

Chlorine Isotope Effects on Chemical Reactions

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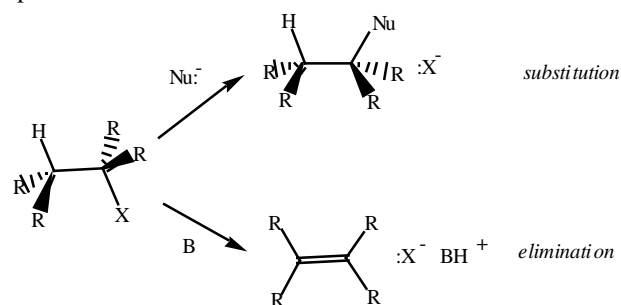
Abstract: A critical review of applications of chlorine kinetic isotope effects in studies of organic reactions mechanisms is presented. Reports from the last five years are considered. During this time chlorine kinetic isotope effects were applied mainly in studies of elimination reactions and nucleophilic substitution reactions. In several cases chemical models were also used as models of the intrinsic kinetic isotope effects in several enzymatic reactions. Both experimental and theoretical approaches have been used.

Chlorine kinetic isotope effects (CI-KIEs) played an important role some 30 years ago in learning the details of mechanisms of organic reactions and understanding the rules that govern isotope effects. The experimental side of these studies was possible due to developments in high-precision measurements of chlorine isotopic composition of methyl chloride. The theoretical aspect was connected with the BEBOVIB program [1], a tool that allowed systematic studies of relationships between geometries, bond orders and the resulting isotope effects. A large number of these studies [7] was carried out on solvolytic reactions [2].

Recent years brought CI-KIEs back to the spotlights. There are two reasons for this. Firstly, new experimental techniques have been developed for measurements of the chlorine isotopic composition that allow these measurements to be carried out directly on silver [3,4] or caesium chloride [5] without the need for tedious conversion to volatile methyl chloride. While they do not offer improvement in the precision, the simplicity of these procedures permits their application to biological systems, such as for example enzymatic reactions. Since chloroorganic compounds constitute the largest group of environmental pollutants their (bio)degradation becomes one of the major ecological problems [6]. Understanding the mechanisms of chemical and biochemical dehalogenation can be greatly enhanced by studies of chlorine isotope effects. Secondly, modern computational tools allow us to scrutinize origins and details of isotope effects in much more precise and unbiased way than the studies performed within the BEBOVIB framework. Enormous increase in the speed of computers together with the simplicity of use and availability of quantum-chemical software resulted in reinvestigations of the foundations of CI-KIEs.

Herein we are going to critically discuss reports on CI-KIEs on organic reactions published in recent years. Since we concentrate here on kinetic isotope effects, it is worth mentioning an elaborated paper on calculations of chlorine partition ratios that provides a key to the predictions of equilibrium isotope effects and may be very useful in studies of chlorine isotopic fractionation processes [7]. An attempt has been made to make this review as comprehensive as

possible which of course doesn't warrant that all literature has been covered. In a few cases the material related to biological processes will be presented although this area has been covered in recent reviews [8]. With quantum-mechanical calculations being now an accepted tool of organic chemistry we have chosen to organize the presentation following analysed mechanisms rather than differentiating experimental and theoretical approaches. Only two types of reaction mechanisms have been studied in the past 5 years by means of CI-KIEs, elimination and nucleophilic substitution (Scheme 1). Throughout this paper we will use the symbol B in case of elimination reactions and Nu:⁻ in case of substitution reactions for the attacking species.



Scheme 1.

1. CHLORINE ISOTOPE EFFECT ON THE ELIMINATION REACTIONS

Depending on the timing of bond making – bond breaking events elimination reactions can be formally classified into a variety of mechanisms, the most common being the E1cB mechanism proceeding through a carbanionic intermediate, the concerted one-step E2 elimination, and the E1 proceeding through a carbocationic intermediate, as shown in Fig. (1).

1.1 The E2 Pathway

Saunders [9] studied heavy atom isotope effects in a number of E2 reactions including chlorine isotope effects. From the point of view of this review the reactions of ethyl chloride with a number of different bases are of interest. Both *syn* and *anti* (Scheme 2) structures of the corresponding transition states were considered. This investigation does not aim at the comparison of experimental and calculated KIEs, but rather explores how calculated heavy-atom KIEs vary

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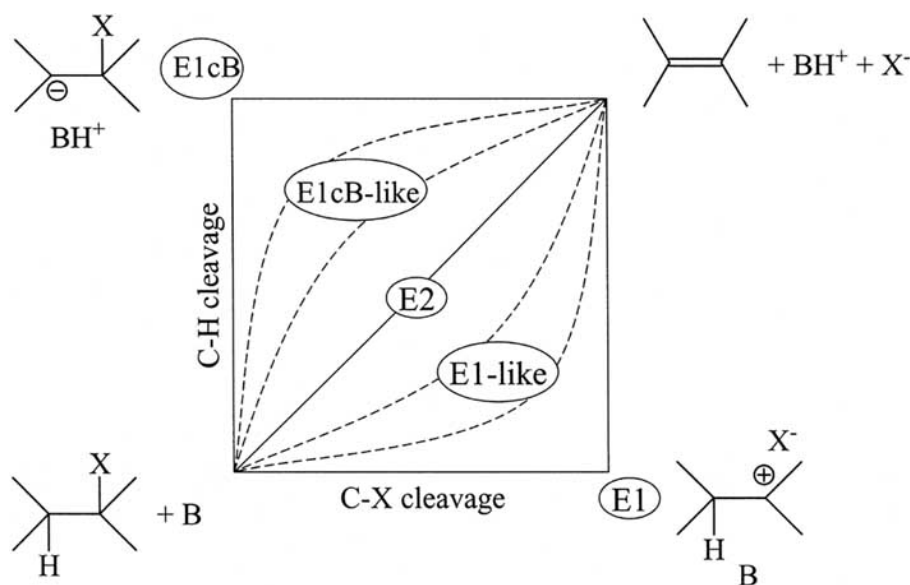
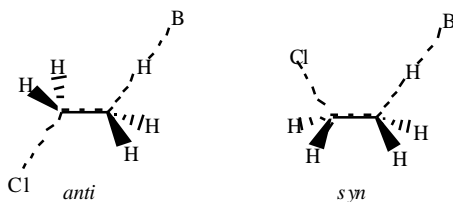


Fig. (1). Formal classification of elimination mechanisms.

with bond orders and charge distributions in transition state structures.



Scheme 2.

All calculations were performed at the MP2/6-31+G(d) level [10-12]. Harmonic frequencies calculated at 298 K

were scaled. Isotope effects without the tunnelling correction were calculated from vibrational frequencies, masses and moments of inertia from the Bigeleisen-Mayer equation [13]. The measures of transition state structure were bond orders and partial atomic charges, the latter calculated by the natural population analysis (NPA) method. Bond orders were calculated from bond distances by the Pauling equation (1) [14]:

$$n = \exp[(r^0 - r^n) / c] \quad (1)$$

where n is bond order, r^0 – length of the single bond, and r^n – length of the n -order bond. A value of 0.3 for the constant c has been used (see, however, discussion in subsection 2.1). Table 1 lists selected properties of the studied systems, for which Cl-KIEs were calculated.

Table 1. Transition state bond orders, changes of partial charges, energetic parameters, and isotope effects for the elimination of HCl from ethyl chloride.

