Kinetics of Small Ising Systems: Deviations from Internal Equilibrium in a Tetrahedral Model*

(cooperativity/protein complex/conformational change/nerve membrane)

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ABSTRACT Computer modeling is used to derive some of the exact kinetic properties of a small Ising system.

This paper has two purposes: (a) to extend remarks concerning kinetic departures from internal equilibrium, in small Ising systems, that were contained in an earlier paper by Hill and Chen (1) on K^+ transport across the nerve membrane; and (b) to serve as an introduction to a more detailed paper (part II) on this subject to be published elsewhere. We confine ourselves here to one model and one kinetic scheme, as an illustration. Our point of view is essentially "experimental": we report on some of the exact kinetic properties of the chosen system, as calculated by computer; we do not offer any grand generalities. In particular, we concentrate on the extent to which the actual transient state of the system deviates, at any time, from the nearest internal equilibrium state.

We refer the reader to a few other somewhat related papers on the kinetics of small (2-5) and large (6-8) Ising systems. In general, large kinetic Ising systems have to be treated by one or another kind of approximation, even in one dimension.

THE MODEL

The model we investigate is Scheme 1, Eqs. 1 and 2 of ref. 1. For the convenience of the reader, we repeat the basic equations and definitions here.

We consider an ensemble of identical systems. Each system contains four identical subunits (e.g., protein molecules), arranged in a tetrahedron. Each subunit can be in one of two conformations, i and ii . The conformational change for a hypothetical isolated (i.e., independent) subunit follows first-order kinetics with intrinsic rate constants α (for $i \rightarrow ii$) and β (for $ii \rightarrow i$). Let w_{11} be the interaction energy between two subunits in conformation i , w_{12} between an i and an ii , etc. There are six pair-interactions in the system (of four subunits). Let Q_1^4 be the (canonical) partition function for the system in state [0] and Q_2^4 for state [4], where $[j]$ denotes the state with j subunits in conformation ii . Then the probabilities (i.e., fractions of systems in the ensemble) p_j^e of the five states, at equilibrium, are

$$p_0^e = Q^4 \xi^{-1}, p_1^e = 4s^3 Q^3 \xi^{-1}, p_2^e = 6s^4 Q^2 \xi^{-1}, \\ p_3^e = 4s^3 Q \xi^{-1}, p_4^e = \xi^{-1} \quad (1)$$

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where

$$\xi = Q^4 + 4s^3 Q^3 + 6s^4 Q^2 + 4s^3 Q + 1 \\ Q = Q_1/Q_2 = \beta/\alpha \\ s = e^{w/2kT}, w = w_{11} + w_{22} - 2w_{12}.$$

Clearly, the p_j^e are determined by the parameters Q and s . "Cooperativity" occurs when $w < 0$ and $s < 1$, and "anti-cooperativity" when $w > 0$ and $s > 1$. The subunits are independent when $w = 0$ and $s = 1$; this is the "Hodgkin-Huxley" case (1, 2) in the nerve membrane problem. Q is the intrinsic i/ii partition function ratio, or equilibrium constant (for $ii \rightleftharpoons i$).

An indefinite number of kinetic schemes can be consistent with this equilibrium model. We consider here the "symmetrical" scheme

The kinetic behavior of the ensemble is governed by a set of five (four independent) linear first-order differential equations in the p_j 's, which can be solved on a computer by standard matrix methods. For example, from Eq. 2, we have

$$\frac{dp_0}{dt} = \beta s^{-3/2} p_1 - 4\alpha s^{3/2} p_0, \quad (3)$$

etc.

In a typical "experiment," we start with an ensemble of systems at equilibrium with a given (initial) Q_i and observe the time evolution of the $p_j(t)$ to a final equilibrium at a different (final) Q_f . Eq. 1 gives initial and final probability distributions. To be more explicit: $\beta/\alpha = Q_f$ in Eq. 3 and the boundary conditions at $t = 0$ are $p_j(0) = p_j^e(Q_i, s)$ (we suddenly switch from "initial" to "final" rate constants, α and β , at $t = 0$, but the value of s remains unchanged).

A particular kinetic case, or "experiment," is characterized by values of Q_i , Q_f , and s . To be complete, though, we would still have to specify, say, $\alpha + \beta$ (to fix the time scale), but instead of doing this we shall use for the time variable $t/t_{1/2}$, where $t_{1/2}$ (in each case) is the value of t at which

$$\bar{j}(t) \equiv \sum_{j=0}^4 j p_j(t) \quad (4)$$

has undergone half of its total change. Thus

$$\bar{j}(t_{1/2}) = \bar{j}(0) + (1/2) [\bar{j}(\infty) - \bar{j}(0)]. \quad (5)$$

The complete range of $\bar{j}$ is $0 \leq \bar{j} \leq 4$; $\bar{j}$ = mean number of subunits in conformation ii .

There is a rather obvious symmetry between a case with Q_i, Q_f, s and one with Q_i^{-1}, Q_f^{-1}, s . Hence, it suffices to discuss cases with $0 \leq Q_f \leq 1$, for which $2 \leq \bar{j}(\infty) \leq 4$.

