A Qualitative Method for Prediction of Amine Oxidation in Methanol and Water

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ABSTRACT: We have developed a predictive method, based on quantum chemical calculations, that qualitatively predicts N-oxidation by hydrogen peroxides in drug structures. The method uses linear correlations of two complementary approaches to estimate the activation barrier without calculating it explicitly. This method can therefore be automated as it avoids demanding transition state calculations. As such, it may be used by chemists without experience in molecular modeling and provide additional understanding to experimental findings. The predictive method gives relative rates for *N,N*-dimethylbenzylamine and *N*-methylmorpholine in good agreement with experiments. In water, the experimental rate constants show that *N,N*-dimethylbenzylamine is oxidized three times faster than *N*-methylmorpholine and in methanol it is two times faster. The method suggests it to be two and five times faster, respectively. The method was also used to correlate experimental with predicted activation barriers, linear free-energy relationships, for a test set of tertiary amines. A correlation coefficient $R^2 = 0.74$ was obtained, where internal diagnostics in the method itself allowed identification of outliers. The method was applied to four drugs: caffeine, azelastine, buspirone, and clomipramine, all possessing several nitrogens. Both overall susceptibility and selectivity of oxidation were predicted, and verified by experiments. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:1409–1420, 2015

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INTRODUCTION

The shelf life of a drug product is an important consideration in the drug development process, and part of the work involved in formulation and packaging development is aimed at obtaining a sufficient stability. The drug substance itself may be prone to degradation via autoxidation, heat, or exposure to light. It is also frequently found that moisture and formulation ingredients, including small amounts of impurities, cause degradation of the drug substance via oxidation or hydrolysis. As these degradation reactions decrease the efficacy of the product and may also result in toxic impurities, it is important to understand the underlying mechanisms so that degradation can be controlled.

Oxidation of amines is a ubiquitous degradation reaction. Amines are common substructures in drugs, and some of them, but not all, are prone to oxidation. They frequently react with hydroperoxides, which may exist as impurities in the formulation or may form in other degradation reactions, that is, autoxidation. Hence, there is a risk for degradation of amines in drugs, which is taken into account and carefully examined in the drug development process, both experimentally and theoretically. The understanding of factors that make the amines prone to undergo oxidation by hydroperoxides is important. During a drug discovery process, many compounds are examined and investigated to bring forth a drug candidate. To be able to predict the stability of these compounds early in the process will save time and money. It is thus important to be able to predict whether nitrogen in a drug molecule will undergo oxidation and at what approximate rate, both early in the drug development process, as well as for the choice of formulation later in the process.

As part of a long-term project to predict degradation of active compounds in formulations, the current investigation aims at finding a tool by means of quantum chemical calculations to predict whether a drug is sensitive to amine oxidation. Today, advanced quantum chemical methods can be used to estimate activation barriers and reaction energies accurately, provided the reaction conditions are known. However, the more complicated the reaction, and the larger and more flexible the system, the more complicated calculations need to be performed where many degrees of freedom in the system make the calculations more difficult and more technical demanding. This is especially the case in transition state searches, which has not yet been automated to a point where a nonspecialist can reliably succeed. The purpose of this work is to circumvent the need for transition state calculations by using a predictive tool that can be automated so that it is easy to use also for a noncomputational chemist.

BACKGROUND

The amine oxidation mechanism was discussed for the first time by Wieland[1] and since then several mechanisms have been considered for oxidation of different kinds of amines by hydrogen peroxides.[2,3] In its simplest form, the oxidation of amines may be considered as an amine reacting with a hydrogen peroxide molecule (H_2O_2) without any other species present (see Scheme 1). This is of course a simplification, as these reactions

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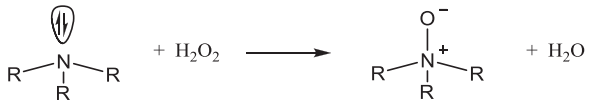

Scheme 1. Oxidation of amine by H_2O_2.

commonly take place in the presence of a solvent that may act as a catalyst as it may stabilize the charge separation in the activated complex.[4,5] The mechanism behind the reaction can be understood as follows: the neutral nucleophilic nitrogen donates its lone pair to the empty σ* orbital of one of the oxygens in the peroxide. Water is released after proton transfer, and an oxide is formed. Hence, the nucleophilic capacity of the nitrogen is important for the rate of the reaction.

