Research Article

Three-dimensional structure-activity relationship modeling of cross-reactivities of a polyclonal antibody against pyrene by comparative molecular field analysis

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Abstract

Immunoassays have been regarded as a possible alternative or supplement for measuring polycyclic aromatic hydrocarbons (PAHs) in the environment. Since there are too many potential cross-reactants for PAH immunoassays, it is difficult to determine all the cross-reactivities (CRs) by experimental tests. In this study, the quantitative structure-activity relationship (QSAR) technique, comparative molecular field analysis (CoMFA), was applied to predict the CRs of a polyclonal antibody against pyrene. The CoMFA model developed shows that the CRs of the compounds are correlated to their 3D structure (n = 14, q^2=0.527, r^2=0.944, and Standard Error (SE) = 0.22478). The contributions of the steric and electrostatic fields to CRs are 95 and 5%, respectively. The results of the correlation predicted the CRs with actual CRs of randomly selected test compounds and showed that the developed model has good prediction ability. The QSAR model has been applied to predict the CRs of other 17 PAHs. The 3D-QSAR model and its respective contour plot could be useful tools to further understand the molecular nature of antibody-antigen interactions.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds containing two or more fused aromatic rings constituted of carbon and hydrogen atoms. PAHs have received considerable attention because of their rich chemistry, physical properties, technological and industrial applications, aromaticity, and the role as a major class of environmental pollutants and carcinogens (Schleyer 2001, Portella *et al.* 2005, Poater *et al.* 2003, Wiberg 1997, Howard & Krygowsk 1997, Suresh & Gadre 1999). PAHs rarely occur as individual compounds; they are usually found as a complex mixture of various compounds, which makes their analysis difficult. Conventional analytical methods for PAHs, such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), are time-consuming and cumbersome (Poster *et al.* 2006, Moret & Conte 2000, Boer & Law 2003). Immunochemical methods, such as enzyme-linked immunosorbent assays (ELISAs) and immunosensors, are fast, selective, and inexpensive analytical methods that complement traditional chromatographic analytical procedures in the environmental analysis of PAHs (Kramer 1998, Barcelo *et al.* 1998, Fahnrich *et al.* 2002, Szekacs *et al.* 1999, Li *et al.* 1999, Zhang *et al.* 2006).

An immunoassay is a very specific analytical method, but it is also known for its cross-reactivities (CRs), i.e., it responds to compounds structurally related to the analyte. CRs are expected not only in polyclonal immunoassays, but also in monoclonal ones. Although CR affects the specificity of the immunoassay and can result in biased test results, it can sometimes be used as a tool to identify metabolites or structurally similar compounds as well as for class-specific immunoassays.

The key step in developing an immunoassay for PAHs is the design and preparation of optimum haptens for immunogens and competitors. The performance of the antibody within the assay is greatly affected by the property of the hapten. Some haptens for PAHs have been reported (Spier *et al.* 2009, Fahnrich *et al.* 2002, Li *et al.* 1999), however, the antibody specificity resulting from the newly designed hapten is often unpredictable, and this knowledge comes only after time-consuming and laborious animal experiments. As there are too many potential cross-reactants

and some of them may not be available for testing, it is not easy to determine all the CR values of the cross-reactants for these immunoassays. It would thus be desirable to be able to predict the specificity of the antibody obtained with a given hapten. As antigen-antibody recognition is based on the steric criteria and the interactions resulting from the electronic properties of the molecules, molecular modeling may be helpful as it allows the determination of the volumes and charges of the compounds (Delaunay-Bertoncini *et al.* 2003).

In this article, comparative molecular field analysis (CoMFA) was used to investigate the relationship between the CRs and the structure of PAHs. The objective of this study is to determine the possibility of predicting the CRs of the PAHs in ELISAs by the quantitative structure-activity relationship (QSAR) method.

