Computational study of substrate isotope effect probes of transition state structure for acetylcholinesterase catalysis

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Abstract Secondary isotope effects for carbonyl addition reactions of methyl thioacetate, acetone and acetaldehyde have been calculated by *ab initio* quantum mechanical methods in an effort to interpret measured β-deuterium isotope effects on acetylcholinesterase-catalyzed hydrolysis of acetylthiocholine. The calculated β-deuterium isotope effect for equilibrium addition of methanol to methyl thioacetate is $^{D3}K_{eq} = 0.965$, and the corresponding effect for addition of methoxide ion to methyl thioacetate wherein three waters are hydrogen bonded to the carbonyl oxyanion is $^{D3}K_{eq} = 1.086$. Neither of these calculated isotope effects is as inverse as the experimental β-deuterium isotope effect for acetylcholinesterase-catalyzed hydrolysis of acetylthiocholine, $^{D3}k_E = 0.90 \pm 0.03$. Structural comparisons show that the water-solvated methoxide adduct of methyl thioacetate is more expanded than is the neutral methanol addition adduct, and suggest that the degree to which the isotope effect is inverse (i.e. less than) is inversely correlated to the degree of expansion of the adduct. A similar correlation of α-deuterium and β-deuterium secondary isotope effects with the degree of expansion of the adducts is found for equilibrium additions of methanol and methoxide ion to acetaldehyde. These computational results suggest that the markedly inverse β-deuterium isotope effect for the acetylcholinesterase reaction arises from enzymic compression of the transition state.

Key words acetylcholinesterase • carbonyl addition reactions • enzyme mechanisms • quantum mechanical calculations • secondary isotope effects • transition state structure

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Introduction

It has long been known that enzymes produce their impressive catalytic power by stabilizing the transition state(s) of their reaction mechanisms [15, 18]. Consequently, determination of transition state structure is a fundamental problem in developing an understanding of enzyme catalysis. The enzyme acetylcholinesterase (AChE) accelerates the hydrolysis of the physiological substrate acetylcholine by at least 10^{13}-fold, which ranks among the most impressive of catalytic accelerations [16]. In an earlier publication [13], we postulated a structure for the transition state of the acylation stage of AChE-catalyzed hydrolysis of acetylthiocholine that was based on measured β-deuterium secondary isotope effects on the second-order rate constant $k_E \equiv k_{cat}/K_m$. In particular, the measured $^{D3}k_E = 0.90$ was interpreted in terms of a transition state that structurally resembles a tetrahedral intermediate. However, this model was inferred from relating the measured isotope effect to an estimate of the equilibrium isotope effect, wherein the estimate was generated from the measured isotope effect for hydration of 1,3-dichloroacetone [10]. Recently, we have initiated an effort to interpret the $^{D3}k_E$ value in a more rigorous manner by computing equilibrium and kinetic β-deuterium isotope effects for nucleophilic addition to thioesters, a process that mimics the formation of the tetrahedral intermediate in the acylation of AChE catalysis. This article focuses on these computational efforts.

Methods

Geometries of nucleophilic addition adducts of methyl thioacetate, acetaldehyde and acetone were optimized in the gas phase and with solvation as modeled by the Onsager self-consistent reaction field [8, 14] by using the program GAUSSIAN 98 [5]. Equilibrium secondary deuterium isotope effects were determined from harmonic vibrational frequencies calculated in GAUSSIAN 98 by using the Bigeleisen equation [3, 4] (Eqs. (1)–(3) below), with the isotopic ratio of MMI terms described by the Redlich-Teller product rule [17] (Eq. (2)).

$$(1) \qquad {}^{S}K = K_H/K_D = \mathrm{MMI} \times \mathrm{EXC} \times \mathrm{ZPE}$$

$$(2) \qquad \mathrm{MMI} = VP = \frac{\prod_i^{n_{final}} u_{iH}/u_{iD}}{\prod_i^{n_{initial}} u_{iH}/u_{iD}}, \quad u_i = \frac{h\nu_i}{kT}$$

$$(3) \quad \mathrm{ZPE} = \frac{\prod_i^{n_{final}} e^{u_{iD}/2}/e^{u_{iH}/2}}{\prod_i^{n_{initial}} e^{u_{iD}/2}/e^{u_{iH}/2}}, \mathrm{EXC} = \frac{\prod_i^{n_{final}} \left(1-e^{-u_{iD}}\right)/\left(1-e^{-u_{iH}}\right)}{\prod_i^{n_{initial}} \left(1-e^{-u_{iD}}\right)/\left(1-e^{-u_{iH}}\right)}$$

The leading superscript S in Equation (1) depends on the particular isotope effect that is being considered. The following designations are used herein for α-deuterium and β-deuterium isotope effects, respectively, on the equilibrium constant: ${}^{\alpha D}K_{eq}$, ${}^{D3}K_{eq}$. For β-deuterium substitution, the number in the leading superscript indicates the number of equivalent isotopic positions that contribute to the isotope effect. The designations α or β refer to situation of the isotopically varied atoms on the carbonyl carbon or one carbon removed from the carbonyl carbon, respectively.

