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Hidden assumptions in a maximum-entropy method stacking analyses

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Abstract

Two models have been used to describe indefinite self-association (stacking). In the more popular isodesmic model addition of molecules to the growing stack occurs with the same equilibrium constant, while in the attenuated model successive equilibrium constants decrease in value. In an attempt to choose between the two models application was made of the maximum-entropy method. This paper points out that the conclusions drawn from this method are not proven as the application assumed specific limiting values for experimental values of a molecule in the interior of a stack, and these values are not identical in the two models for the two systems considered.

Keywords: Self-association; Stacking; Maximum-entropy method

1. Introduction

In a recent comprehensive review of models for describing indefinite self-association [1], two models were considered in detail. In the more popular isodesmic model the addition of a molecule to a stack occurs with the same free energy and equilibrium constant, *K,* as for addition of previous molecules. This model was called the *equal K* or EK model. In the second model the enthalpy of addition to the growing stack remains constant and subsequent additions should be increasingly less probable and hence less favored entropically, so that successive equilibrium constants should taper off in value. This model was labeled the *attenuated K* or AK model. Rarely invoked, both models may be refined by allowing for the equilibrium constant for dimer formation to vary from the sequence for addition of subsequent molecules to a stack. Assumptions involved in both models were described in the review. For both systems considered here it was shown that next-nearest neighbor interactions are negligible and only nearest neighbor interactions need be considered. In the review, a non-linear least squares analysis interpreted data in terms of both the EK and AK models for comparison. Except for simpler mathematics and better handling by the non-linear least squares analysis of sometimes limited data by the EK model, from the analysis comparison there was no strong reason to prefer one model to the other.

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Building upon the formulations in the review, Douglas Poland proposed differentiating between the EK and AK models by a maximum-entropy conversion of moments into a cluster distribution function [2]. He considered stacking in two systems: daunorubicin which was deemed to be described by the EK model, and $Mg(ATP)^{2-}$ by the AK model. The purpose of this note is to point out hidden assumptions in application of the maximum-entropy method that render its conclusions in these two cases unproved.

Even the name of the method: maximum-entropy, suggests a strong bias in favor of the AK model where the addition of subsequent monomers to an elongating stack become less probable with a tapering off of the equilibrium constant.

Association was followed by the concentration dependence of the intensity of the circular dichroism spectra in daunorubicin and by proton chemical shifts in $Mg(ATP)^{2-}$. The formalism describing dimerization and stacking for these two techniques is identical. Several parameters are fitted in performing the non-linear least squares analysis: among them an equilibrium constant and the limiting intensity or chemical shift values of a monomer and of a molecule within a long stack. The last two quantities should be independent of the model used, EK or AK, but disconcertingly the intensity or chemical shift of a molecule within a long stack, is usually significantly different in the two models. Unlike that of the monomer, the value for the molecule within a stack depends upon a long extrapolation as even in the most concentrated solutions most molecules are not in long stacks. At least one of the two models, EK or AK, must be an inappropriate formulation for those systems with a different value of the limiting parameter for a molecule within a long stack.

The original paper on daunorubicin interpreted the results in terms of a dimer [3]. (For some strongly metal ion binding complexes the second daunorubicin binds to the 1:1 complex with up to 100 times the equilibrium constant of the first [4].) Without additional input, the dimer and isodesmic or EK models are formally indistinguishable for intensity or chemical shift measurements. For daunorubicin the limiting molar intensity shifts from monomer to molecule within a stack are 1.7 in the EK model and 2.1 in the AK model, a 24% difference. In his paper Poland [2] describes equation 64 which uses the 1.7 difference as ‘at the heart of our method.’ It is not surprising that using the difference calculated from the EK model that he obtains a result consistent with the EK model. Until the greater difference of 2.1 from the AK model result is tested, no final conclusions should be drawn for the daunorubicin system.

The situation with $Mg(ATP)^{2-}$ is considerably more complicated. In his equation 66 Poland quotes an incorrect H2 chemical shift for a molecule within a long stack of 6.95 ppm [2], where the value from the original paper is 7.35 ppm [5]. However, Poland’s Figure 14 shows a more reasonable limiting value for this system. The original paper deduces a limiting chemical shift difference between monomer and molecule within a stack as 0.93 ppm [5], and we assume that Poland used a value close to that in his critical equation 64. Poland than shows that for the two and three moment distributions the successive values of the equilibrium constants for stacking decrease, but not as much as required by the full fledged attenuated K model. However, the more refined analysis shows that the equilibrium constant for the first step in the self-association of $Mg(ATP)^{2-}$, dimer formation, is only 0.41(instead of 1.00) of the subsequent additions in the EK model and a corresponding amount in the AK model [1]. A further consequence of this result is that the limiting chemical shift differences are not 0.93 as above, but only 0.68 in the EK model and 0.80 in the AK model. With these complications the $Mg(ATP)^{2-}$ system becomes one of the poorest to test appropriateness of models by the maximum-entropy method, and no conclusions may be drawn.

We conclude that in both systems tested by Poland he used incomplete or inappropriate choices for the limiting values for molecules within a stack. The hidden assumptions refer to the choice of values to employ and not to the maximum entropy method itself. Until a more thorough analysis is performed no conclusions may be drawn as to the efficacy of the maximum-entropy

method to differentiate between the EK and AK models.

Are there systems that appear especially amenable to testing the maximum-entropy method as a differentiator? Non-linear least squares analysis of stacking in 6-methylpurine gave identical limiting H2 and H8 chemical shift values for both the EK and AK models, as shown in Table 3 of Ref. [1]. The observed concentration range extends to almost one molal, only nearest neighbor interactions are significant, and the equilibrium constant for dimer formation is identical with that for subsequent additions to the stack. It would be interesting to learn the results of a maximum-entropy analysis of stacking in 6-methylpurine.

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