Finding of a Characteristic Reactive Region Common to Some Series of Chemical Carcinogens

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Quantum chemical calculations were carried out to explain how the electronic states of some series compounds vary with metabolic activation. Compounds studied included aromatic amines and amides, polycyclic hydrocarbons, and a few alkylating agents that do not require metabolic activation. The 1,2 and 4 positions forming the *trans*-butadiene frame of a molecule, henceforth referred to as "the *trans* 1,2,4 region", have seen to be important positions for the prediction of carcinogenic activity of these compounds. It is also evident that their electrophilic properties increase with metabolic activation. That is the sum of π-electron densities of the trans 1,2,4 region in the lowest unoccupied molecular orbital (LUMO) has been found to increase in the order of precarcinogens < proximate-ones < the carbocation ultimate-ones. This is consistent with the fact that chemical carcinogens become more strongly electrophilic with activating. This region not only provides a unified view of structurally diverse carcinogens, but also predicts uniformity in their reactive sites. Accordingly, we suggest that an understanding of the trans 1,2,4 region would be helpful in elucidating the mechanism of carcinogenesis.

1. Introduction

One of the major advances in recent cancer research has been the discovery of a great variety of chemical carcinogens. Included in this large group are polycyclic aromatic hydrocarbon (PAH) and related heterocyclics, biological alkylating agents, and aromatic amines and azocompounds. Numerous attempts have been made, both chemically and physically, to account for the differences in carcinogenic activity of various compounds and thereby to unfold the riddle of carcinogenesis due to those substances.[1-4] But no satisfactory explanation has ever been given for the exact nature of carcinogenic compounds. In 1939, Schmidt explained for the first time the carcinogenic activity of aromatic compounds from quantum mechanical view. He pointed out the possible importance of L-region in the tumor production by these compounds (Figure 1). After that Pullman emphasized the importance of K-region in the incidence of tumor by PAH.[6,7]

The K-region is an area which has the greatest double bond-like character. In many aromatic hydrocarbons there was a rough correlation between high reactivity in the K-region and carcinogenicity. In 1971, it was determined experimentally that any addition of cellular constituents to a hydrocarbon occurred in the K-region. This K-region theory was accepted as a powerful one in the explanation of carcinogenicity of PAH.[8]

In 1962, Fukui and co-workers applied frontier electron theory for the explanation of carcinogenic activity of non-substituted aromatic hydrocarbon.[9] They designated two carbon atoms corresponding to K-region as principal carcinogenophore (PC) and anthracene meso-type carbon in proximity to PC as the subsidiary carcinog-enophore (SC), and calculated the values of approximate superd elocalizability (Sr^s) which take into account only the distribution of the frontier level to superdelocalizability (Sr) at PC and SC. From the calculated values, they concluded that the existence of SC is a necessary condition, though in itself insufficient, for respective carcinogenicity. In other words, they did not take into account the explicit value of Sr^s at SC, but explained carcinogenic activity of chemical carcinogens with only the values of Sr^s at PC.

More recently, it has been proposed that the carcinogenicity of a PAH is due to metabolic activation of benzo-ring epoxide located near the bay region of the hydrocarbon.

Miller and co-workers introduced the concept that chemical carcinogens, which are not chemically highly reactive, exert their carcinogenic activity through metabolites which are sufficiently reactive to modify macromolecules such as DNA, RNA, and protein. Because the PAH's are relatively inert, a specific class of metabolite is presumed to be responsible for their carcionogenic activity.[10] Accordingly, many workers have concentrated attention on the identification of the reactive metabolites fromed from benzo (a) pyrene and related hydrocarbons.[11-13]

Jerina *et al.* have postulated, based on their quantum mechanical calculations and many other experimental results, that bay-region metabolic activation plays a more important role than the K-region one in carcinogenesis.[12-15]

The reactivity of all the above theories have their respective critical values corresponding to PAH series, aromatic amine series, azo-compound series and so on. Furthermore, the L-, K-, and bay-region theories are applicable only to PAH series. Accordingly, it is desirable to find a unified reactivity index and a new reactive region which can be applied to all series of compounds for a unified mechanism of chemical carcinogenesis.