Calculations were performed at the 6-31G* level of theory with inclusion of electron correlation via Becke-Perdew--Wang density functionals [2, 11]. This level of theory was arrived at by comparing the agreement of calculated and experimental secondary deuterium isotope effects. Calculated isotope effects were produced at various basis set levels, with and without the use of density functional approaches. The method adoped herein gave the best agreement between calculated and experimental effects, a result that accords with the demonstration of Wong [20] that density functional methods and the 6-31G* basis set give the best overall agreement between calculated and experimental frequencies for a large number of organic molecules. Calculations were performed on an IBM system that consists of the following components: five 4-CPU RS/6000 44P 270 workstations, one with 4 GB RAM/54 GB scratch disk, the rest each with 2.5 GB RAM/36 GB scratch disk.

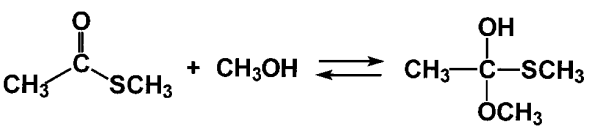


Fig. 1. Equilibrium addition of methanol to the carbonyl function of methyl thioacetate.

Results and discussion

Initial calculations of β-deuterium secondary isotope effects utilized a model in which CH_3O^- adds to the carbonyl carbon of methyl thioacetate in the gas phase. However, this model failed to produce a tetrahedral addition adduct; i.e. the mechanism is an S_N2-like direct displacement of the methylthiolate leaving group. Consequently, the model shown in Fig. 1, which involved equilibrium addition of CH_3OH to the thioester carbonyl, was explored. The corresponding calculated β-deuterium secondary isotope effect was ${}^{D3}K_{eq} = 0.965$, a value that is more than two standard deviations less inverse than the experimental kinetic isotope effect, ${}^{D3}k_E = 0.90 \pm 0.03$ [13]. The model of Figure 1, *vis-á-vis* that erstwhile tried for direct CH_3O^- attack, represents the addition of a proton at the carbonyl oxygen. Therefore, electrophilic assistance at the carbonyl oxygen is sufficient to cause a tetrahedral addition adduct to form.

The fact that the calculated isotope effect is less inverse than the experimental effect suggests that AChE compresses the acylation transition state. This hypothesis was explored by calculating a model of the tetrahedral addition adduct in which three water molecules interact with the incipient oxyanion of the ester carbonyl function. This alternate model, which represents weaker electrophilic interaction with the oxyanion, gives a calculated ${}^{D3}K_{eq} = 1.086$. A possible reason for the conversion from inverse to normal isotope effect arises from comparing the structures of the two tetrahedral adducts (Table 1). It is apparent that the tetrahedral adduct in which the oxyanion interacts with three waters is more expanded than is the oxyanion-protonated adduct. In particular, the bond distances for bonds b, c and d in Table 1 are markedly longer for the water-solvated methoxide adduct than for the neutral methanol adduct. The greatest difference in bond lenght, 0.103 Å, is noted for the bond between the carbonyl carbon and the thiolate leaving group.

The calculated isotope effects discussed in the preceding two paragraphs establish that protonation at the thioester carbonyl oxygen products a compressed tetrahedral adduct and a more inverse equilibrium β-deuterium isotope effect.

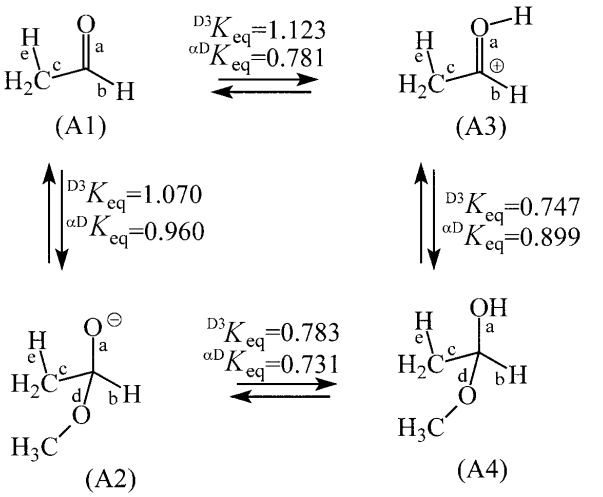
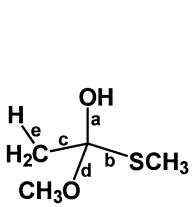
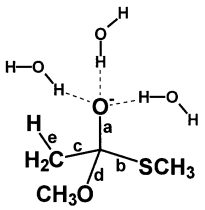


Fig. 2. Equilibrium addition of methanol to the carbonyl function of acetaldehyde. Secondary isotope effects are given for the individual steps of two pathways for the overall addition reaction.