Base	Stereochemistry	$n(\text{C-Cl})^a$	$Q(\text{Cl})^b$	H^c	H_R^d	k^{35}/k^{37}	(k^{12}/k^{13})	(k^{12}/k^{13})
OH ⁻	<i>anti</i>	0.562	-0.271	-48.6	-211.2	1.0046	1.0169	1.0140
OH ⁻	<i>syn</i>	0.702	-0.175	-15.1	-211.2	1.0034	1.0164	1.0218
LiOH	<i>anti</i>	0.838	-0.088	106.9	-105.0	1.0023	1.0110	1.0319
LiOH	<i>syn</i>	0.216	-0.513	28.5	-105.0	1.0044	1.0160	1.0133
NaOH	<i>anti</i>	0.789	-0.118	76.6	-111.7	1.0034	1.0151	1.0364
NaOH	<i>syn</i>	0.277	-0.475	24.6	-111.7	1.0046	1.0170	1.0151
Cl ⁻	<i>anti</i>	0.098	-0.703	91.3	1.5	1.0076	1.0419	1.0114
Cl ⁻	<i>syn</i>	0.143	-0.615	138.2	1.5	1.0069	1.0427	1.0160
F ⁻	<i>anti</i>	0.341	-0.431	-176.2	-294.3	1.0062	1.0487	1.0153
F ⁻	<i>syn</i>	0.431	-0.336	-125.6	-294.3	1.0062	1.0430	1.0326

a – bond order between α -carbon and chlorine atom,

b – charge difference on the chlorine atom between the transition state and the reactant,

c – enthalpy of activation,

d – enthalpy of reaction.

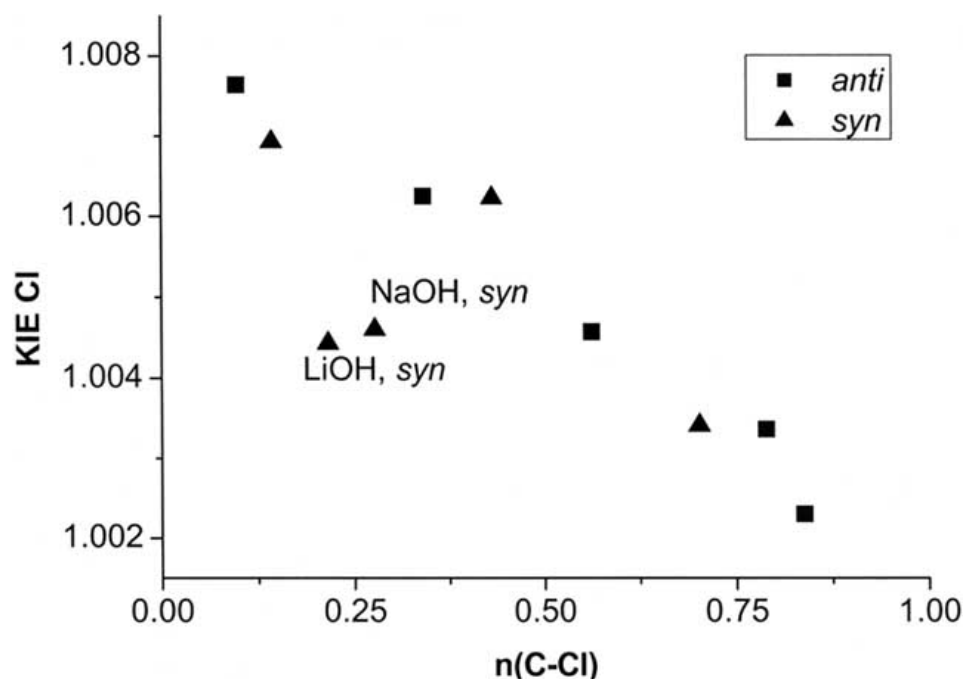


Fig. (2). Chlorine KIE vs. $n(\text{C-Cl})$ for ethyl chloride elimination.

In the hydroxide ion reactions, the *anti* transition state is coplanar, indicating developing *p*-orbital overlap between the two carbon atoms. In contrast, the *syn* transition state is not coplanar. Apparently eclipsing effects cost more than any energy gained from the overlap. The situation is reversed with a metal hydroxide as the base. The metal ion can coordinate with the leaving group as the hydroxide attacks the β -hydrogen, significantly stabilizing the *syn* transition state structure. The *anti* transition state does not experience such stabilization and is adversely affected by the weaker basicity of the hydroxide coordinated with the metal ion.

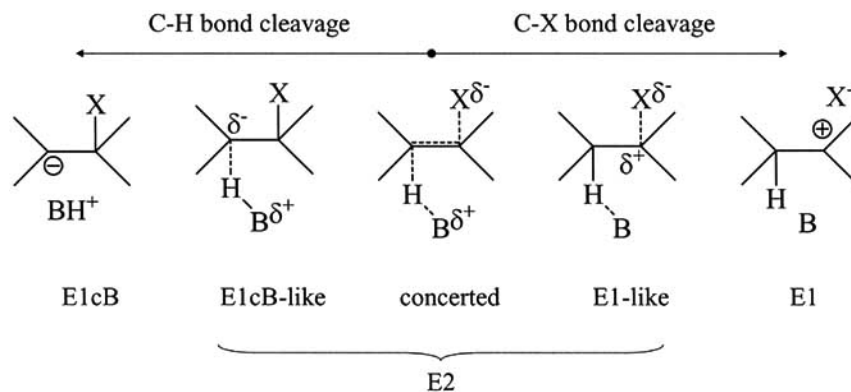
The leaving group KIE is larger for *anti* than for *syn* eliminations in all cases where the barrier to *syn* elimination is higher. The *syn* elimination barrier is lower for the reactions with LiOH and NaOH, and the associated leaving group KIEs are smaller for the *anti* elimination. The reason for the *syn* elimination being favoured energetically is that the metal ion can assist the departure of the leaving group as

the hydroxide attacks the β -hydrogen. The KIE at C-2 is usually larger for the *syn* than for the *anti* reactions, again except for the LiOH and NaOH reactions.

Fig. (2) shows the results for ethyl chloride reactions with different bases, in both *anti* and *syn* systems. The leaving group KIE increases as the charge on the leaving group increases and as $n(\text{C-Cl})$ decreases. Equally good dependence is obtained when Cl-KIE is plotted against $Q(\text{Cl})$. Only two points that correspond to the *syn* eliminations promoted by LiOH and NaOH do not fit the dependence.

1.2 Borderline Between E1cB and E2 Mechanism

While the bond-breaking bond-making events may occur in a synchronic fashion, they do not necessarily have to be concerted. These subtle differences in timing can lead to borderline mechanisms, as shown in Scheme 3 that are often difficult to differentiate experimentally.



Scheme 3.

The borderline between the stepwise E1cB mechanism and the one-step E2 mechanism is of particular interest and has for a long time been a controversy between organic chemists. Fig. (3) illustrates the energy profiles for such cases.

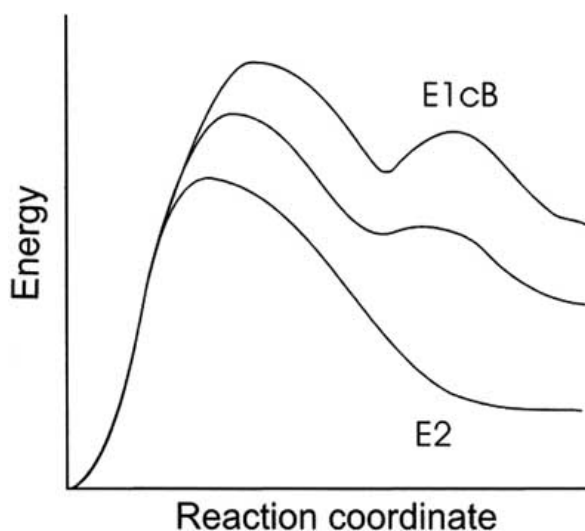
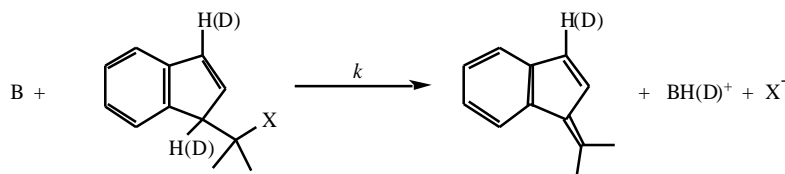


Fig. (3). Schematic diagrams showing the potential energy reaction coordinate diagrams for the E1cB-E2 merging at the mechanistic borderline. After ref [15].