In 1980, we found that the sum of LUMO electron densities of the trans 1,2,4 region is commonly above 0.5 for some series of compounds.[16]

Extending this, we examined the LUMO electron densities of the above trans-butadiene 1,2 and 4 positions to see if some

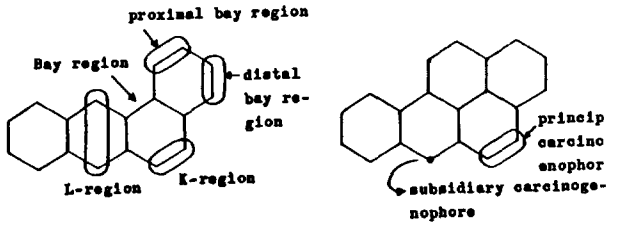

Figure 1. Schematic representation of K, L and Bay-region.

series of carcinogens also have any effect on the carcinogenic activity or on electrophilic nature with activation.

Specifically, our objectives were to determine (1) Is there any difference between the π-electronic states of those chemical carcinogens that require metabolic activation and that do not; (2) Are there the essential differences between the reactive regions of PAHs which have K-, L- and bay regions and the reactive regions of other carcinogens which have none? (3) Can the increase in the electrophilic states of the metabolites of carcinogenic parent compounds be explained on the basis of electronic states of the K-, L- and bay regions?

2. Calculation and Model

In general, chemical carcinogens may be divided into two broad classes, those which act directly and those that require metabolic activation. The former group includes nitrogen and sulfur mustards, ethylene imines, diepoxybutane, and many other compounds that have non-planar structure.

The second group, those requiring activation, compasses most of the environmentally important carcinogens, including PAH, aromatic amines, aminoazo dyes, and others. These have nearly planar skeletons; the metabolites of this latter group are also non-planar.

We calculated π-electron densities of some positions in the in pre-, proximate-, and ultimate carcinogens by the simple Hückel method for the planar compounds and the extended Hückel method or the CNDO/2 method for the non-planar ones. Adopted parameters were taken from original references.[17,18]

Model. According to one of Pullman's fundamental propositions, if a molecule contains both K-and L-region, a supplementary condition requires that L-region should be rather inactive. This implies that there exists the mutual dependence between L-and K-regions.

On the other hand, Fukui *et al.* mentioned in their introduction, the necessity of the existence of SC in addition to PC. The PC and SC correspond to the K-region of Pullman and the L-region in proximity of the K-region respectively. That is, they suggested a supplementary role of the L-region in addition to the K-region for the explanation of carcinogenic activity of PAH but did not introduce any explicit reactivity index in a molecule.

A portion of the L-through K-regions constitutes the *trans*-butadiene frame in PAH, whose 1,2 and 4 positions correspond to two atoms of the K-region and one atom of L-region respectively. The trans 1,2, and 4 positions could be extended to apply to other compounds as well (Figure 2).

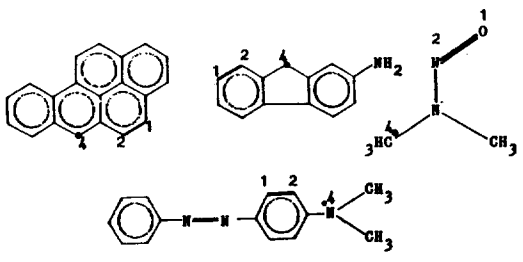

Figure 2. Some illustrations of 1,2 and 4 positions *trans*-butadiene frame in molecules.

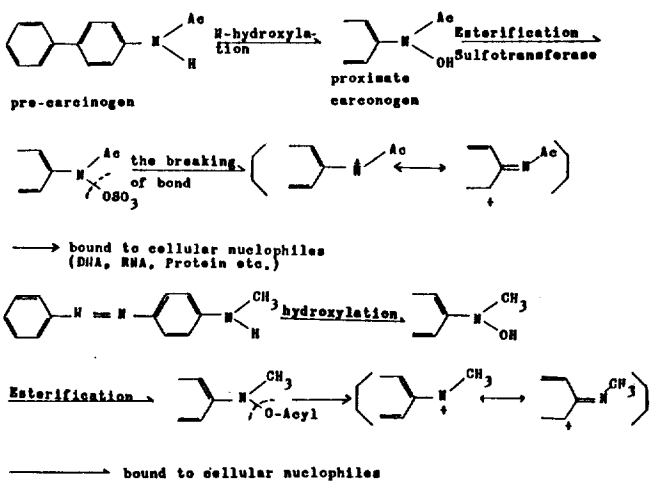

Figure 3. Proposed mechanism of carcinogenesis of N-2-fluorenyl acetamide and methylamino azo benzene.

