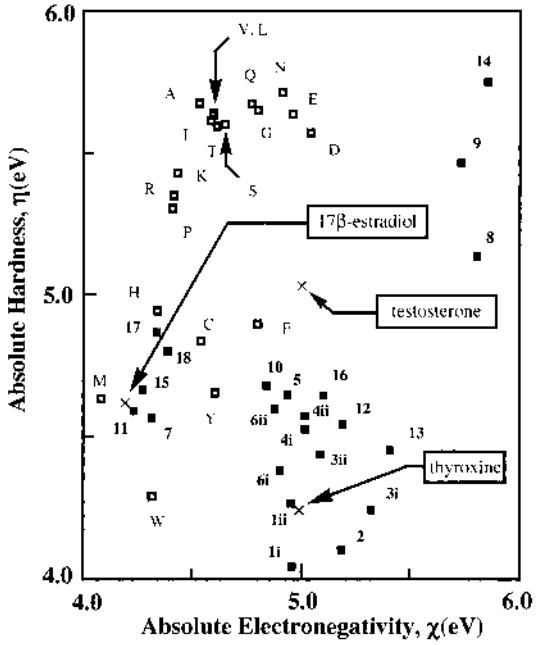

Fig. 8. Correlation of the Electronic Structures between Natural Amino Acid Residues and Environmental Hormones[a—c]

a) AM1 level. *b*) Name of amino acid residues, one-letter symbol. *c*) **1i**, 2,3,7,8-TCDD; **1ii**, 1,4,6,9-TCDD; **2**, 2,3,7,8-TCDF; **3i**, 3,4,5,3′,4′,5′-HCB; **3ii**, 2,3,6,2′,3′,6′-HCB; **4i**, *p,p′*-DDT; **4ii**, *o,p′*-DDT; **5**, *p,p′*-DDD; **6i**, *p,p′*-DDE; **6ii**, *o,p′*-DDE; **7**, DES; **8**, chlordecone; **9**, mirex; **10**, chlorfenethol; **11**, bisphenol A; **12**, 4,4′-sulfobisphenol; **13**, PCP; **14**, α-HCH; **15**, nonylphenol; **16**, dicofol; **17**, *cis*-styrene dimer; **18**, *trans*-styrene dimer. *d*) Symbols ■, □ and × represent the coordinate (χ, η) of electronic structures of environmental hormones, natural amino acid residues, and 17β-estradiol and thyroxine, respectively.

structures of amino acid residues is observed in this diagram. There is a non-distribution area of amino acid residues, and this blank area is complementary to the distribution of environmental hormones, as seen in the Fig. 8. We can predict whether or not this blank area would be related to biological activity, toxicity and estrogen activity, of environmental hormones.

From the results shown in Fig. 8, it is very significant to elucidate the correlation between amino acid residues and environmental hormones, because the environmental hormone–receptor complex, *e.g.* dioxin–Ah receptor or BPA–estrogen receptor complexes, move into the nucleus, and the gene expression is induced.[22] Here, the plot of environmental hormones does not overlap with the plot of amino acid residues except for thyroxine, 17β-estradiol, DES, and BPA. In addition, it was found that the blank area from amino acid residues is covered by environmental hormones. That is, environmental hormones are distributed complementarily to amino acid residues. No amino acid residues are softer acids than toxic 2,3,7,8-TCDD**1i**, 2,3,7,8-TCDF**2**, and 3,4,5,3′,4′,5′-HCB**3i** or harder acids than chlordecone **8** and HCH **14**.

The significance of Fig. 8 is that based on the hardness concept, soft acids prefer soft bases, and hard acids prefer hard bases. Accordingly, the results express characteristic reactivity for the interaction between amino acids (receptors) and environmental hormones. The environmental hormones are classified into electronic structures of three types: 17β-estradiol, thyroxine, and HCH, described above, which have different biological activities. It is possible to predict that

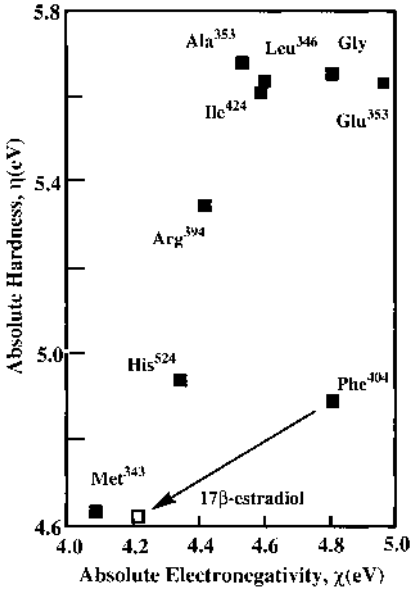

Fig. 9. Plot of an η–χ Activity Diagram for Electronic Structure of Amino Acids and 17β-Estradiol around Ligand 17β-Estradiol Binding Site

dioxins and DDTs act as agonists (thyroxine) to the thyroid receptor, and DES and BPA act as agonists (17β-estradiol) to the estrogen receptor. We showed that the relationship between chemical structure and biological activity of environmental hormones is dependent on the magnitude of the η values. The absolute hardness values would be the measures of stability of complexes formed between receptors and environmental hormones, and of the toxicity. Absolute hardness contributes to the stabilization of reactions with environmental and sex hormones.

