

Calculations

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ADJUSTMENT FOR DEUTERIUM LOSS

Although some loss of deuterium label from the peptic fragments during peptic digestion and HPLC is unavoidable in these experiments, simple adjustments can be made for these losses. These adjustments are based on analysis of appropriate controls, a 0% deuterium control and a 100% deuterium control (Zhang, 1995; Zhang & Smith, 1993). Control samples representing 0% and 100% exchanged protein were analyzed with each set of samples. The 0% control was protein that had never been exposed to deuterium and the 100% control was protein in which all exchangeable hydrogens have been replaced with deuterium (see Materials above for preparation). The adjustment for loss of deuterium is explained with the help of Figure 1. Panel a is an example of data for a segment of protein, displaying the 0% and 100% references. The equation used to apply the adjustment is shown in Figure 1b where D is the adjusted deuterium level, m is the experimentally observed mass, $m_{0\%}$ is the 0% or undeuterated control, $m_{100\%}$ is the totally deuterated control and N is the total number of amide hydrogens in the fragment. Since the adjustment is based on the assumption that loss of deuterium from a totally deuterated fragment is proportional to the loss from the same fragment when partially

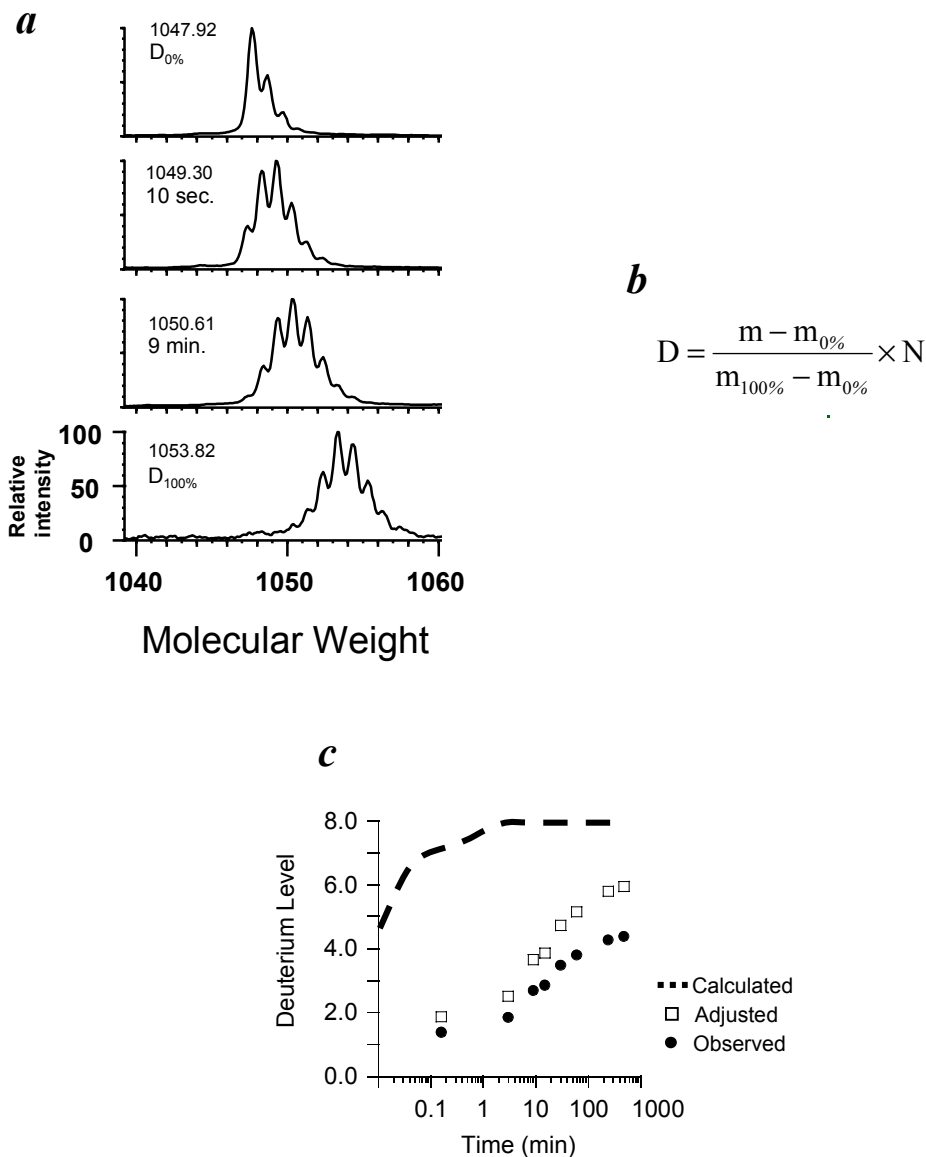


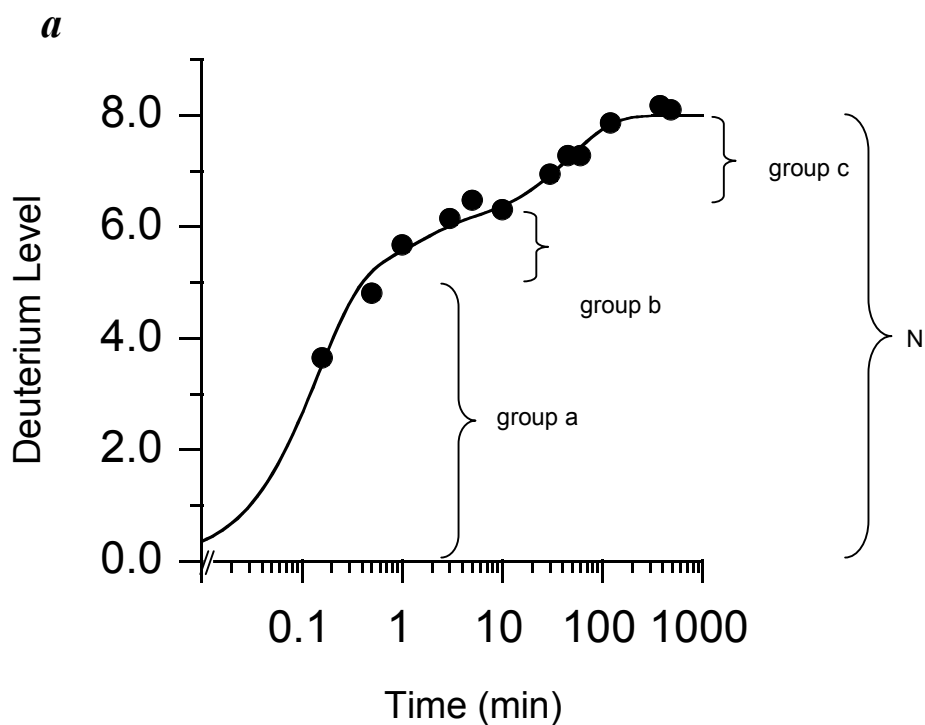
Figure 1 -- Example of data processing steps for adjusting the value of incorporated deuterium. **(a)**. The centroid value of each isotopic distribution representing a given peptide is determined and adjusted with the equation in **(b)** for loss of deuterium during analysis. See text for definition of terms in **(b)**. Panel **(c)** compares raw data for a segment of protein. Observed: measured amount of deuterium taken-up at the given time; Adjusted: amount of deuterium in the peptide after adjusting the data with the equation in **(b)**; Calculated: amount of deuterium in an unstructured peptide with the sequence of this segment of protein under identical exchange-in conditions. See text for more details.

deuterated, the accuracy of the adjustment depends on the sequence of the peptide (Zhang & Smith, 1993). The average error that results from the equation in Fig 1a is about 5%, with 92% of fragments having an error <10%, 3% having an error >15% and 0.5% having an error >25% (Zhang & Smith, 1993). Figure 1c shows the deuterium level plotted versus exchange-in time for a segment of protein before and after the adjustment for back-exchange was considered. The absolute number of deuterium is affected, but the profile of the curve is not. For comparison, a curve for exchange of the same sequence in an unstructured peptide [calculated from the parameters in (Bai et al., 1993)].

Adjustment is most useful when determining the actual amount of deuterium incorporated in a fragment. However, when the levels of deuterium in the same fragment are compared (such as comparing the amount of deuterium in a given region in the free or ligand bound state) the correction is not required as long as the analysis conditions are identical.

