A Dichotomy in the Lipophilicity of Natural Estrogens, Xenoestrogens, and Phytoestrogens

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Using two independent analyses, it is demonstrated that natural (e.g., estradiol) and some xenoestrogens (e.g., methoxychlor metabolite) are characterized by a lipophilic region that is absent in nonestrogens as well as in phytoestrogens. It is suggested that this lipophilic region affects binding to specific receptors and may, in fact, differentiate harmful from beneficial estrogens. — Environ Health Perspect 105(Suppl 3):665-668 (1997)

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Introduction

Recently, we identified a 6Å 2-dimensional distance (2D) descriptor associated with the carcinogenicity in mice of estrogens (e.g., diethylstilbestrol and 17β-estradiol) (1). This descriptor (biophore) was originally recognized during structure-activity relationship (SAR) studies of diethylstilbestrol (2) and tamoxifen and toremifene (3), using the SAR expert systems computer automated structure evaluator (CASE) and multiple case (MULTICASE). This biophore was derived from the CASE/MULTICASE learning set of murine carcinogens (4-8). Based on its presence in carcinogenic estrogens, we suggested that the 2D biophore represented a ligand binding site on an estrogen receptor (1). This hypothesis was supported by the realization that the biophore was derived from estrogens in the carcinogenicity database and the fact that CASE/MULTICASE had been programmed to recognize 2D biophores that possess lipophilic centers as well as moieties capable of hydrogen bonding. These characteristics are associated with ligands that bind to cellular receptors. Hence this finding is consistent with an estrogen possessing a hydrogen-bonding moiety at one end and a lipophilic moiety on the other. In fact, CASE/MULTICASE identified it as a lipophilic, anchored, p-substituted phenol moiety (Figure 1). This 2D descriptor is absent from the vast majority of nonestrogens.

Using CASE/MULTICASE, we identified a number of chemicals, including many estrogens and xenoestrogens, that possess this 2D moiety (Table 1), as well as a number of estrogens that lack it. However, some of the estrogens devoid of this moiety acquire it following metabolic activation, e.g., tamoxifen metabolism to 4-hydroxytamoxifen; the latter is the metabolite thought to be responsible for the estrogenicity of the parent molecule (9). On the other hand, phytoestrogens, as a group, lack this descriptor (Table 1). These findings suggest that the presence of the 2D descriptor could be used to classify estrogens with respect to possible risk to humans and to the ecological biota, or even to distinguish between harmful (xenoestrogens) and potentially beneficial estrogens (e.g., phytoestrogens).

While we do not expect this 2D biophore to provide a unifying principle that accounts for the action of estrogens, it might provide further insight into their mechanism of action. In the present study we expand the definition of the 2D biophore, especially with respect to its putative lipophilicity.

Methods

The CASE/MULTICASE methodologies have been described on a number of occasions (10,11). The 6Å moiety (above) identified by CASE/MULTICASE involves phenol substitution at the p-position with a carbon atom. The specific lipophilicity of the p-substituent is specified by CASE/MULTICASE to include carbon atoms that are four bonds away from heteroatoms. By this criteria 17β-estradiol was identified as possessing the appropriate lipophilic moiety while the carbon para to the phenol in genistein was found to lack it. To clarify the lipophilicity of the 2D biophore, we analyzed a group of molecules with Molecular Modeling Pro (MMP) (12) for the presence, location, and characteristics of their lipophilic regions.

Briefly, MMP assigns values for the lipophilicity of each atom of a molecule using the procedure of Hansch and Leo (13). For example, a value of 0.23 is assigned to hydrogens, 0.13 to carbons with one hydrogen, 0.22 to carbons with two or more hydrogens, −1.14 to hydroxy groups, and −2.24 to keto oxygens. Each atom is also modified by its neighbors. The value of atoms α are multiplied by 0.5, β by 0.25, γ by 0.125, and δ by 0.0625. These values are totaled and added to the value of the atom of interest. After all calculations are completed, atoms with negative values are designated “hydrophilic” and those with positive values as “lipophilic.” MMP then colors each atom to denote its degree of lipophilicity or hydrophilicity (Figure 1).
Results and Discussion

Not all estrogens contain the 2D biophore (above) (Table 1). The simplest molecule that contains this biophore, 4-methylphenol (Figure 1), can illustrate the biophore. The 1-position of 4-methylphenol contains the hydroxyl group that is both hydrophilic and capable of hydrogen bonding. The 4-position is occupied by a benzylic methyl group that is in a lipophilic environment. In general, the benzylic carbon can be methyl, methylene, methine, or quaternary. Between the p-hydroxyl group and the lipophilic moiety is a conjugated six-membered ring system that may be substituted at some positions (J). The structure of 4-methylphenol can be superimposed on other molecules for easy identification of the 2D biophore.