Method

CR data set

17 molecules (Table 1) together with experimental CR data (Székács *et al.* 1999) were used to develop a model for predicting the CRs of a polyclonal antibody against pyrene. Pyrene was the reference substance during measurements. The training set contained 14 molecules (No. 1-3, 5, 6, 8, 9, 11-17). The model was validated externally by excluding from the PLS analysis the three randomly selected test compounds (No. 4, 7, and 10), representative of the training set.

CR is normally quantified by comparing the assay's response to a range of similar analytes (cross-reactants) and expressed as a percentage. It is calculated according to Eq.1:

$$CR(\%) = \frac{IC_{50Ag}}{IC_{50Cr}} \times 100 \quad (1)$$

Since activity data used for QSARs should be in molar dimensions, the molar CR (MCR) is defined in this article and used for QSAR analysis. It is 100 times the ratio between the molar IC_{50} value (mass IC_{50} value divided by the molecular weight) of the antigen and that of the cross-reactant, as indicated by Eq.2:

$$\frac{IC_{50Ag}/MW_{Ag}}{IC_{50Cr}/MW_{Cr}} \times 100 = CR(\%)\frac{MW_{Cr}}{MW_{Ag}}$$

MCR(%)=(2)

CR was firstly converted to MCR, and then used for QSAR modeling and prediction. Finally, the predicted MCR obtained by the QSAR model was converted to CR, and compared to the experimental CR value.

CoMFA procedure

The CoMFA procedures were performed with SYBYL X 2.0 (Tripos, St. Louis, MO, USA). The 3D structure of each compound was constructed and energy-minimized to a low-energy conformation using the standard Tripos molecular force field. Atomic partial charges were computed using the Gasteiger-Hückel method. Most of the compounds studied in this work are flat geometrical compounds owing to their fused-ring structure and planarity. The rigid character of these compounds reduced the number of conforma-

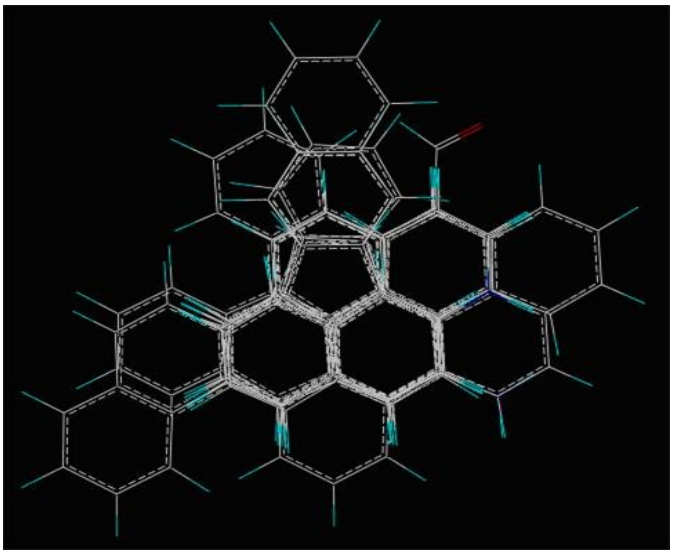


Figure 1. The PAHs in the training set overlay on the template molecule of pyrene.

