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

different numbers of parameters. The parameter statistics (i.e., support plane 95% confidence limits) for k_1 and k_2 provide information on the apparent certainty of the parameter values within the domain space explored.

Shown in Table 1 are the results of the fits using Models I and II for each of the five individual data sets at $[CSH]_0^T = 4$ mM, and varying $[H_2O_2]_0$ and the combined data set. Descriptive information on each data set has also been provided in Table 1, including the first sampling time, the number of time points, and the mass balance with respect to cysteine (i.e., the ratio $([CSH] + 2[CSSC])/[CSH]_0^T)$) averaged over all time points along with the standard deviation. A comparison of the goodness-of-fit statistics (sums of squared deviations and MSC values) reveals that Model I produces smaller sums of squared deviations (i.e., better fits) and larger MSC values for four of the five individual sets. We relied on the combined set in our original analysis of the pH 6 data—a set comprising 182 data points. Model I produces a smaller sum of squared deviations and a larger MSC value for the combined data set than Model II. These statistical data for the combined set provide compelling support for the selection of Model I over Model II for the reaction conditions explored. We find it difficult to imagine the circumstances wherein one would choose Model II instead of Model I in the face of these statistical comparisons.

In only one set (i.e., the 2nd data set at $[CSH]_0^T = 4$ mM and $[H_2O_2]_0 = 4$ mM) did Models I and II produce the same goodness-of-fit statistics. This data set was unique among the five in that the first time point was relatively late (i.e., 1 min vs. 15 s or 30 s in all other sets), and the number of data points ($n = 8$) was smaller than in any of the other sets. This set therefore appears to be the only one to satisfy the conditions described by Ashby and Nagy, as being insufficiently precise to differentiate between the two models.

Also evident in Table 1, the k_1 values determined are nearly the same from experiment-to-experiment regardless of the model chosen for analysis and the 95% support plane confidence limits are very narrow for k_1. This is not the case for k_2, however, which is quite variable from one set to another, and the accompanying 95% confidence limits are generally quite broad. In the one set in which Models I and II were indistinguishable, confidence limits for the parameters could not be generated. The fluctuations in k_2 in fits of the individual sets and the relatively wide 95% con-

Table 1. Descriptive Information and Results of Fits Using Models I and II for Five Individual pH 6.0 Data Sets at $[CSH]_0^T = 4$ mM and Varying $[H_2O_2]_0$ and for the Combined Set

Data Set	First Time Point (s)	# of Time Points	Average Mass Balance	Sum of Squared Deviations		Model Selection Criterion (MSC)		k_1(/Ms) (95% Conf. Limits)		k_2(/Ms) (95% Conf. Limits)	
				Model I	Model II	Model I	Model II	Model I	Model II	Model I	Model II
C4H2	15	17	0.993 (±0.008)	1.07E-08	1.92E-08	8.17	7.59	14.7 (14.5, 14.9)	14.4 (14.3, 14.6)	316 (202, 430)	— —
C4H4-1	15	15	0.99 (±0.006)	3.01E-08	1.01E-07	7.43	6.22	15.0 (14.6, 15.4)	14.5 (13.9, 15.0)	311 (243, 380)	— —
C4H4-2	60	8	1.000 (±0.010)	7.53E-08	7.51E-08	6.32	6.31	14.5 —	14.5 (14.0, 15.1)	6842480 —	— —
C4H8	30	12	1.002 (±0.014)	3.81E-07	4.91E-07	6.59	6.34	14.3 (13.1, 15.5)	13.5 (12.6, 14.4)	718 (161, 1275)	— —
C4H9.2	15	10	0.970 (±0.018)	2.66E-08	1.25E-07	9.37	7.84	14.8 (14.4, 15.2)	14.2 (13.6, 14.8)	761 (590, 931)	— —
Combined set				5.89E-07	8.57E-07	7.39	7.01	15.2 (14.8, 15.5)	14.7 (14.4, 14.9)	720 (540, 890)	— —

fidence limits support the expectation that k_2 is indeed difficult to determine precisely because the overall reaction rate is largely controlled by the first reaction step. However, it is the application of Model I that is necessary to illustrate this point!