To clarify the relationship of electronic structure between the ligand and the eighteen amino acid residues, we prepared an η–χ activity diagram of eighteen amino acid residues bound around the ligand, as shown in Chart 2. Hydrophobic amino acid residues such as Leu, Ala, Ile are located in the upfield area of 17β-estradiol, and Glu, Arg, and His form hydrogen-bonding with the 3-hydroxyl or 17-hydroxyl of 17β-estradiol which are chemically softer than the three amino acid residues. Similarly, the coordination pattern in the η–χ activity diagram is different from that of the agonist/antagonist diagram.

In general, it appears that the results for electronic structures between the ligand and binding site of receptor can be demonstrated by the coordinates in the η–χ activity diagram, as shown in Fig. 9. The electronic structure (χ_1^a, η_1^a) of 17β-estradiol (□) is different from that of the binding site of the hERαLBD (■) (χ_{LBD}^0, η_{LBD}^0), and the relationship clearly differ with the correlation of the electronic structure between agonist and antagonist (Fig. 7). This suggests that the LBD conditions must be $\Delta\chi=\chi_1^a-\chi_{LBD}^0 \leqq 0.0$ and $\Delta\eta=\eta_1^a-\eta_{LBD}^0 \leqq 0.0$. In the hER$\alpha$LBD, we especially focused on the role of Phe^{404}, because the calculated value (ΔQ) of the quantity of charge transfer between 17β-estradiol and Phe^{404} is about +0.03084, and the ΔQ value is larger than that (+0.01867) of charge transfer between 17β-estradiol and Leu. This means that the side chain of Phe^{404} interacts with the phenolic group in 17β-estradiol and environmental hormones (group I in Fig. 6).

Conclusion

We have demonstrated that the prediction of the toxic potency, estrogen activity, and chemical structures of the environmental hormones is possible by using the hardness concept. The estrogen activities of environmental hormones also are dependent on not only the molecular shapes but on the absolute hardness (or softness) η (or S) of the chemicals. The η values thus would be a new means of predicting the toxicity and binding with estrogen receptor of chemicals. The receptor binding affinity of the sex hormones is also due to the η values. The electronic structure coordinate (χ, η) between environmental and sex hormones can be easily compared using the η–χ activity diagram. This diagram indicates that the environmental hormones can be classified into four groups: 17β-estradiol (group I), testosterone (group II), thyroxine (group III), and HCH (group IV) types. Such information should be helpful in predicting the chemical structures which act as environmental hormones.

Our findings suggest that the smaller the η values are, the softer the compounds are chemically, and the activity of environmental hormone increases. From the η–χ activity diagram, we have concluded the following:

(i) The environmental hormones belonging to electronic coordinates in group I have estrogen activity. A necessary condition is that a phenol-like aromatic system be contained in the molecule.

(ii) The condition for the electronic structure between ligand (χ_1^0, η_1^0) and LBD (χ_{LBD}^0, η_{LBD}^0):

$$\Delta\eta=\eta_1^0-\eta_{LBD}^0 \leqq 0.0, \quad \Delta\chi=|\chi_1^0-\chi_{LBD}^0| \leqq 0.0$$

(iii) The condition for the electronic structure among ligand (χ_1^0, η_1^0), agonist (χ_1^a, η_1^a) and antagonist (χ_1^t, η_1^t):

$$\Delta\eta=\eta_1^0-\eta_1^a=\sim\pm 0.0, \quad \Delta\chi=|\chi_1^0-\chi_1^a|=\sim\pm 0.0$$

$$\Delta\eta=\eta_1^0-\eta_1^t=>0.0, \quad \Delta\chi=|\chi_1^0-\chi_1^t|=\sim\pm 0.0$$

(iv) Coordinates of the electronic structure for environmental hormones are located in a position complementarily to that of amino acids (estrogen receptor, *etc.*) in the η–χ activity diagram.

Finally, the η–χ activity diagram may be a very useful method for studies of drug interaction and toxicity. Further studies on this and related chemicals will provide insight to predict their biological activities as environmental hormones.

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