Thibblin and co-workers [15] approached the problem of borderline mechanism using several kinetic tools including chlorine leaving group kinetic isotope effects in case of 1-(2-chloro-2-propyl)indene (**1-Cl**) and 1-(2-acylo-2-propyl)indene (**1-OAc**) reactions shown in Scheme 4.

In methanol a side 1,3-rearrangement is observed for some leaving groups and bases. For example, with AcO^- as the leaving group and TEA or quinuclidine as the base, the 1,3-rearrangement product is formed approximately as fast as the elimination product. With pyridine (the weaker base), the alkene is the minor product.

The elimination reaction of the deuterated acetate is not accompanied by any significant incorporation of protium in



Scheme 4.

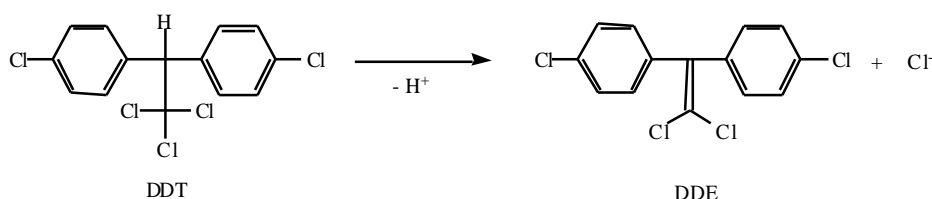
Table 2. Experimental kinetic isotope effects at 30°C and mechanistic assignments for the base-promoted reactions of **1-OAc** and **1-Cl**.

Substrate	1-OAc		1-Cl		
	k^H/k^D	Mechanism	k^H/k^D	k^{35}/k^{37}	Mechanism
pyridine	n.d. ^a	E1cB	5.6	n.d.	E1cB
TEA	7.3	E1cB	8.4	1.0101	E2
MeO ⁻	6.5	?	7.1	1.0086	E2

a) n.d. – not determined

the alkene product. The measured kinetic deuterium isotope effects for competitive reaction are all large and equal 7.3 (30 °C) with TEA, 7.1 (20 °C) with quinuclidine, and 6.5 (30 °C) with methoxide ion. With the more efficient leaving group Cl^- , rearrangement product is only observed with weak bases. The measured kinetic deuterium isotope effects with pyridine as the base at 30 °C also strongly indicate coupled reactions through a common hydrogen-bonded carboanion intermediate. The measured isotope effects for the elimination with the stronger bases (TEA and methoxide ion) at the same temperature are large and no rearrangement product is formed. These large kinetic deuterium isotope effects are consistent with the irreversible E1cB reaction mechanism as well as with the concerted E2 mechanism. The change in mechanism with change in leaving group, from irreversible E1cB for **1-OAc** to synchronous, concerted E2 for **1-Cl**, does not correspond to merging of mechanisms; the transition states close to the borderline are not similar in structure. The results do not give clear information about the mechanism of methoxide-promoted elimination of **1-OAc**; the reaction can be of either the E1cB or the E2 type. There is also a change in mechanism for the reaction of **1-Cl** with change of base, from irreversible E1cB for pyridine to synchronous, concerted E2 with TEA. This mechanistic change is also of non-merging type. The experimental results with pyridine require coupled reactions through a common carboanion intermediate, but this does not exclude the fact that a significant part of the alkene is formed by a parallel E2 pathway.

The chlorine KIEs for methoxide- and triethylamine(TEA)-promoted reactions are 1.0086 and 1.0101, respectively. This was taken as the evidence that the reaction with these two bases involves extensive, but not complete, cleavage of the C-Cl bond in the rate-limiting transition state and thus the E2 mechanism has been assigned to these reactions. Additionally, the leaving group chlorine isotope effect for the E2 reaction with TEA is somewhat larger than that of the reaction with MeO^- . This is in accordance with the variable transition state theory for a concerted E2 reaction with a diagonal transition state. The measured kinetic isotope effects and the conclusions about the mechanisms are summarized in Table 2.

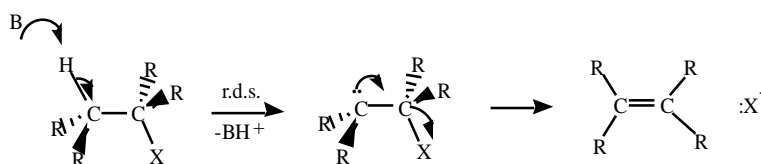


Scheme 5.

Thus the large Cl-KIEs as those measured for the reaction of 1-Cl with strong bases, combined with the large hydrogen KIEs, are strongly indicative of the concerted E2 mechanism.

Another example of the studies, where the mechanism of an elimination process is uncertain has been presented by Reddy *et al.* [16]. They have measured Cl-KIE for the abiotic decomposition of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT), the process illustrated by Scheme 5.

On the basis of the experimentally determined chlorine isotope effect of 1.009 the E1cB mechanism has been postulated. The measured value of Cl-KIE does not, however, support the E1cB mechanism, which follows the pathway illustrated in Scheme 6 with the rate determining (r.d.s.) removal of the hydrogen atom and subsequent dehalogenation step:



Scheme 6.

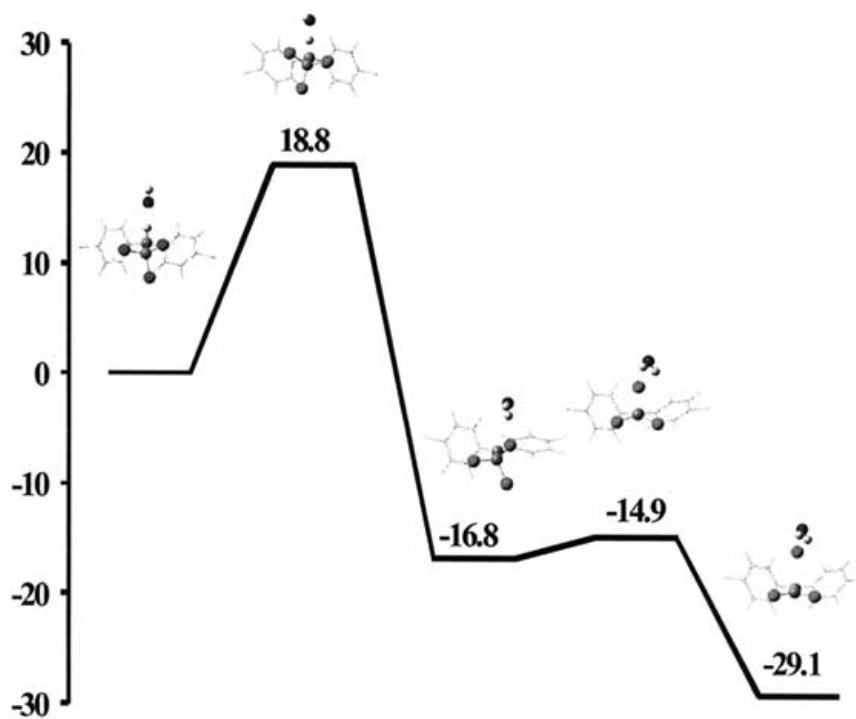
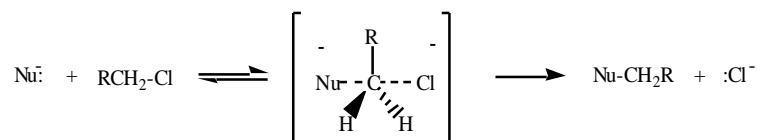


Fig. (4). AM1 energetic profile of DDT dehalogenation.