The major aim of this investigation was to visualize and confirm, using MMP, that in fact the MULTICASE biophore is indeed anchored in a lipophilic region. This is readily demonstrated (Figure 1). All the chemicals shown in Figure 1 possess the physical distance requirements of the biophore (i.e., 6A from phenol to benzylic carbon); however, the chemicals that lack the biophore have a benzylic carbon atom located in a region that is either hydrophilic or only somewhat lipophilic. For example, diethylstilbestrol (DES) and 17β-estradiol, which possess the 2D biophore, have a large lipophilic region that encompasses the p-substituted carbon. On the other hand, dietary estrogens such as coumestrol and genistein, which lack the biophore, have the corresponding carbon embedded in a region intermediate between lipophilic and hydrophilic (Figure 1).

For chemicals to have a lipophilic area at the alkyl end of the 2D biophore, heteroatoms (e.g., oxygen atoms) must be sufficiently distant from the p-carbon. Thus chemicals such as the dietary estrogens with their intra- and extracyclic oxygens produce an environment that is not very lipophilic and hence the biophore is absent.

The 2D biophore was originally identified from a carcinogenicity database.
Table 1. Distribution of the 2D biophore among selected estrogenic and antiestrogenic chemicals.

| Chemical                                                      | Type       | 2D |
|---------------------------------------------------------------|------------|----|
| 2',4',8',6'-Tetrahydroxydihydrochalcone (phloretin)         | Phytoestrogen | -  |
| 5,7-Dihydroxyflavone (chrysin)                               | Phytoestrogen | -  |
| 3,5,7-Trihydroxyflavone (galangin)                          | Phytoestrogen | -  |
| 4',5',7-Trihydroxyflavone (apigenin)                         | Phytoestrogen | -  |
| 3',3',4',7-Tetrahydroxyflavone (fisetin)                     | Phytoestrogen | -  |
| 2',4,4',6'-Tetrahydroxydihydrochalcone (apigenin)           | Phytoestrogen | -  |
| 3,3',4',5,7-Pentahydroxyflavone (kaempferol)                 | Phytoestrogen | -  |
| 3,5,7-Trihydroxy-4'-methoxyflavone (kaempferide)             | Phytoestrogen | -  |
| 3',3',4',5,7-Pentahydroxyflavone (quercetin)                 | Phytoestrogen | -  |
| 2',3',4',5,7-Pentahydroxyflavone (morin)                     | Phytoestrogen | -  |
| 4',5,7-Trihydroxynaringenone (naringenin)                    | Phytoestrogen | -  |
| 3',5,7-Trihydroxy-4'-methoxyflavone (hesperidin)             | Phytoestrogen | -  |
| 3,3',4',5,7-Pentahydroxyflavone (taxifolin)                  | Phytoestrogen | -  |
| 4',7-Dihydroxyisoflavone (diadzein)                          | Phytoestrogen | -  |
| 3,3',4',5,7-Pentahydroxyflavanone (biochanin A)              | Phytoestrogen | -  |
| Coumestrol                                                    | Phytoestrogen | -  |
| 4',4'-Dihydroxyisoflavone                                   | Phytoestrogen | +  |
| α-Sitosterol                                                   | Phytoestrogen | +  |
| Zearalenone                                                   | Phytoestrogen | +  |
| Tetrahydrocannabinol                                          | Phytoestrogen | +  |
| **Xenoestrogens and therapeutics**                           |            |    |
| α,α'-DDE                                                      | Xenoestrogen | -  |
| Chlordecone                                                   | Xenoestrogen | -  |
| 4-Nonylphenol                                                 | Xenoestrogen | +  |
| 4-tert-Butylphenol                                            | Xenoestrogen | +  |
| DES                                                           | Estrogen    | +  |
| Indenol A                                                     | DES metabolite | +  |
| 4',4'-Diethylstilbestrol quinone                             | DES metabolite | -  |
| TMX                                                           | Antiestrogen | -  |
| 4-Hydroxytamoxifen acid                                      | TMX metabolite | +  |
| TRM                                                           | Antiestrogen | -  |
| 4-Hydroxytamoxifenhydroxytoremifene                          | TRM metabolite | +  |
| ICI 164,384                                                   | Antiestrogen | +  |
| ICI 182,780                                                   | Antiestrogen | +  |
| LY 117018                                                    | Antiestrogen | -  |
| MER 25                                                       | Antiestrogen | -  |
| 17β-Estradiol                                                 | Estrogen    | +  |
| 17α-Ethynyl estradiol                                         | Estrogen    | +  |
| Benzoestradiol                                                | Estrogen    | +  |
| Dienesol                                                     | Estrogen    | +  |
| Estrone                                                      | Estrogen    | +  |
| Estradiol                                                    | Estrogen    | +  |
| Hexoestradiol                                                 | Estrogen    | +  |
| Megoestradiol                                                 | Estrogen    | -  |
| Noroestradiol                                                 | Estrogen    | -  |
| Noroestrone                                                   | Estrogen    | -  |
| Phenol red                                                    | pH indicator | -  |
| Bis(4-hydroxyphenyl)(1,2-phenoxysulfonylphenyl)methane*       | Xenoestrogen | -  |

Abbreviations: TMX, tamoxifen; TRM, toremifene. *This chemical has been identified as the estrogenic impurity of commercial phenol red preparations (14).

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