Of the four compounds illustrated in Figure 3, only benzo (a) pyrene constitutes the exact *trans*-butadiene frame. Here we see that the 1-position is located trans to the 4-position. The L-and K-region theories fail to account for carcinogenic activity of alkylating agents, aromatic amines, and azo compounds, although they do account for the carcinogenic activity of the PAHs.

The sum of π-electron densities in the LUMO at the above 1,2 and 4 positions will be seen to be a measure of carcinogenicity of some series of compounds including metabolites, whose carcinogenicity will be explained without all most exception. (see next section.) Since electron density in the LUMO at the position in a molecule provide a measure of ability of accepting electrons from other molecule, it is natural that electron density in the LUMO can be adopted as a measure of carcinogenicity. Accordingly, the trans 1,2,4 region should be regarded as the reactive one common to some series of carcinogenic compounds.

3. Results and discussion

Aromatic Amines and Amides. Extensive studies on aromatic amines and amides show that they are enzymatically N-hydroxylated as the first step for carcinogenesis and the resultant N-hydroxy amines and amides are again esterified. The latter induce tumors via cationic species by the breaking of the bonds indicated Figure 3.[19-22]

The sum of π-electron densities of the trans 1,2,4 region in LUMO ($q_{1,2,4}^{LUMO}$) of the above mentioned compounds and related one in a similar fashion have been calculated and summarized in Table 1 and Table 2 for pre-carcinogens, proximate ones, and the putative ultimate ones. Table 1 and Table 2 show that $q_{1,2,4}^{LUMO}$ is increased in the order, pre- < proximate- < ultimate-carcinogens, through some exceptions which are non carcinogenic compounds and weak carcinogenic ones. This agrees with the fact that chemical carcinogens are converted into electrophilically stronger ones through metabolic activiation. So far, this generalization has failed not only to provide a unified view of structually diverse chemical carcinogens but also to predict uniformity in the regions on them susceptible to the attack of the cellular macromolecules. However, the sum of LUMO elec-

TABLE 1: $q^{LUMO}_{1,2,4}$ Values of Pre-Carcinogens(H), Proximate Ones(OH), and Ultimate Ones (Cation) for Various Aromatic Amines and Amides

Compound	Precarcinogen (H)	Proximate carcinogen (OH)	Ultimate carcinogen (cation)
2-Fluorenamine	0.5886	0.5912	0.9384
N-2-Fluorenylacetamide	0.5761	0.5744	1.1507
N-4-Fluorenyl acetamide	0.6532	0.6591	1.0783
4-(*o*-Tolyazo)-*o*-	0.2246	0.3277	0.7255
1-Naphthylamine	0.8127	0.7915	1.1316
2-Naphthylamine	0.9841	0.9992	0.7173
1-Anthramine	0.5727	0.5419	0.9946
2-Anthramine	0.7492	0.7601	0.5144
2-Aminobiphenyl	0.4727	0.4916	0.3318
4-Aminobiphenyl	0.2604	0.2768	0.6411
2′, 3-Dimethyl-4-aminobiphenyl	0.2650	0.2751	0.6404

TABLE 2: $q^{LUMO}_{1,2,4}$ Values of Pre-Carcinogens, Proximate ones, and Ultimate ones (Cation) for DAB and Substituents