This scenario is also supported by our calculations performed at the AM1 semiempirical level [17] with the continuum solvent model SM5.2 [18]. The energetic profile for the HCl elimination from DDT by OH⁻ is shown in Fig. (4), the r.d.s. causes only minimal changes to the C-Cl bonds of CCl₃ group and therefore its sensitivity to chlorine isotopic composition should be negligible. Thus no or minor Cl-KIE should be expected for the E1cB mechanism. The value of 1.009 is relatively large (see Fig. (2)) and rather suggests intervention of the E2 mechanism.

2. CHLORINE ISOTOPE EFFECT ON THE NUCLEOPHILIC SUBSTITUTION REACTION

Second group of chemical reactions, which mechanisms have been studied recently using chlorine kinetic isotope effects are nucleophilic substitution reactions. Most studies



Scheme 7.

Table 3. C-Cl bond lengths in the transition state and chlorine leaving group KIEs on the S_N2 reaction between ethyl chloride and cyanide ion calculated using semiempirical methods and other quantum levels that yield values within one standard deviation from the experimental value (1.0070 ± 0.0003 at 30°C in DMSO).

Methods	r(C-Cl)	k ³⁵ /k ³⁷
Semiempirical		
AM1	2.113	1.0075
SAM1	2.173	1.0045
SAM1D	2.154	1.0058
PM3	2.100	1.0039
PM5	2.016	1.0042
PM6	1.975	1.0023
MNDO	1.988	1.0054
MNDO-d	1.989	1.0070
Hartree-Fock		
HF/MIDI!	2.357	1.0073
HF/6-31G(d)	2.305	1.0072
Post Hartree-Fock		
MP2/6-31+G(d,p)	2.250	1.0068
MC-QCISD/ML	2.208	1.0071
DFT		
B3LYP/6-31G(d)	2.134	1.0069
B1LYP/6-31G(d)	2.239	1.0069
mPW1PW91/6-31G(d)	2.198	1.0071
PCM-UAHF/mPW1PW91/6-31G(d)	2.227	1.0071
MPW1K/6-31+G(d,p)	2.240	1.0067
MPW1KK-SRP/6-31+ G(d,p)	2.232	1.0071
B1LYP/aug-cc-pVDZ	2.296	1.0070
B3LYP/aug-cc-pVDZ	2.291	1.0070
C-PCM-UAHF/B3LYP/aug-cc-pVDZ	2.314	1.0067
B3LYP/aug-cc-pVTZ	2.295	1.0069

addressed questions regarding details of the S_N2 mechanism (Scheme 7).

We start the discussion with two studies that aimed at the problem of the position of the transition state on the reaction coordinate and its relation to Cl-KIEs and have important bearings on the discussion.

2.1 Quantum Mechanical Models of S_N2 Reactions

Paneth and co-workers [19] examined the S_N2 reaction between tetrabutylammonium cyanide and ethyl chloride in DMSO at 30°C. They have determined experimentally the secondary ²D-deuterium, the secondary ³D-deuterium, the chlorine leaving group, the nucleophile nitrogen, the nucleophile ¹²C/¹³C carbon, and the ¹¹C/¹⁴C ¹-carbon kinetic isotope effects. All KIEs were then calculated using 39 popular theoretical levels in some cases including continuum solvent models. Results for Cl-KIEs are presented in Table 3 and illustrated graphically in Fig. (5).

Fig. (5) shows that post-Hartree-Fock and DFT levels give Cl-KIE values closest to this found experimentally. Interestingly, in general, inclusion of a solvent model in calculations does not improve the Cl-KIE (while it is mandatory for proper calculations of the energetics). For the purpose of this review we have calculated Cl-KIEs with a few semiempirical methods that were not included in previous studies. These calculations show poor performance of the semiempirical MNDO, PM_x (x = 3, 5, 6¹), and SAM1 Hamiltonians. They yield Cl-KIE substantially smaller than the experimental value (see Table 3). Clearly these methods are not capable of modelling chlorine KIEs. The AM1 calculated Cl-KIE, on the other hand, is in reasonable agreement with the experimental value. The comparison of SAM1 results with those obtained using SAM1D, as well as

¹ PM6 Hamiltonian is not yet finalized thus the result reported here is only preliminary.

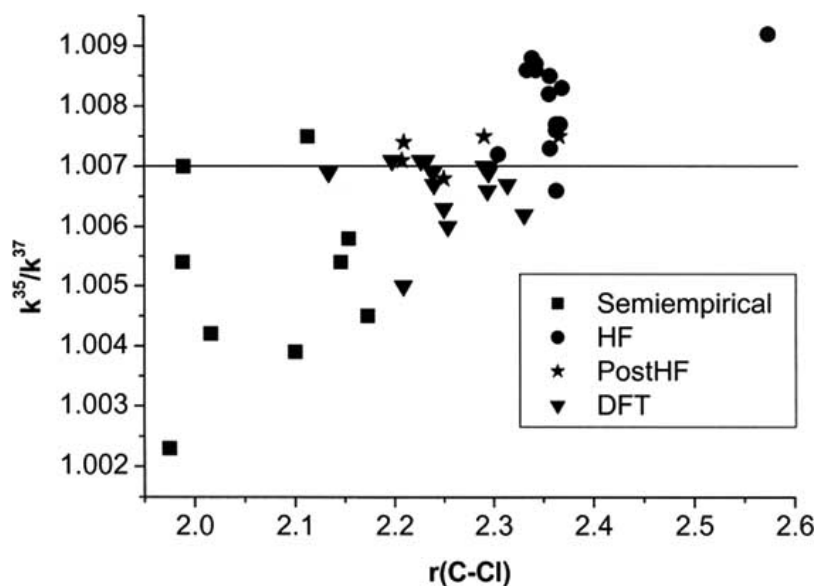


Fig. (5). Dependence of the chlorine leaving group KIEs and C-Cl bond length for ethyl chloride-cyanide ion S_N2 reaction; horizontal line represents the experimental value.

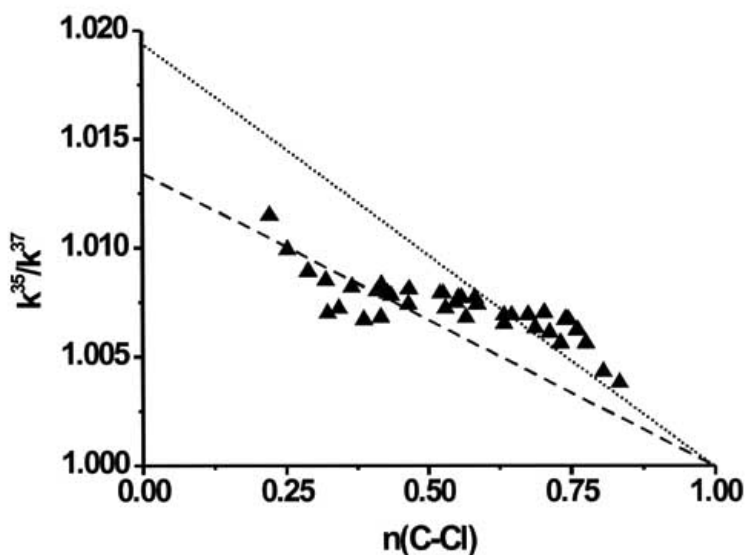


Fig. (6). The dependence of the Cl-KIE on the Wiberg C-Cl transition state bond order for the S_N2 reactions of methyl chloride with different nucleophiles. Lines represent estimates using the BEBOVIB approach (see text).