Table 1. Structural comparison of nucleophilic addition adducts of methyl tioacetate.



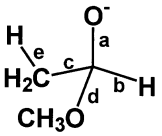
E1



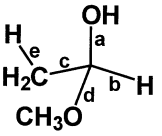
E2

	Bond lengths (Å)	
	Adduct E1	Adduct E2
Bond a	1.419	1.308
Bond b	1.865	1.968
Bond c	1.523	1.539
Bond d	1.411	1.466
Bond e	1.094	1.096

Table 2. Structural comparison of nucleophilic addition adducts of acetaldehyde.



A2



A4

	Bond lengths (Å)	
	Adduct A2	Adduct A4
Bond a	1.366	1.420
Bond b	1.118	1.104
Bond c	1.531	1.515
Bond d	1.443	1.412
Bond e	1.100	1.098

Consequently, electrophilic interaction with the carbonyl oxyanion could be the mechanism through which AChE compresses the transition state. Electrophilic interaction is provided by a three-pronged oxyanion hole in AChE that is comprised of the H-bond donating peptide NH functions of Gly-118, Gly-119 and Ala-201 [6]. The isotope effect on the AChE-catalyzed reaction, $^{D3}k_E = 0.90$, is more inverse than that calculated for the addition of methanol to methyl thioacetate. Consequently, if the compression of the transition state arises solely from oxyanion hole interaction, then the oxyanion hole is yet more effective than full protonation at the carbonyl oxyanion in providing electrophilic compression of the transition state. This electrophilic compression of the transition state is perhaps less troublesome than an imagined mechanical compression mechanism, since the later is by nature destabilizing and therefore does not provide a rationale mechanism for catalysis.

To judge the accuracy of calculated equilibrium secondary isotope effects, nucleophilic additions to additional carbonyl-containing molecules were explored. The first reaction that was studied is the formation of the dimethyl ketal of acetone. The calculated isotope effect for this reaction is $^{D6}K_{eq} = 0.7562$ (0.9545 per D), in excellent agreement with the experimental isotope effect $^{D6}K_{eq} = 0.72\pm0.05$ (0.947 ± 0.008 per D) [19]. Equilibrium addition of CH_3OH to acetaldehyde was more extensively explored, as outlined in Fig. 2. The net isotope effects for the A1 to A4 conversion are $^{D3}K_{eq} = 0.838$ and $^{\alpha D}K_{eq} = 0.702$. For the hydration of acetyldehyde (i.e. addition of H_2O), the calculated isotope effects are $^{D3}K_{eq} = 0.895$ and $^{\alpha D}K_{eq} = 0.665$. The calculated values of the α-deuterium secondary isotope effects are in excellent agreement with the reported values for hydration of acetaldehyde, $^{\alpha D}K_{eq} = 0.73\pm0.02$ [12], and of pentanal, $^{\alpha D}K_{eq} = 0.72\pm0.02$ [7] and for equilibrium formation of the ethyl hemiacetal of pentanal, $^{\alpha D}K_{eq} = 0.73\pm0.02$ [7]. The calculated $^{D3}K_{eq}$ value agrees nicely, on a per deuterium basis, with various experimental values in the literature for equilibrium conversion of sp^2 carbonyl reactants to sp^3 adducts [9, 10, 19]. Revealing additional information comes from breaking the CH_3OH addition in Figure 2 into steps. Intermediate A3 represents protonation of the carbonyl oxygen before CH_3O^- addition. The corresponding calculated effect for the conversion of A1 to A3 is $^{D3}K_{eq} = 1.123$. This value agrees in direction (i.e. greater than 1, a normal isotope effect) with the more substantial value reported by Arnett *et al.* [1], $^{D3}K_{eq} = 1.29\pm0.10$ for equilibrium protonation of acetophenone. Germane to the hypothesis that a more expanded adduct gives isotope effects that are less inverse are calculations for the conversion of A1 to A2, the oxyanionic tetrahedral adduct. As Figure 2 shows, $^{D3}K_{eq}$ is normal and $^{\alpha D}K_{eq}$ is only slightly inverse for this equilibrium conversion. Moreover, the structural comparisons in Table 2 show that the anionic adduct A2 is more expanded than is the neutral adduct A4. This trend agrees nicely with the calculated results, discussed above, for tetrahedral adduct formation for thioesters.

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