Compound	Precarcinogen	Proximate carcinogen (OH)	Ultimate carcinogen
Dimethylaminoazobenzene (DAB)	0.5841	0.6473	0.7557
2′-Methyl DAB	0.5065	0.5508	0.8403
3′-Methyl DAB	0.5414	0.5844	0.8887
4′-Methyl DAB	0.5079	0.5521	0.8069
2-Methyl DAB	0.4753	0.5158	1.0492
2-Fluoro DAB	0.5761	0.5158	1.0534
2-Hydroxy DAB	0.4502	0.4906	1.1879
2′-Hydroxy DAB	0.4899	0.5353	0.6479
4′-Hydroxy DAB	0.4917	0.5368	0.6082
4-Amino DAB	0.5060	0.5517	0.0586
3′,4′-Dimethyl DAB	0.5070	0.5514	0.7628
3′-Methyl-4′-hydroxy DAB	0.507	0.4787	0.5240

tron densities of the trans 1,2,4 region makes it possible to provide the above unified view and prediction of uniformity in the sites because the trans 1,2,4 region is commonly applicable to all kinds of carcinogens treated here. In Table 1 and Table 2, it is particularly remarkable that $q^{LUMO}_{1,2,4}$ values approach unit for ultimate carcinogens.

Alkylating agents. The *trans* 1,2,4 regions of diazomethane, β-propiolactone, and dimethylnitrosoamine are depicted along with the LUMO electron densities in Figure 4.

As seen Figure 4, the $q^{LUMO}_{1,2,4}$ value is above unit in itself. Especially, β-propiolactone and diazomethane which do not require metabolic activation can be seen to be near two in $q^{LUMO}_{1,2,4}$ values. Alkylating agents can be thought to act as potent electrophilic reactants in themselves. Many authors have documented their reactivities non-enzymatically with nucleophilic regions in proteins and nucleic acid.[23,24]

It can be concluded from our results that the *trans* 1,2,4 region plays a pivotal role in the prediction of carcinogenicity of compounds and that the π-electron density in the LUMO can be

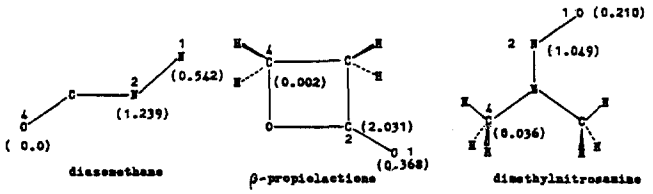

Figure 4. LUMO Electron Densities of Alkylating agents and Dimethylnitrosamine.

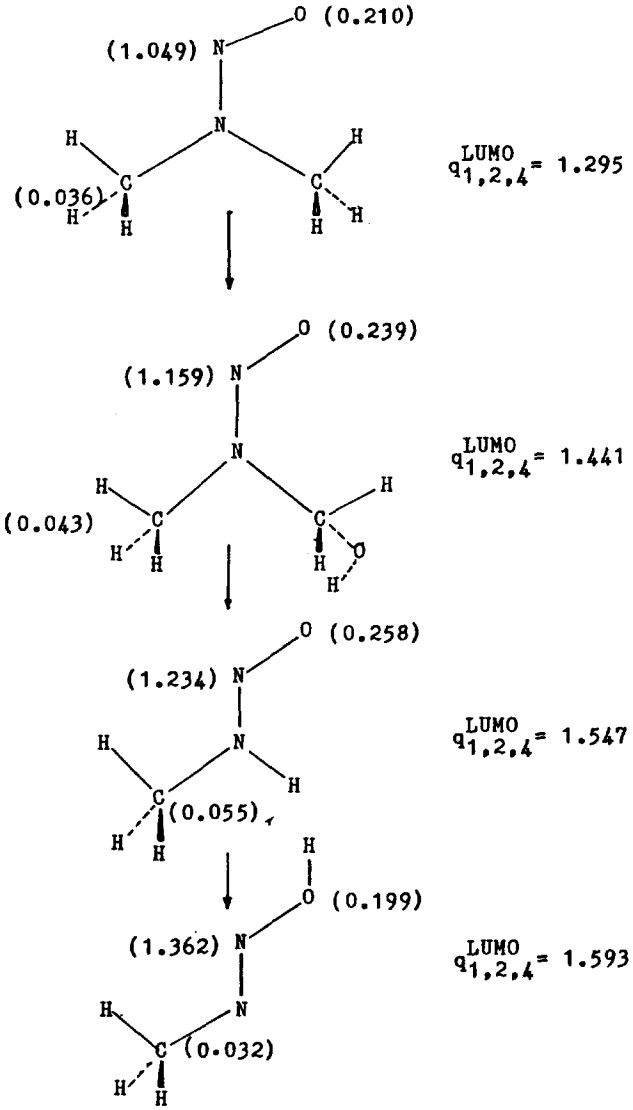

Figure 5. Metabolic pathway of dimethylnitrosoamine.

regarded as a measure of electrophilicity.