MNDO with MNDO-d results, indicates that inclusion of d-orbitals for chlorine substantially improves calculations of Cl-KIEs with semiempirical Hamiltonians. In particular the value obtained using MNDO-d matches exactly the experimental value.

The comparison of the experimental and theoretical results for all KIEs led the authors to the conclusion that B3LYP/aug-cc-pVDZ and B1LYP/aug-cc-pVDZ levels of theory are most successful in predicting kinetic isotope effects. These studies also point out substantial discrepancy between the qualitative interpretation of KIEs within the

BEBOVIB framework and the results of quantum calculations, both approaches leading to significantly different predictions regarding the localization of the transition state along the reaction coordinate. In a further attempt at solving this discrepancy the theoretical calculations of Cl-KIEs on the reaction between methyl chloride and a number of nucleophiles were carried out at the B1LYP/aug-cc-pVDZ level [20]. 25 nucleophiles of different properties (including radicals, neutrals and anions) were used in these calculations. Solvent continuum model COSMO [21] was included in several cases.

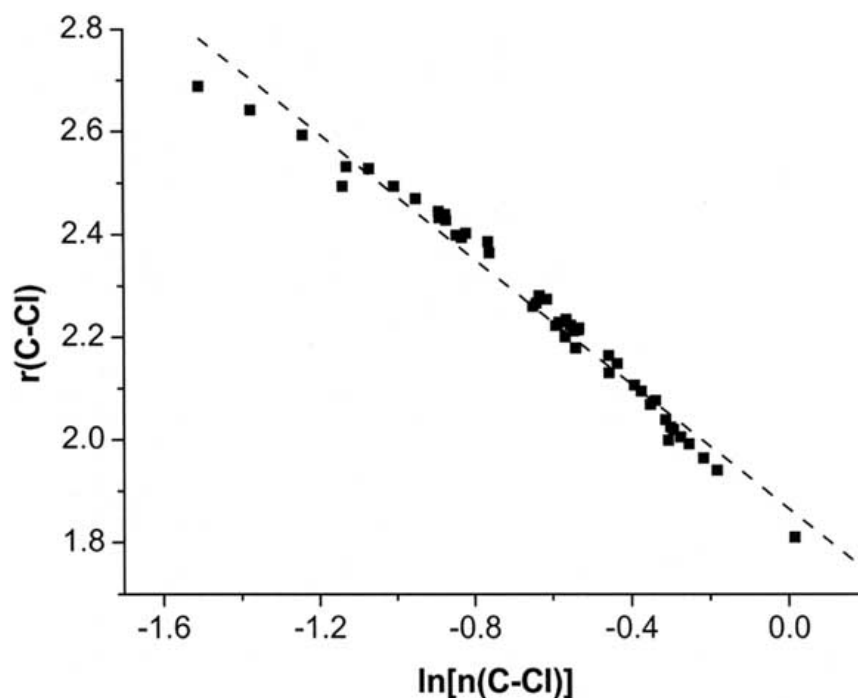


Fig. (7). C-Cl bond length in the transition state versus natural logarithm of the Wiberg bond order.

The dependence of the Cl-KIEs on the Wiberg C-Cl bond order in transition states is presented in Fig. (6). It illustrates the main result of this study, namely that Cl-KIEs are NOT related to the C-Cl bond order in the transition state! As can be seen Cl-KIEs increase only slightly with the lowering of the C-Cl bond order in the transition state in the wide range from 0.3 to 0.8, covering values for most typical nucleophiles. Only at extremes there is a sharp decrease (for large bond orders) or increase (for small bond orders) of Cl-KIEs. These results should, however, be treated with caution since they were obtained using continuum solvent models with artificially lowered apparent dielectric constant. Several other aspects of this study are worth mentioning:

- There is discrepancy in literature regarding the maximum value of the Cl-KIE for the S_N2 reactions. Several estimates of the maximum chlorine KIE have been made. Maccoll [22] and Buddenbaum and Shiner [23] have suggested the value of 1.014 at 25 °C assuming that the maximum value should approach the equilibrium isotope effect. Thus these maxima do not include the contribution from the tunneling or the imaginary frequency. The value of 1.019 has been reported by Paneth [24] on the basis of simple BEBOVIB calculations. In addition, Sims and co-workers [25] also suggested a maximum chlorine leaving group KIE in an S_N2 reaction would be greater than 1.02. We have addressed this problem quantum-mechanically; the maximum contribution from real modes corresponds to fully broken C-Cl bond so that only vibrational contribution from the reactant influences the isotope effect. This value is 1.0086 at B1LYP/aug-cc-pVDZ level in case of methyl chloride. The largest

contribution from the imaginary frequency obtained is 1.0081 and corresponds to a case in which an electron acts as the nucleophile. Together these values yield the Cl-KIE of 1.0167. The C-Cl bond order in the transition state of the reaction with the electron is 0.737. Thus one might expect that the maximum Cl-KIE should be even larger since the contribution from the imaginary frequency should increase with the decrease of this bond order reaching a maximum for small a C-Cl bond order.ⁱⁱ

- The linear dependence of Cl-KIE on the transition state C-Cl bond order, frequently used in the interpretation of isotope effects, is based on the Pauling's relationship (equation 1). The coefficient value of 0.3 has been used. Quantum calculations [26] suggested the value of 0.6 for calculating the bond orders in TSs. The same value comes from the calculations on the S_N2 substitution of chloride discussed herein, if the Pauling relationship is assumed to be valid. The choice of the value for the coefficient c changes the range of bond orders in the TSs. From the dependence of the calculated bond lengths on the natural logarithm of the corresponding Wiberg bond orders it is, however, apparent that the dependence is not linear (linear fit is shown as a dashed line in Fig. (7)). Thus equation 1 can be treated only as a first approximation. Second order polynomial fit gives much better results.

ⁱⁱ Note that as the bond order approaches extreme values (either zero or unity) the imaginary frequency decreases and thus its contribution to kinetic isotope effect should tend to unity.

- As already pointed out in literature [27] although not widely appreciated, tunnelling contribution can be significant for heavy atom KIEs. In the case of the reaction between methyl chloride with various nucleophiles Cl-KIE increases with lowering mass of the nucleophile reaching largest values (accounting for over 10% of the observed chlorine leaving group KIE) of 1.0009, and 1.0026 for BH_4^- (hydride being the effective nucleophile), and electron, respectively. One might expect even larger contribution from tunnelling since the Wigner correction underestimates tunnelling under the potential barrier [28] and does not include other tunnelling pathways [29].
- The influence of anharmonicity on KIEs has not been well documented thus far. It was evaluated for the reaction of ammonia with methyl chloride. The contribution from the imaginary frequency that includes anharmonicity has been found to be only slightly smaller than that obtained within the harmonic approximation (1.0010 versus 1.0012, respectively). Anharmonicity in the transition state causes the chlorine KIE to be smaller by the factor of 0.0017. However, this is mostly compensated by the similar effect in the reactant resulting in the overall lowering of the Cl-KIE by 0.0005.

2.2 Influence of Substituent and Solvent Model on the Transition State Structure

Westaway's group [30] examined incoming nucleophile KIEs for the $\text{S}_{\text{N}}2$ reactions between sodium borohydride and several *para*-substituted benzyl chlorides in DMSO at 30°C ($\text{Nu}^- = \text{BH}_4^-$). Values of chlorine KIEs are collected in Table 4.