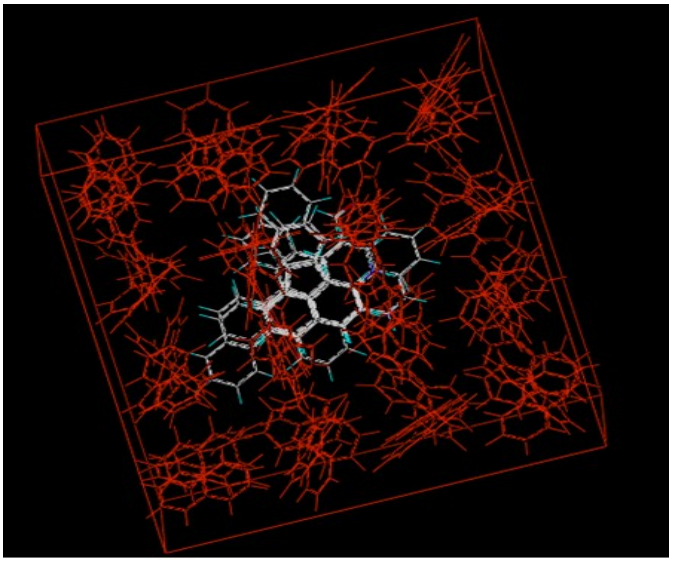
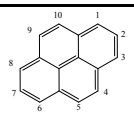
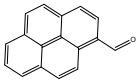
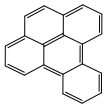
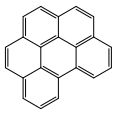
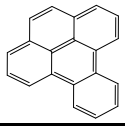
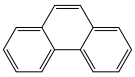
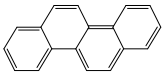
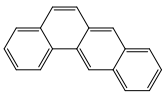
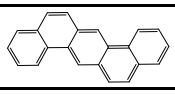
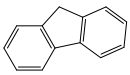
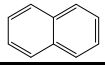
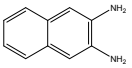
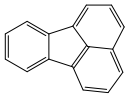
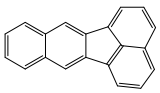
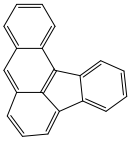
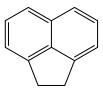
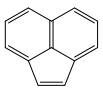


Figure 2. The PAHs in the training set were placed in a 3D cubic lattice.

Table 1. Experimental and predicted CR data of PAHs in the training and the test sets

No.	Structure	Compounds	CR (%)		MCR (%)
			Predicted	Experimental	
1		Pyrene	37.0	100	100
2		Pyrene-1-carboxaldehyde	19.3	25.1	28.58
3		Benzo[a]pyrene	1.6	3.2	3.99
4*		Benzoic[g,h,i]perylene	0.9	1.0	0.16
5		Benzo[e]pyrene	1.8	0.9	1.12
6		Phenanthrene	15.9	7.1	6.26
7*		Chrysene	1.1	1.2	1.75
8		Benzo[a]anthracene	0.6	0.2	0.23
9		Dibenz (a,h) anthracene	0.1	0.2	0.28
10*		Fluorene	1.1	1.5	0.44
11		Naphthalene	0.2	0.2	0.13
12		2,3-Diaminonaphthalene	0.2	0.2	0.16
13		Fluoranthene	0.2	0.2	0.20
14		Benzo[k]fluoranthene	0.1	0.2	0.25
15		Benzo[a]fluoranthene	0.2	0.2	0.25
16		Acenaphthene	2.5	2.3	1.75
17		Acenaphthylene	0.4	0.6	0.44

*Randomly selected test compounds

tional degrees of freedom, thus eliminating the need to select from numerous conformations for the alignment procedure. With the use of an atom-fit alignment scheme, the compounds were aligned to the template molecule of pyrene.

Following alignment, the PAHs and related compounds were placed in a 3D cubic lattice with 2-Å spacing. Steric (van der Waals) and electrostatic (Coulombic) field descriptors were calculated for each molecule at all lattice points using a probe represented by a sp^3-hybridized carbon atom with +1.0 charges.

We assumed that 0.2% was a low enough CR value to describe low levels of cross-reactions in this ELISA and considered the CR value "lower than 0.2%" to be " equal to 0.2%". We then included these compounds in the data set subjected to CoMFA modeling. The log MCR data of 14 compounds were correlated with the CoMFA-generated steric and electrostatic fields using the statistical method of partial least-squares regression.

