COMMENTARY

Application of an Exact Mathematical Model and the Steady-State Approximation to the Kinetics of the Reaction of Cysteine and Hydrogen Peroxide in Aqueous Solution: A Reply to the Ashby and Nagy Commentary

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To the Editor,

In their "Commentary on the Kinetics and Mechanism of the Reaction of Cysteine and Hydrogen Peroxide in Aqueous Solution," Ashby and Nagy take issue with our application of the exact mathematical equation (Model I) to estimate the individual rate constants (k_1 and k_2) for the two sequential reaction steps in Scheme 1 of our original study.[1] They suggest that our data "afford no insight into the mechanism that follows the rate-determining step" and offer a "simpler" model based on applying the steady-state approximation to the same reaction scheme thus producing a best-fit estimate only for k_1. Later, they suggest that, "To conclude that Model I is valid, the experimental data must be sufficiently accurate to differentiate it from the simpler Model II."

We disagree. Our analysis requires no assumptions as to the approximate value of the rate constant for the 2nd reaction step. Rather, we rely on the exact model to provide such insight. In this response, we first present logical arguments favoring the use of Model I rather than Model II for the data analyses in question. Next we discuss the statistical results that establish the superiority of Model I over Model II. Finally, we discuss additional evidence shown in Figure 7 of our original study that demonstrates the superior predictive accuracy of Model I over Model II outside the range of concentrations explored in our original kinetic studies.

WHICH MODEL IS "SIMPLER?"

Ashby and Nagy assert that their Model II is simpler. Evidently they are invoking Ockham's razor, a principle attributed to William of Ockham, a 14th century Franciscan monk. Ockham's razor has been interpreted in various ways, the most common of which is, "when deciding between two models which make equivalent predictions, choose the simpler one."[2] Another interpretation of Ockham's razor consistent with the principle of uncertainty maximization is "from your data, induce that model which minimizes the number of additional assumptions."[3]

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Ashby and Nagy's assertion that Model II is simpler is misleading. The underlying mechanism as described in Scheme 1 is the same for both Models I and II. Thus, Models I and II do not represent conflicting mechanisms. Rather, Model II is nested within Model I, which includes among its possible outcomes those values of k_2 that would be necessary to justify the steady-state assumption. The only difference between the two models is that Model II requires the *additional assumption* that k_2 must have certain values relative to k_1 such that $d[CSOH]/dt \ll k_1[CS^-][H_2O_2]$ whereas Model I contains no such additional constraints. According to the second interpretation of Ockham's razor (above), Model I should be preferred because it minimizes the number of additional assumptions.

The steady-state assumption is always an *approximation* to the *exact* mathematical description of the underlying mechanism being tested (see, e.g., Connors[4]). Given the power of computers and software available today, why would one include this constraint in a nonlinear regression analysis if the exact equation will produce estimates for all parameters along with statistics, from which one can then determine whether or not the parameter estimates are reliable and whether or not the steady-state assumption or another assumption would have been justified?

If one were to assume (incorrectly) for the sake of this discussion that Models I and II were not nested models (i.e., the exact and approximate solutions, respectively, to the same underlying mechanism), but rather representations of completely independent mechanistic hypotheses, then perhaps the former interpretation of Ockham's Razor—"when deciding between two models which make equivalent predictions, choose the simpler one"—would be a useful guide in selecting the best model. However, Ashby and Nagy have demonstrated in their Figure 2 that the two models do not make equivalent predictions of [CSSC] versus time over the $[H_2O_2]_0$ concentration range of 2–9.2 mM. Rather, Model I predicts a reduction in the percent formation of [CSSC] with increasing $[H_2O_2]_0$, while Model II does not. Figure 3 in Ashby and Nagy's commentary demonstrates that, even though the largest discrepancy between the two models occurs at early stages in the reaction, a significant discrepancy persists throughout the reaction. Thus, at the end of the reaction for $[CSH]_0^T = 4$ mM and $[H_2O_2]_0 = 9.2$ mM, approximately 4% [CSOH] remains *as the final product* in these simulations! The two models therefore do not make equivalent predictions, which forces Ashby and Nagy to base their preference for Model II on a suspicion that our data were not sufficiently precise to differentiate between the two mechanisms. An unfortunate attribute of Model II is that it requires poor precision in the underlying data or insufficient data to be competitive with the exact Model I.

Visually the two models may produce similar curves because the magnitude of the error in making the steady-state approximation may be small. Statistical analysis may tell us more because in statistics, the size of the mistake matters. The 95% confidence limits for k_2 generated using Model I, for example, may be expected to provide the approximate range of values that k_2 must have to be consistent with the experimental data. This information cannot be gleaned from the data using Model II because an assumption regarding the value of k_2 is already built into the model in advance of the data analysis.

STATISTICAL COMPARISONS—MODEL I VERSUS MODEL II

This brings us to the actual experimental data analyzed in our original study. We will focus on the pH 6.0 data (available on request *via* an E-mail to the corresponding author) as have Ashby and Nagy. Three of the five sets of curves fit simultaneously were shown previously in Figure 3 of the original study. (There was an error in the reported concentrations of $[H_2O_2]_0$ used in the original study. The paper listed the $[H_2O_2]_0$ concentrations as 2, 4, 6, 8, and 9.2 mM, but the actual $[H_2O_2]_0$ concentrations used and analyzed were 2, 4, 4, 8, and 9.2 mM.) In response to the concerns of Ashby and Nagy, we refit the five data sets individually and as a combined set using both models. The goodness-of-fit statistics that were employed to compare the models were the sums of squared deviations of the observed versus the calculated data, and the model selection criterion (MSC). The MSC is a modified Akaike information criterion (AIC)[5–7] that provides the largest number for the most appropriate model.[8] It gives the same rankings between models as the AIC, but it has been normalized so that it is independent of the scaling of the data points. Because they quantify how much better the goodness-of-fit should be for the model with more parameters to be considered more appropriate, the MSC and AIC are useful for comparing models with