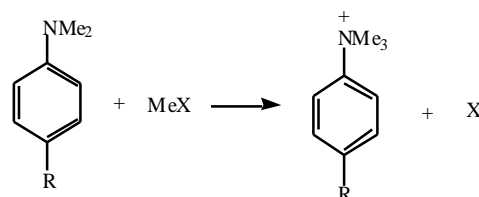
Table 4. Chlorine leaving group KIEs for $\text{S}_{\text{N}}2$ reactions between sodium borohydride and *para*-substituted benzyl chlorides in DMSO at 30°C.

Substituent	k^{35}/k^{37}
CH_3	1.0076 ± 0.0004
Cl	1.0078 ± 0.0006
NO_2	1.0036 ± 0.0004
H	1.0074 ± 0.0003

With the exception of the *p*-nitrobenzyl chloride, all the chlorine KIEs are large. This was taken as the evidence of significant elongation of the C-Cl bond in the transition state. However, as indicated in the previous chapter, this conclusion is not supported by the results of calculations since Cl-KIEs of this magnitude are obtained for the broad range of C-Cl bond lengths. Even less supported is further discussion on bond lengths differences between *p*-methyl, *p*-chloro and benzyl chlorides. Apart from the above-mentioned results of calculations, all these results are within the experimental error and therefore they are statistically indistinguishable. Significant decrease in Cl-KIE observed for the *p*-nitro derivative is consistent with suggested earlier, on the basis of analogous reactions, asymmetry of the transition state, with a short H-C bond and a long C-Cl bond.

The influence of the substituents has also been studied for another type of $\text{S}_{\text{N}}2$ substitution, namely quarternization of amines. While in the previously discussed reaction the nucleophile is negatively charged, in case of quarternization neutral reactants yield ionic products. For several reasons this, so called, Menshutkin reaction is one of the best targets of such studies. Firstly, its mechanism is well established, a condition necessary for a good model. Secondly, a wealth of data on solvents effects, substituent effects, and isotope effects for many kinds of these reactions is readily available in literature.

Paneth and co-workers [31] analysed the Menshutkin reaction between methyl halides and *N,N*-dimethyl *para*-substituted aromatic amines (Scheme 8) based on nitrogen, deuterium, halogen and carbon kinetic isotope effects (including chlorine KIE) using quantum mechanical methods.



Scheme 8.

The reaction is a single step $\text{S}_{\text{N}}2$ reaction, first order in each reactant. Geometry optimisations, frequency calculations for reactants and transition states were performed at the HF/6-31G(d) level of theory augmented by the C-PCM continuum solvent model for several solvents. Results of calculations of Cl-KIEs are presented in Table 5.

Despite good correlations between Gibbs free energy of activations and geometrical parameters (e.g., C-N bond elongation and C-Cl bond shortening) of the studied reactions with the ρ parameter for the *para*-substituent, there is no such correlation with calculated Cl-KIEs. Furthermore, changes are small, within typical experimental errors. Thus, contrary to previous suggestions, these results indicate that Cl-KIEs on Menshutkin reactions cannot be used to predict the position of the transition state on the reaction pathway.

The lower part of Table 5 illustrates the influence of the solvent on the Cl-KIE. As can be seen chlorine KIE is increasing with the increase of solvent polarity. However, this trend is also well within the typical experimental error and thus not detectable experimentally.

2.3 Influence of Inert Salts on the Structure of the Transition State

Westaway and co-workers [32] studied the dependence of kinetic isotope effects on the $\text{S}_{\text{N}}2$ reaction between *n*-butyl chloride and thiophenoxide ion in methanol and DMSO on the transition state structure with and without inert salts. The results of these studies are summarized in Table 6.

While the authors interpret minor changes in chlorine KIEs in the reactions with and without the added salt, yet again these are identical considering the experimental errors. The chlorine KIEs indicates that the C-Cl bond is practically the same or only very slightly longer when

Table 5. Chlorine kinetic isotope effects for the reaction between methyl chloride (X=Cl) and *N,N*-dimethylaniline calculated at 300K.

	R	p	G (kcal/mol)	r(N-C) (Å)	r(C-Cl) (Å)	k ³⁵ /k ³⁷
Substituent	2,6-diCH ₃	-	49.7	2.168	2.382	1.0086
	4-NO ₂	0.78	40.1	2.148	2.335	1.0082
	4-CN	0.66	37.4	2.155	2.334	1.0084
	4-F	0.06	33.2	2.174	2.310	1.0084
	H	0	32.9	2.179	2.307	1.0084
	4-CH ₃	-0.17	32.5	2.183	2.293	1.0081
	4-OH	-0.37	32.4	2.186	2.298	1.0084
Solvent ^a	cyclohexane	0	41.6	2.056	2.371	1.0082
	THF	0	34.1	2.153	2.320	1.0083
	DMSO	0	32.0	2.184	2.304	1.0084
	ethanol	0	31.7	2.179	2.307	1.0084
	H ₂ O	0	30.8	2.189	2.303	1.0085

^a unsubstituted *N,N*-dimethylaniline (R=H)

Table 6. Effect of ionic strength on the secondary α -deuterium and chlorine leaving group KIEs for the S_N2 reaction between *n*-butyl chloride and thiophenoxide ion.

Solvent	Ionic strength NaNO ₃ [M]	k ³⁵ /k ³⁷	(k ^H /k ^D)
DMSO	0	1.0078	1.103
	1.05	1.0078	1.065
Methanol	0	1.0083	1.057
	0.21	1.0084	0.954

sodium nitrate is present in both DMSO and methanol. But it is rather deuterium KIE that allows discussion of the position of the transition state along the reaction coordinate. The fact that secondary α -deuterium isotope effect is smaller for both solvents when the inert salt is present, suggests that the S_N2 transition state is tighter and more product-like, with a shorter S-C and slightly longer C-Cl bond. The salt effect on the reaction in methanol where the reacting nucleophile is the solvent-separated ion-pair complex is much larger than the salt effect on the reaction in DMSO where the reacting nucleophile is a free ion. This larger change in transition state structure upon addition of the inert salt found for methanol is consistent with the solvation rule for S_N2 reactions. The larger change in the S-C bond is predicted by the bond strength hypothesis. The rationale for the changes found in the transition state structure when the inert salt is present is proposed for both reactions; with the nucleophile being the free-ion and the ion-pair. The S_N2 transition state for the free-ion nucleophile (Fig. 8 top) allows solvation at both negatively charged species, the nucleophile and the leaving group. As the result, the relative nucleophilicity of both moieties that carry fractional negative charge does not change significantly upon salt addition. In the reactions involving ion-pair complex (Fig. 8 bottom) where the nucleophile is a tight solvent-separated ion pair, sodium ion, which has a full positive charge is mostly solvated and prevents solvation of the partially charged sulphur anion.

The change of the solvent from DMSO to methanol raises chlorine kinetic isotope effect by about 0.0005.