Results and Discussion

Figure 1 shows the results of the 14 PAHs in the training set aligned to the template molecule of pyrene and Figure 2 shows the PAHs in the training set placed in the 3D cubic lattice. A final CoMFA model in the training set, that included all the experimentally tested compounds with defined stereochemistry, was then generated. A tabulation of the actual and model predicted CRs for the final models is shown in Table 1.

The result of the partial least-squares regression shows that the model requires four principal components to explain the variance of logMCR. The non-cross-validated square of the correlation coefficient (r^2) is 0.91. The CoMFA model obtained was internally validated by leave-one-out cross-validation, and the cross validated square of the correlation coefficient (q^2) was found to be 0.595. Since a QSAR model with a value of $q^2>0.5$ is normally considered to possess significant predictive ability, this CoMFA model can be said to show fairly good predictive ability. As illustrated in Figure 3, a significant linear correlation was obtained between the corresponding CoMFA-predicted and experimental logarithmic CR values (r=0.9075, n=14, SE=0.310657).

The basic principle of CoMFA is that if a group of similar compounds react on a receptor in the same way, their biological activities depend on the difference of the surrounding molecular field of each compound. This molecular field can reflect the characteristics of the nonbonding interactions between the molecules and the receptors. Because the antigen-antibody interaction is such an interaction, using CoMFA to predict CR can be more accurate and successful, even though it needs more complex modeling steps. CoMFA also predicts the effects of structure changes on the CR of the molecule.

Figure 4 depicts steric and electrostatic contour plots from the CoMFA analysis that show where changes in the steric and electrostatic fields are associated with CR. Greater CR values are correlated with more bulk near the green contours and with less bulk near the yellow contours (Figure 4a), whereas a more positive charge is correlated with the blue contours, and a more negative one with the red contours (Figure 4b).

As illustrated in Figure 4a, increasing the volume of position 9 of the pyrene ring would increase CR, and increasing the volume of position 6 and 10 would decrease CR. As illustrated in Figure 4b, a positive charge near the blue contour regions, at positions 3 to 6, or a negative charge near the red contour regions, at positions 1 and 2, would favor increased CR.

The contributions of the steric and electrostatic fields to CR, as indicated by the log MCR value, are 95 and 5%, respectively. This shows that, in this ELISA, steric interactions between PAHs and the antibody play a more important role than electrostatic interactions.

The pyrene derivatives (No. 2-5), have the most common structure with the pyrene reference compound, among the training set compounds (Table 1).

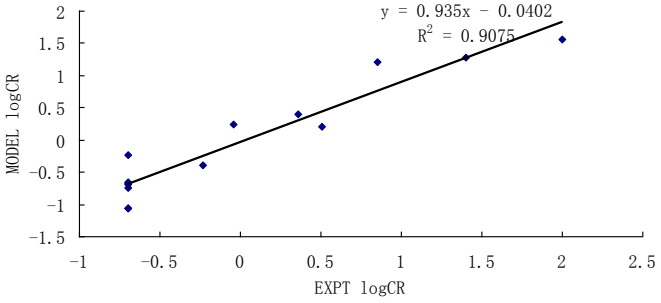


Figure 3. Plot of experimental versus predicted log CR values derived from the CoMFA model.