In Figure 5, the value of $q^{LUMO}_{1,2,4}$ can be seen to increase and approach two as dimethylnitrosoamine is activated. This also allows us to explain experimental facts that carcinogens become gradually electrophilically stronger through metabolic activation.

Metabolites of Benzo(a)pyrene. Many studies have been carried out on the metabolism of PAH, particularly of benzo (a)pyrene(BP). It has been shown through many experiments that K-region arene oxide of BP is not the ultimate carcinogenic metabolite but its 7,8-diol-9, 10-epoxide is.[26-27] A unique aspect of the BP 7,8-diol-9, 10-epoxide is that the epoxide forms part of a "bay region" of the hydrocarbon. Jerina has provided a quantum mechanical basis for the bay region theory as the

results of carbonium ion formation from diol epoxide of PAH's.[25]

We did a CNDO/2 calculation for the portion of the compound below the dashed line in Figure 6 for limitation of computer capacity. We have examined the value of $q_{1,2,4}^{LUMO}$ of parent BP, its 7,8-diol, and 7,8,9-triol-10-carbonium ion at the benzylic positions to see how they change with metabolic activation. The calculated values are summarized in Table 3.

From Table 3 and Figure 6, it can be seen that the value of $q_{1,2,4}^{LUMO}$ for the BP 7,8,9-triol-10-carbocation is higher than that for parent BP, but the value of $q_{1,2,4}^{LUMO}$ for the BP 7,8-diol is slightly lower than that for parent BP. It is therefore, thought that the central role of BP 7,8-diol is activated only 9,10 sites without influencing the 1,2,4 region. In Table 3, the value of $q_{1,2,4}^{LUMO}$ can be seen to increase again, when the BP 7,8-diol forms the putative ultimate carbocation followed BP 7,8-diol-9, 10-epoxide.

This agrees with other experimental evidence that metabolic activation causes these carcinogens to become more electrophilic, and hence more carcinogenic. It is, therefore, believed that the *trans* 1,2,4 region is of key importance in the prediction of carcinogenic activity. In our previous report, we showed that the sum of LUMO electron densities of any *trans* 1,2,4 region in a molecule is commonly above 0.5 in the critical value for some series of compounds.[16]

Now we report the discovery of a common reactive region in structually diverse chemical carcinogens which explains the carcinogenicity of these compounds, while taking into account the possibility of their increased electrophilicity through metabolic activation. Thus, we concluded that the carcinogenicity of these compound and their mechanism of action are directly related to the electrophilicity of the trans 1,2,4 region in these molecules.

4. Conclusions

It has been found that any trans 1,2,4 region in a molecule is a characteristic reactive region common to various kinds of chemical carcinogens and the sum of their LUMO electron densities is a reliable reactivity index to predict carcinogenicity of carcinogens.

The electron densities in LUMO of *trans* 1,2,4 region increased with activation in the order, pre- < proximate- < ultimate carcinogen (carbocation) whose approach unit for carcinogens requiring metabolic activation, while those of direct acting agents were greater than unit in itself. This is consistent with the facts that carcinogens become stronger electrophiles by activation, and that electron density in LUMO can be regarded as a measure of the electrophilic nature of a certain position in a molecule. Also, it has been found that there exists a correlation between K-region and bay region theories, since 1 and 2 positions of the *trans* 1,2,4 region correspond to the K-region. Accordingly, we believe that the *trans* 1,2,4 region could help elucidate mechanism of chemical carcinogenesis.