Even though the 95% confidence limits for k_2 generated from the combined data are broad, indicating that the reported value for k_2 is far less certain than the value for k_1, it is noteworthy that the application of Model I enables the determination of such limits, even for k_2. We analyzed the reliability of these limits further because the confidence ranges listed in Table 1 were produced by an algorithm that assumes the parameters to have a linear influence on the sum of squared deviations near the optimum, an assumption that may not be sufficiently rigorous.[8] Shown in Figure 1 are plots of the sums of squared deviations versus the distance of k_2 from its optimum ($\pm 2\sigma \approx \pm 95\%$ conf. limits) for two of the data sets described in Table 1 (C4H4-1 and C4H9.2). The dashed and solid lines represent the sum of squared deviations at the support plane 95% confidence boundaries defined by the F distribution. (As a point of reference, note that the sum of squared deviations for these data sets using Model II exceeds 1×10^{-7}.) A rigorous determination of the support plane confidence limits for k_2 requires setting k_2 at various distances from the optimum value, re-fitting k_1 to a new optimum, and determining the point at which the sum of squared deviations for the adjusted k_2 value crosses the 95% confidence boundary. Figure 1 illustrates that the 95% confidence limits are not symmetric around the optimum value for either set of conditions. The lower 95% confidence intervals are slightly narrower than those listed in Table 1, while the upper 95% confidence intervals are significantly (but less than twofold) wider. Nevertheless, this more rigorous analysis demonstrates that there appears to be sufficient information content in the underlying data to support both lower and upper 95% confidence boundaries for k_2—a finding that is not possible to explore using Model II.

PREDICTIVE ACCURACY OF MODEL I VERSUS MODEL II

According to Forster, the "goal of model selection is to maximize the predictive accuracy of the best fitting cases drawn from rival models" when the models are used to predict unseen data beyond the range of the observed data.[7] Whereas the best fits of Models I and II produce curves that are visually similar over the domain of prediction (i.e., over the range of $[CSH]_0^T$ and $[H_2O_2]_0$) explored in our study, how well do the two models perform if we extrapolate beyond the range of this study? Figure 7 of our original study explored this question by examining the predicted percent of CSSC formed at the completion of the reaction

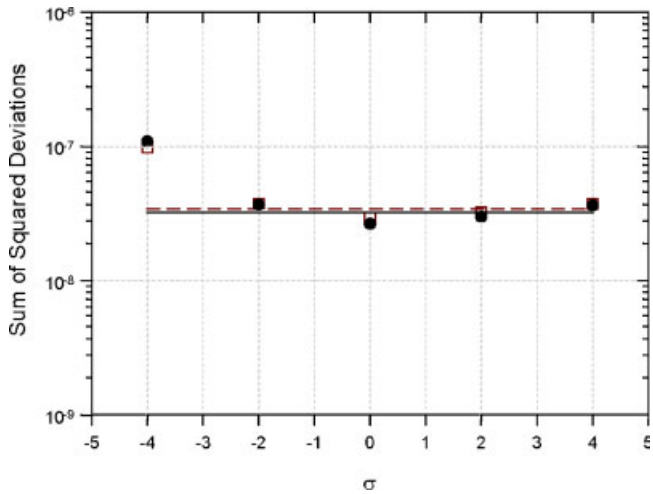

Figure 1. Plots of the sums of squared deviations versus the distance of k_2 from its best-fit value ($\pm 2\sigma \approx \pm 95\%$ conf. limits) for two of the data sets described in Table 1 (C4H4-1 (□) and C4H9.2 (●)). The dashed and solid lines represent the sum of squared deviations at the support plane 95% confidence boundaries for the C4H4-1 and C4H9.2 data sets, respectively, as defined by the F distribution.

according to Model I with ratios of $[H_2O_2]_0/[CSH]_0^T$ ranging from 1 to 1000. The dashed line in that figure was the predicted curve using Model I and the best-fit values for k_1 and k_2 generated from studies over a much narrower $[H_2O_2]_0/[CSH]_0^T$ range (0.5–2.3). Model I predicts a decrease in percent of CSSC formation with increasing $[H_2O_2]_0/[CSH]_0^T$ due to a depletion in concentration of CSH necessary to convert CSOH to CSSC at high $[H_2O_2]_0/[CSH]_0^T$ resulting in a predicted buildup of the reactive intermediate, CSOH, whereas Model II predicts no such effect. In proposing Model II, Ashby and Nagy appear to have given inadequate consideration to our Figure 7, as Model II is incompatible with Figure 7.