CALCULATION OF RATE CONSTANTS

From exchange-in time-course data, one can estimate the distribution of rate constants describing isotope exchange at each linkage within a peptide. An example of fitting the data is shown in Figure 2. Real data for a fragment of a protein are shown in panel a. A smooth line corresponding to the equation that best fits the experimental data was calculated using the equation in panel b. Rate constants are fit to the experimental data with a series of first order rate expressions where D is the number of



b

$$D = N - \sum_{i=1}^N \exp^{-k_i t}$$

c

$$D = 8 - \left[\underbrace{(5 (e^{-(m1) \cdot t}))}_{\text{group a}} + \underbrace{(1 (e^{-(m2) \cdot t}))}_{\text{group b}} + \underbrace{(2 (e^{-(m3) \cdot t}))}_{\text{group c}} \right]$$

$m1 = 7.21 \pm 0.82 \text{ min}^{-1}$ $t_{1/2} = 0.096 \text{ min}$	$m2 = 0.77 \pm 0.32 \text{ min}^{-1}$ $t_{1/2} = 0.90 \text{ min}$	$m3 = 0.021 \pm 0.003 \text{ min}^{-1}$ $t_{1/2} = 33.0 \text{ min}$
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Figure 2 -- Example of the data processing steps involved in determining the distribution of amide hydrogens within a peptide fragment and the rate constants for these amide hydrogens. (a). An example of curve-fit data from a protein. It is derived by fitting the data with the equation in (b). Details of the terms in (b) are explained in the text. (c). Final form of the equation in (b) for the example data in (a). Amide hydrogens have been grouped (according to similar exchange rates) into the categories a, b, c [also shown in (a)]. The rates of exchange of each of these groups, (m1, m2, m3, respectively) are also shown.

deuterium present in a peptide, N is the number of peptide amide linkages in a segment and k_i are the hydrogen-deuterium exchange rate constants for each peptide linkage (Zhang et al., 1996). Although single amino acid resolution is not generally possible, exchange rates describing groups of hydrogens with similar exchange rates can be calculated. Therefore, a three-term exponential equation was used where the amide hydrogens are divided into fast, medium and slow categories according to their exchange rates. Figure 2c shows the actual equation used for the data in Fig 2a. The number of amide hydrogens that exchange at similar exchange rates is calculated for each term of the equation. At the same time the average rate constant for each group is calculated. The results for fitting the experimental data are shown in the bottom of panel c where the number of amide hydrogens that exchange at a given rate are tabulated along with the average rate constant calculated for each group. Software packages such as Kaleidograph (Synergy Software) or SigmaPlot (Jandel) can be used for fitting.

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Zhang, Z., Post, C. B., & Smith, D. L. (1996). Amide hydrogen exchange determined by mass spectrometry: application to rabbit muscle aldolase. *Biochemistry* **35**, 779-791.

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