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TABLE 3: Values of $q_{1,2,4}^{LUMO}$ and $q_{1,2,4}^{LUMO}$ for Parent BP and Its Metabolites

		Parent BP	BP-7,8-diol	7,8,9-troil-10-cation
trans	1	0.310	0.243	0.331
	2	0.100	0.065	0.105
	4	0.346	0.243	0.368
$q_{1,2,4}$		0.756	0.555	0.803
	9	0.272	0.284	0.011
	10	0.050	0.088	0.007

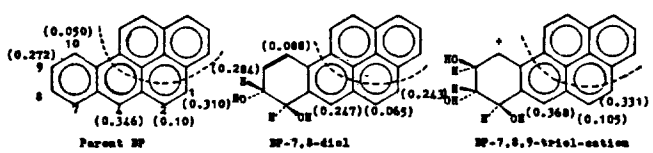

Figure 6. Graphic representations and electron densities in LUMO of *trans* 1,2,4 region.

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Theory of the Chemical Bond. Bond Ionicities and Bond Energies of Diatomic Molecules

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A revised simple method is proposed to calculated the ionicities and bond energies of diatomic molecules including hydrogen halides, interhalogen compounds, alkali hydrides, and alkali halides. The relative nuclear quadrupole coupling constants are evaluated to check the further validity of this method. It is shown that calculated values are consistent with available experimental values.

Introduction

Several attempts have been made to calculate the bond properties of diatomic molecules. Each method has been proposed to calculate a particular property of the bonds, which includes bond energy, dipole moment, or nuclear quadrupole coupling constant. Although most of the results are in good agreement with experiment, some systematic discrepancies are observed and a simple general treatment has not nearly developed.

The principle of electrongeativity equalization provided by Sanderson[1,2] has been used to calculate bond distances and energies of diatomic systems. However, as a result the charge distribution calculated from this principle is invariant with respect to bond distance, obviously an absurd result. Pauling[3] assigned electronegativity values to the elements in such a way that electronegativity difference could be used to predict the ionic character and the strength of a polar bond. But his method did not give a quantitatively good result. Rittner[4] proposed the polarizable spherical charge distribution method to describe the bond energies and dipole moments of alkali halide molecules, but nearly all the calculated values are slightly too low compared to available experimental data.

Klopman[5] has proposed that a molecule will reach its maximum stability when the potentials around each atom in each orbital are equalized; it is the total energy of the molecule which must be at a minimum for the equilibrium charge distribution. Ferreira[6] calculated the ionicities and bond energies of diatomic molecules by utilizing the idea of Klopman's, and partitioned the bond energy into a sum of a homopolar term and a heteropolar term. Later, Evans and Huheey[7] reexamined this method to describe the reactivity of hard and soft acid-base interactions in predominantly ionic compounds. However, these approaches do not give a quantitatively good result.

Also, the quantum mechanical techniques such as exchange perturbation theory[8] and implicit perturbation theory[9] have been used to analyze the dipole moments and bond energies of alkali halides. Recently, Parr *et al.*[10] have applied the Hohenberg-Kohn density functional theory[11] to describe the bond properties of diatomics, but many further studies are called for by this analysis.

In the present work, we have utilized the method of Evans and Huheey's[7] to a conceptually simple model of bonding and calculated the bond properties such as bond energies, dipole moments and nuclear quadrupole coupling constants of various diatomics. One of the primary purposes of the present work is to evaluate the effective net charges of atoms in diatomic molecules and to extend this method to the calculation of those in polyatomic molecules.

Methods

Total Bond Energy. The total bond energy E_{tot} is the sum of the covalent energy E_c, the electrostatic energy E_e, and the charge transfer energy E_x;[7]

$$E_{tot} = E_c + E_e + E_x \quad (1)$$

Covalent Energy. It is reasonable to assume the covalent bond formed between atoms of different sizes will overlap to an extent which is intermediate between those of the constituent atoms in homopolar bonds. This "overlap" factors (C) can be estimated from the square roots of the adjusted homopolar bond energies of the elements involved according to Pauling's assumption.[3] The contribution of nonbonding lone pairs to the destablization of the bonding, resulting from Pauli repulsion should be also considered in the calculation of the covalent bond energy. Thus, Evans and Huheey[7] have calculated the "repulsive" factors (R) from the difference between the extrapolated values and the experimental bond energies.

The contribution of covalent energy to a bond is inversely related to the ionicity of the bond. For a diatomic molecule AB