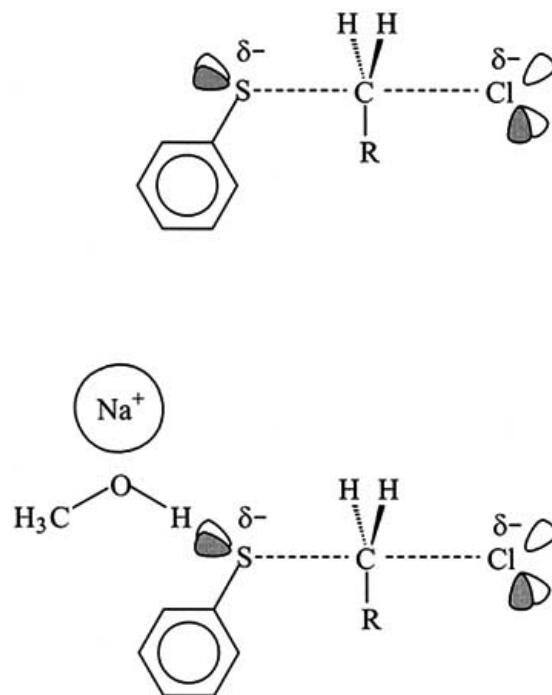
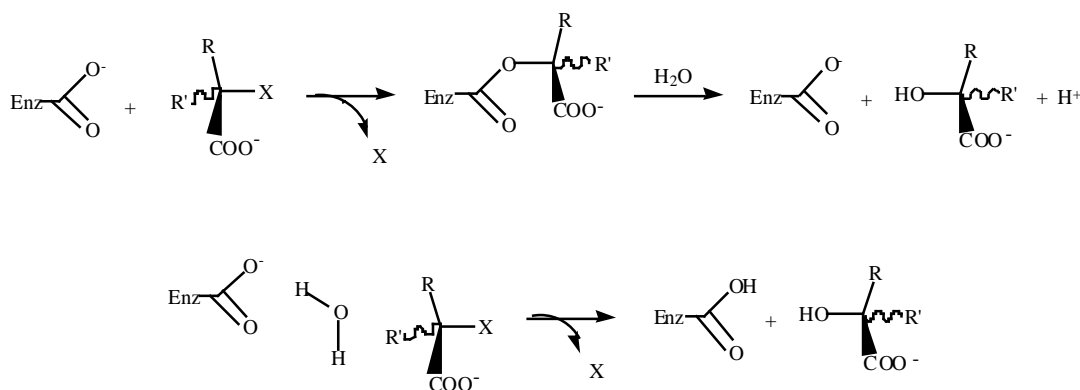


Fig. (8). Interactions between transition states and solvents. After ref. [32].

3. CHLORINE ISOTOPE EFFECTS IN CHEMICAL MODELS OF ENZYMATIC PROCESSES

As pointed out in the introduction, chlorine kinetic isotope effects and chlorine isotopic fractionation have been applied recently to systems of biological interest. Experimental values of Cl-KIEs for enzymatic reactions can be lower than the isotope effects of the chemical step in



Scheme 9.

active site (so called intrinsic isotope effects) because of the complexity of the mechanism. Therefore, theoretical calculations of the intrinsic isotope effects and experimental measurements at various reaction conditions, mutated enzymes, “slow” reactants, or on spontaneous reactions are frequently used to determine the size of the intrinsic isotope effects. For the completeness of this review we briefly present below such cases. For the dehalogenation processes that constitute the majority of these studies two mechanisms have been identified. In the first (upper pathway in Scheme 9) there are two sequential nucleophilic substitutions, first of the carboxyl moiety of the enzyme on the reactant leading to covalent intermediate complex that is subsequently hydrolyzed by an enzyme-activated water molecule. In the second mechanism enzyme-activated water molecule acts directly as a nucleophile on the reactant (lower pathway in Scheme 9).

3.1 Models of the Reaction Catalysed By Fluoroacetate Dehalogenase

Fluoroacetate dehalogenase catalyses dehalogenation of haloacetate ions. With chloroacetate as the reactant Lewandowicz *et al.* [33] have found chlorine KIE to be equal to 1.0082 ± 0.0005 . Since the crystal structure of this enzyme is not known it was not possible to model the intrinsic isotope effect. In order to learn if the measured Cl-KIE corresponds to the intrinsic value or if it is diminished due to other steps being partly rate-determining they have measured Cl-KIE for the spontaneous S_N2 chlorine displacement in chloroacetate by hydroxyl at pH=11 and 34 °C. Experimental Cl-KIE was found to be equal to 1.0079 ± 0.0004 . This reaction was also modelled theoretically at the AM1 semiempirical level, which was found to be reasonably good in predicting chlorine kinetic isotopic effects (see Table 3). Fig. (9) illustrates the transition state calculated for this reaction.

The calculated value of 1.0084 is in excellent agreement with the experimental Cl-KIE. Together both these results suggest that Cl-KIE observed for the enzymatic reaction is equal to the intrinsic chlorine kinetic isotope effect and therefore the dehalogenation step is solely rate determining.

3.2 Models of the Reaction Catalysed by Dh1A Haloalkane Dehalogenase

Lewandowicz *et al.* [34] determined experimental chlorine kinetic isotope effects on the dehalogenation

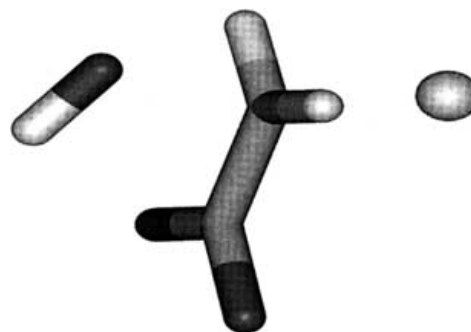


Fig. (9). Transition state model for the spontaneous dehalogenation of chloroacetate.

catalyzed by haloalkane dehalogenase Dh1A. These isotope effects are 1.0045 ± 0.0004 for physiological substrate, 1,2-dichloroethane, and 1.0066 ± 0.0004 for “slow” substrate, 1-chlorobutane. In order to examine intrinsic isotope effects several models of the active site were constructed based on the crystal structure of this enzyme. Calculations were carried out using ONIOM approach [35] with DFT levels for the higher layer and semiempirical Hamiltonians for the lower layer. Justification of the truncation of the model was achieved by varying the size of the higher layer as illustrated by Fig. (10).

Results of these calculations are summarized in Table 7. As can be seen neither the size of the higher layer in the model, neither the theory level, nor the reactant used in calculations influences significantly the calculated value of the intrinsic Cl-KIE, which is about 1.006 – 1.007. This supports the conclusion that Cl-KIE measured for the “slow” reactant, n-butyl chloride, corresponds directly to the intrinsic KIE. The first line in Table 7 reports results obtained for the gas-phase reaction between n-butyl chloride or dichloroethane and methoxy anion in methanol. The Cl-KIEs are larger than those obtained for the previous models indicating the importance of active site residues in modelling of the intrinsic KIEs for enzymatic processes. Recently, Gao and Devi-Kesavan [36] calculated the same chlorine KIE using the whole solvated enzyme in the MM part of the QM/MM model. They have obtained the value of 1.0031, much smaller than the observed Cl-KIE. This result is not

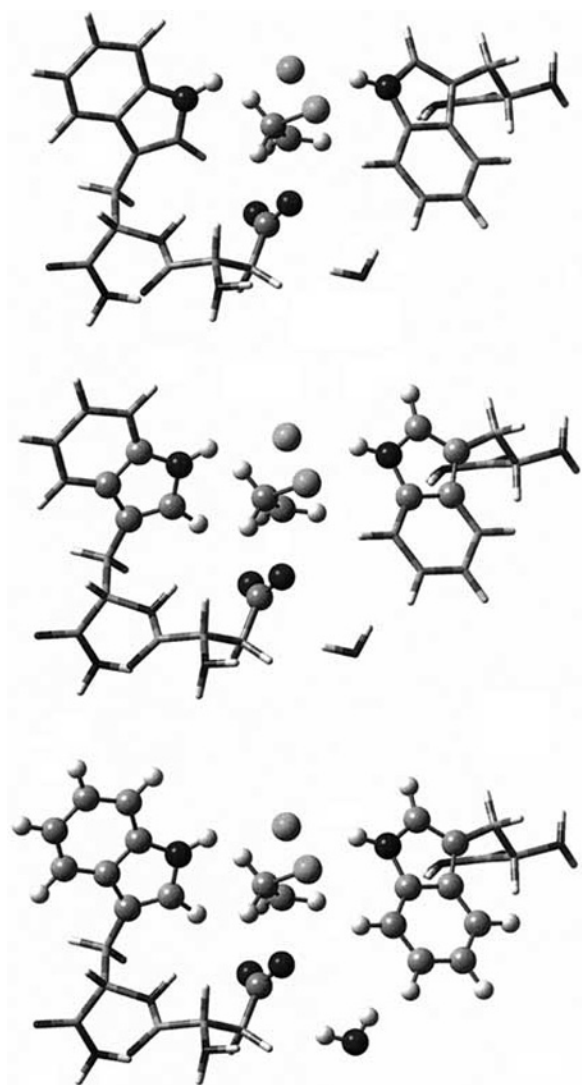


Fig. (10). Models of the dehalogenation reaction in the active site of haloalkane dehalogenase. Top to bottom: “NH₃”, “pyrrole”, and “indole” models.

surprising since they have used PM3 Hamiltonian for the QM part of the model (compare Table 3).