Importantly, the discrepancy between the two models becomes quite large as $[H_2O_2]_0/[CSH]_0^T$ is increased beyond the range covered in our experiments, as illustrated in Figure 2. Panel A of Figure 2 illustrates simulated data based on Model I (data points) for CSSC formation at $[CSH]_0^T = 1$ mM and $[H_2O_2]_0 = 0.5$, 1.0, and 2.5 mM, a range that produces $[H_2O_2]_0/[CSH]_0^T$ ratios similar to those explored in our experiments. The solid lines are the fits of Model II. In this range, the two models produce similar predicted CSSC formation curves versus time. Panel B includes two additional $[H_2O_2]_0$ concentrations of 5 and 10 mM. Here the ability of Model II to fit the data deteriorates markedly.

As pointed out in our original study, our value of k_2 *qualitatively* predicts a decline in percent of CSSC formation with increasing $[H_2O_2]_0/[CSH]_0^T$, but it fails to predict Figure 7 *quantitatively*. A somewhat larger value of k_2 would produce a more accurate prediction of the data in Figure 7, although other hypotheses may also account for the disparity, such as one proposed in our original study. We suggest that additional kinetic studies at higher $[H_2O_2]_0/[CSH]_0^T$ ratios may help to improve the estimate of k_2, but as we and Ashby & Nagy noted previously, Scheme 1 is incomplete and a more complex model that includes formation of cysteine sulfinic acid, cysteine sulfonic acid, and perhaps other products may be necessary at high $[H_2O_2]_0$ to account for the fate of CSOH when CSSC is not the only final product.

In conclusion, we vigorously defend the approach of (1) using an exact mathematical model representing the proposed reaction mechanism rather than an approximation involving an additional assumption and introducing error that becomes increasingly apparent with an improvement in quality of the underlying data; and (2) relying on goodness-of-fit statistics and confidence limits for the parameter estimates to gain insight into the respective contributions of each reaction step. Statistical analysis indicates that (1) Model I is statistically superior to Model II; and (2) 95% confidence limits can be ascertained for both parameters, but the estimated value for k_2 is much less certain than that for k_1. Finally, we have demonstrated that the predictive accuracy of Model I is superior to Model II for reaction conditions beyond the range upon which our fits were obtained.

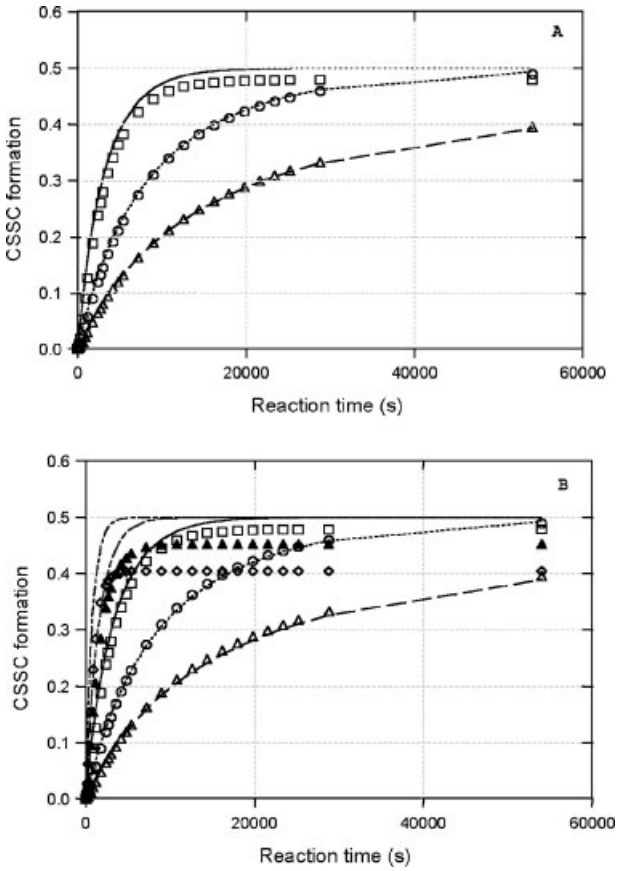

Figure 2. Simulated data based on Model I (data points) for CSSC formation versus reaction time. The lines are the fits of Model II. Panel A: $[CSH]_0^T = 1$ mM and $[H_2O_2]_0 = 0.5$ mM (- -△- -), 1.0 mM (· · ○ · ·), and 2.5 mM (—□—). Panel B: $[CSH]_0^T = 1$ mM and $[H_2O_2]_0 = 0.5$ mM (- -△- -), 1.0 mM (· · ○ · ·), 2.5 mM (—□—), 5 mM (- -▲- -), and 10 mM (– · ◇ – ·).

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