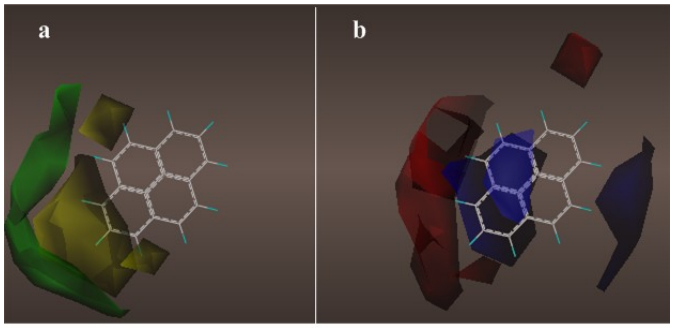
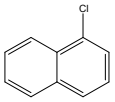
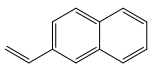
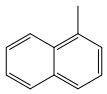
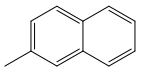
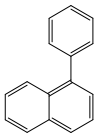
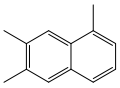
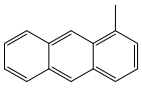
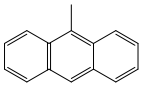
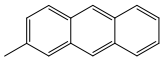
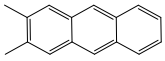
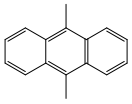
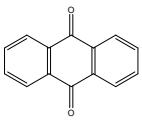
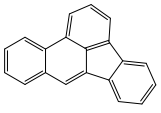
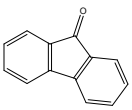
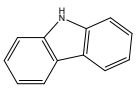
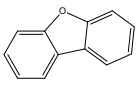
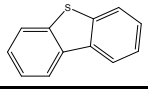


Figure 4. CoMFA contour plots of (a) steric field and (b) electrostatic field contributions of CR.

Table 2. The CRs predicted for other 17 molecules

Compound	Structure	CR (%) Predicted
1-Chloronaphthylene		0.07
2-Vinylnaphthalene		0.11
1-Methylnaphthalene		0.09
2-Methylnaphthalene		0.10
1-Phenylnaphthalene		0.22
2, 3, 5-Trimethylnaphthalene		0.06
1-Methylanthracene		0.10
9-Methylanthracene		0.08
2-Methylanthracene		0.14
2, 3-Dimethylanthracene		0.09
9, 10-Dimethylanthracene		0.06
Anthraquinone		0.08
Benzo [b]fluoranthene		0.12
9-Fluorenone		0.10
Carbazole		1.36
Dibenzofuran		0.15
Dibenzothiophene		0.09

As they have the negative charge close to the blue contour regions, at positions 3 to 6, their CRs are decreased. Phenanthrene and its derivatives (No. 6-9), have a less common structure with the reference compound. They have smaller CRs. Since compounds 8 and 9 have the negative charge near the blue contour regions, at positions 3 to 6, their CRs are further decreased. Compound 10 (fluorine) has a similar structure to phenanthrene. Its CR is therefore similar to the latter. In addition, naphthalene and its derivatives (No. 11-17) have a less common structure with the pyrene. As a result, they have smaller CRs.

The ability of the model to properly predict the CRs of the molecules in the test set served as a measure of its robustness. The residual values correlating the predicted CRs with the actual CRs of the three randomly selected test compounds (No. 4, 7, and 10 in Table 1) were -0.1, -0.1, and -0.4, respectively. These results show that the developed model has good prediction ability. The model was subsequently applied to predict the CRs of other 17 molecules (Table 2) without experimental activities.

Conclusion

PAHs have been designated as priority-pollutants and are regularly measured in polluted areas. In the environment, single PAHs will not be detected. Instead, various PAHs and other related compounds will be found together with the target compound. Immunoassays often aid the environmental analysis of PAHs. CR is an important and useful property of such assays, and directly affects their specificity and selectivity, together with the results of the determination. In this article, the QSAR technology named CoMFA was used to predict the CRs of a polyclonal antibody against pyrene. The QSAR model demonstrated satisfactory predictive ability and exhibited a strong correlation between experimental and predicted CR values. CoMFA is developed on the basis of small molecule-receptor interaction. It can provide an insight into the mechanisms of the antigen and antibody reactions in immunoassays. We believe that the established QSAR model could be employed for CR prediction and specificity evaluation for immunoassays, and it may also help in understanding the mechanisms of cross-reaction and in the performance enhanced antibodies.

Conflicts of Interest

The authors declare no conflicts of interest associated with this publication.

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