3.3 Models of the Reactions Catalysed by DL-2 Haloacid Dehalogenase

Another example of using chemical models for the interpretation of mechanisms of enzymatic reactions that involves modelling of Cl-KIEs is dehalogenation catalysed by DL-2-haloacid dehalogenase. This enzyme dehalogenates both D- and L-haloalkanoic acids but the leaving group chlorine KIE is different for both enantiomers. For S(-)-enantiomer it was found equal to 1.0105 ± 0.0001 while it is 1.0090 ± 0.0005 for R(+)-enantiomer. Both these KIEs are large and the difference between these values is significant. Both reactants bind to the same active site of DL-haloacid dehalogenase. While the difference may arise from different kinetic patterns, with the intrinsic value being the same for both enantiomers, results of modelling support differences in the intrinsic Cl-KIE values. Crystal structure of this enzyme is not yet available but it is known that three amino acid (Thr65, Glu69 and Asp194) are essential for the dehalogenation. On this basis a theoretical model was built and Cl-KIEs calculated using the AM1 Hamiltonian with SM5.4/A solvent model. Three moieties were used to mimic the active site of the enzyme. In the resulting model Asp residue acts as proton acceptor and activator of water molecule that participates in the S_N2 replacement of chlorine atom according to the lower pathway presented in Scheme 10. Two guanidine groups complete the active site model. One is positioned over the forming chloride ion and the other forms hydrogen bonds with carboxyl group of the R(+)-enantiomer. This orientation permits hydrogen bonding between chlorine atom of the transition state and a residue of the active site. The contribution of this hydrogen bond to chlorine causes that Cl-KIE is smaller than in its absence. When the S-stereoisomer binds to this active site, the guanidine moiety faces methyl group rather than carboxyl group of the transition state as illustrated by the structure on the right hand side in Fig. (11). This orientation shifts chlorine atom away from guanidine group and outside hydrogen bonding range. As the result the Cl-KIE for this enantiomer should be larger. In fact theoretical calculations yield chlorine kinetic isotope effects of 1.0085 and 1.0101, for R(+)- and S(-)-enantiomer, respectively, in excellent agreement with experimental values.

Table 7. Theoretical chlorine KIEs on the dehalogenation step for different models of haloalkane dehalogenase reaction.

Methods	Tryptophan models	Reactant	
		ⁿ BuCl	(CH ₂ Cl) ₂
B1LYP/6-31G(d)	-	1.0074	1.0080
B1LYP/6-31G(d):AM1	NH ₃		1.0070
B1LYP/6-31G(d):PM3	NH ₃	1.0063	1.0065
	pyrrole ring	1.0061	1.0063
	indole ring	1.0065	
B1LYP/6-31+G(d):PM3	NH ₃		1.0068

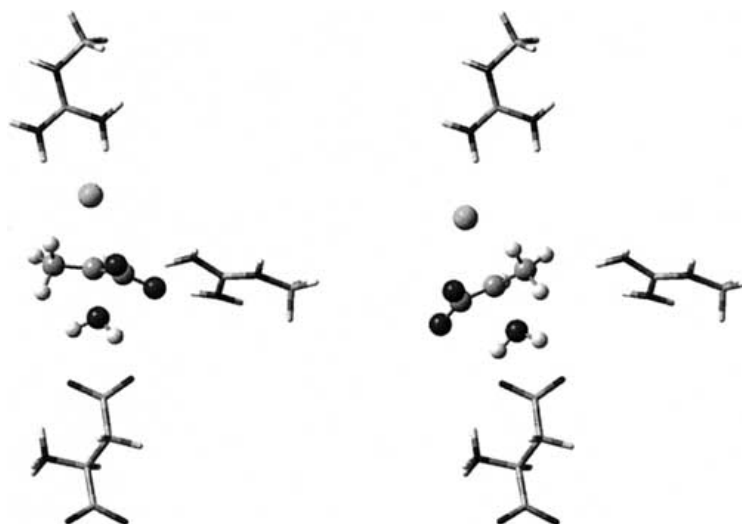


Fig. (11). Models of the DL-2-haloacid dehalogenase active site with bounded R(+)-enantiomer (structure on the left) and S(-)-enantiomer (structure on the right).

3.4 Models of the Reactions Catalysed by Fe(III)-Heme-Chloroperoxydase

While a majority of biological system studied is those in which chloride atom is being displaced, Reddy and co-workers [37] used chlorine KIEs for the interpretation of the enzymatic chlorination processes. They have studied chlorination of 1,3,5-trimethoxybenzene and 3,5-dimethylphenol under both biotic and abiotic conditions. The chemical reactions carried out using sodium hypochlorite yielded Cl-KIEs of 1.0037 and 1.0039 for the above reactants, respectively. It is interesting to note that the corresponding Cl-KIEs for the enzyme-catalysed reactions were much larger; 1.012 and 1.011. In both cases, enzyme-catalysed and non-enzymatic, more than one chlorine atom is being incorporated into the product. Thus the experimental values correspond to multiple KIEs and the chlorine isotope effect on a single step is most likely much smaller.

Usually one expects chemical reaction to mimic the intrinsic kinetic isotope effect assuming that the experimentally determined value is smaller than the intrinsic KIE because of the reaction complexity and participation of the isotopically insensitive steps in the overall rate. Cl-KIEs for the enzymatic process catalysed by Fe(III)-heme-chloroperoxydase are much larger than those for the corresponding abiotic reactions indicating that one needs to be very cautious when applying chemical reactions as models for enzymatic processes. Yet other surprising findings of these studies are large values of Cl-KIEs. Chlorine atom of hypochlorite acts formally as an incoming electrophile rather than the leaving group and therefore its Cl-KIE should be very small. In fact equilibrium chlorine isotope effect calculated at the B1LYP/aug-cc-pVDZ level for OCl^- as the reactant and chlorobenzene as the product is inverse (smaller than unity) and equal to 0.9962. Harmonic stretching O-Cl frequency of 636.2 cm^{-1} is larger than the corresponding C-Cl frequency in chlorobenzene (415.7 cm^{-1}) and the contribution from this mode to the isotope effect is normal (larger than unity). However, the inverse value is not

surprising since there are several isotope sensitive vibrational modes in IR spectrum of chlorobenzene that are not present in hypochlorite ion. Thus the origin of the observed large values of Cl-KIEs, especially in case of the enzymatic reactions, remains unclear.

CONCLUSIONS

Studies discussed herein indicate that chlorine kinetic isotope effects may be very useful in the elucidation of mechanisms of organic reactions. Although small they are very informative. However, with the shift made in recent years from the qualitative evaluation of theoretical values of chlorine kinetic isotope effects (and KIEs in general) to their calculations using reliable quantum-mechanical methods, it is evident that their use in mechanistic studies requires much more caution than thought thus far. DFT and post-Hartree-Fock methods seem to be adequate for the modelling of Cl-KIEs.

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