The rate, v, of this reaction can be written as:

$$v = k\left[H_2O_2\right]\,[\text{amine}] \tag{1}$$

where k is the reaction rate constant. According to Eyring–Polanyi, the reaction rate constant can be expressed as:

$$k = \frac{K_B T}{h} e^{-\Delta G^{\ddagger}/RT} \tag{2}$$

where $\Delta G^{\ddagger}$ is Gibbs free energy of activation, T is the temperature, K_B is Boltsmann's constant, R is the gas constant, and h is Planck's constant.

In water, there is a competing reaction where the nitrogen donates its lone pair to a proton, instead of to the peroxide, whereby the amine becomes protonated. The protonated form of the amine cannot react with the peroxide. Hence, the basicity of the amine is an important factor when estimating the rate of oxidation. The amount of neutral amine at a particular pH can be obtained by applying the Hendersson–Hasselbalch equation:

$$-\log\frac{[B]}{[BH^+]} = pKa - pH \tag{3}$$

$$K_a = \frac{[A^-][H^+]}{HA} \tag{4}$$

Therefore, in order to obtain the rate of reaction in protic solvent, the basicity of the amine must be known. Moreover, the basicity is closely connected to the nucleophilicity of the nitrogen,[6] which in turn is reflected in the activation barrier of the oxidation reaction, that is, a stronger nucleophile lowers the activation barrier.

In this study, the close relationship between the basicity and the nucleophilicity of the amine is used to relate the p*K*a of the amine to the activation barrier of the reaction. Quantum chemical calculations are used to explicitly calculate the activation barrier of a set of different substituted amines as well as determining the p*K*a of the amines. The p*K*a of the amines and the activation barrier are correlated and a linear relation is obtained. The linear relation may then be used to predict the activation barrier of larger and more complicated systems by calculating the p*K*a only. This approach will be applied in two different solvents, water and methanol, both common solvents in pharmaceutical stability testing and stability studies.

As a complement, the Bell–Evans–Polanyi principle,[7–20] which states that there is a linear relationship between the activation barrier ($\Delta G^{\ddagger}$) and the enthalpy of reaction (ΔH) for reactions of the same family, can be employed. However, in the current study, we have used the Gibbs free energy of reaction (ΔG_r) instead of ΔH for this purpose. Thus, ΔG_r was calculated for the amine oxidation by H_2O_2 (see Scheme 1), and correlated to the activation barrier for the same set of substituted amines.

Hence, two different approaches are employed to estimate the activation barrier without calculating the transition states explicitly: (1) by calculating the p*K*a of the amine and relating the p*K*a to the activation barrier of the N-oxidation and (2) by calculating ΔG_r for the N-oxidation by H_2O_2 and relating ΔG_r to the activation barrier.

The use of two complementary approaches is important as it allows us to identify cases where steric factors, hydrogen bonding by adjacent groups, or other factors have strong influences on the activation barriers. This is important as the purpose is to automate the method, and two different but related approaches will allow for internal diagnostics in the method itself to identify cases where the method is expected to be less reliable.

MATERIALS AND METHODS

Computational Details

All calculations were performed using density functional theory[21,22] with the M06,[23] functional as implemented in the Gaussian 09 package.[24] All geometries were fully optimized in vacuum with the 6–31G(d,p) basis sets. Harmonic vibrational frequencies were used to calculate the thermodynamic contribution to the enthalpies and the free energies, as well as to ensure the nature of the stationary points (minima or saddle points). The connectivity between a given transition state and the corresponding reactants and products was verified by following the intrinsic reaction coordinate. Single point calculations of the optimized geometries with the same level of theory were made to describe the solvents effect of water (ε = 78.3) and methanol (ε = 32.6) using the polarizable continuum model,[25,26] PCM. For estimation of the p*K*a of the amines, all the geometries were optimized and the frequency calculations were performed in the PCM model.

Estimation of a Local p*K*a via a Reference System

The purpose of this work is to circumvent the need for transition state calculations when estimating a drug's inherent sensitivity to oxidation. This may be carried out by calculating a relative p*K*a of the amine in the solvent of choice. In this way, the basicity of the amine may be calculated and connected to the nucleophilicity, which reflects the activation barrier of the oxidation reaction. Hence, the only calculations necessary for a more complex system are a geometry optimization in the preferred solvent for the neutral molecule and a geometry optimization for the molecule with the nitrogen atom protonated. If there are several nitrogens in the molecule, one geometry optimization for each protonated nitrogen is necessary, whereby one local relative p*K*a for each nitrogen is obtained. This local relative p*K*a does not reflect the real p*K*a, as it does not take into account the protonation state of the other nitrogens in the molecule. In contrast to other methods that aim to predict p*K*a values using quantum chemical methods, such as Jaguar p*K*a, this method does not aim to determine the absolute p*K*a value,