PROPERTIES OF THE MODEL

Hill and Chen (1) point out that, in kinetic cases of the type described above, in general, although the system starts and finishes with equilibrium sets of p_j 's (given by Eq. 1), intermediate sets of p_j 's—i.e., at arbitrary times—do not correspond to any equilibrium distribution. That is, a single parameter $Q(t)$ does not suffice to determine all the $p_j(t)$'s and thus to characterize an intermediate state of the ensemble. The exceptions (1) are $s = 1$, $s \rightarrow 0$, and $s \rightarrow \infty$.

Our primary interest here is in examining these time-dependent departures from "internal equilibrium." As a measure of this property, we have chosen the function

$$\Delta(t) = 5 \sum_j [p_j(t) - p_j^e(Q_e(t), s)]^2 \quad (6)$$

where the p_j^e are an equilibrium set and $Q_e(t)$ is that particular value of Q in Eq. 1 which, at the specified t , minimizes Δ . That is, $\Delta(t)$ is a measure of the absolute deviation of the actual kinetic state at t from the *nearest* equilibrium state (using a sum-of-squares criterion). In the exceptional cases mentioned above (1), the system passes through equilibrium states only and $\Delta(t) \equiv 0$.

A quantity related to $\Delta(t)$, and used somewhat below, is $\sigma^2(t) - \sigma_e^2(t)$, where $\sigma^2(t)$ is the actual variance at t ,

$$\sigma^2(t) = \bar{j}^2(t) - \bar{j}(t)^2 \quad (7)$$

and $\sigma_e^2(t)$ is the variance of the nearest equilibrium distribution, as found from Eq. 6.

The behavior of the mean, $\bar{j}(t)$, is not particularly interesting or discriminating. A few examples for $s = 1.0, 0.7, 0.5, 0.3$, and 0.1 are shown in Figs. 1-3 of ref. 1. All of these curves have a more or less exponential appearance. The behavior of $\bar{j}(t)$ is exactly exponential for $s = 1$, and virtually so for $s = 0.1$ (1).

FIG. 1. Δ as a function of $t/t_{1/2}$ for fixed Q_i and Q_f , but various $s < 1$. Each curve has its own $t_{1/2}$ value.

FIG. 2. $\sigma^2 - \sigma_e^2$ for the same cases as in Fig. 1.

CALCULATIONS

We turn now to the figures, which provide a sampling of our calculated results. Even such a small and simple system exhibits quite complicated behavior of the relatively sensitive function $\Delta(t)$.

Fig. 1 shows the effect on $\Delta(t)$ of variation of the strength of cooperative interactions ($s < 1$). The values $Q_i = 2.0$ and $Q_f = 0.1$ are chosen the same in all cases; these values require that $Q_e(t)$ pass (decrease) through unity and that $\bar{j}(t)$ pass (increase) through $\bar{j} = 2$. As is apparent from Fig. 1, the largest deviations from internal equilibrium are reached (for these particular values of Q_i and Q_f) at an intermediate value of s , probably about $s = 0.33$ or 0.34 . Correspondingly, Hill and Chen (1) found in their cases that "superposition" was at its worst at approximately $s = 0.4$. Also, it will be noted in this figure that there is an early single peak in $\Delta(t)$ for $s < 0.4$ (strong cooperativity), but that a second later peak arises

FIG. 3. 10Δ as a function of $t/t_{1/2}$ for fixed s and Q_f , but various Q_i . $t_{1/2}$ for $Q_i = 10$ is used for all curves. Curves are translated horizontally to give superposition at large t .

FIG. 4. 10Δ as a function of $t/t_{1/2}$ for fixed s and Q_f , but various Q_i . Each curve has its own $t_{1/2}$ value.

for $s > 0.4$ (weak cooperativity). The vertical scale of the $s = 0.9$ curve in Fig. 1 is expanded by a factor of 10.

The growth of the second peak in Fig. 1 can also be followed in Fig. 2, which shows $\sigma^2(t) - \sigma_e^2(t)$ for the same cases as in Fig. 1. For $0.5 \leq s \leq 1.0$, the minimum in Δ occurs near $\sigma^2 - \sigma_e^2 = 0$, which is not surprising. (This property continues into the region $s > 1$, except in the approximate intermediate range $1.4 \leq s \leq 4$.)

The "false" approaches to equilibrium (minima in Δ) that can be seen in Fig. 1 (see below for more examples) are made

FIG. 5. 100Δ as a function of $t/t_{1/2}$ (each curve) for fixed s and Q_i , but various Q_f .

FIG. 6. 1000Δ as a function of $t/t_{1/2}$ (each curve) for fixed Q_i and Q_f , but various $s > 1$. Compare Fig. 1.

possible by the fact that the rate constants governing the entire kinetic process (Eq. 2) belong to the final equilibrium state, and *not* to intermediate states. Even if an intermediate equilibrium distribution with, say, Q_e' is approached very closely (as, for example, in the $s = 0.9$ case in Fig. 1), this state will not be maintained because $\beta/\alpha = Q_f \neq Q_e'$. In effect, in such a case, the system merely starts over again with a new $Q_i \cong Q_e'$.

Hill and Chen (1) discussed "superposition" (or lack thereof), using the variables $p_i(t)$ and $\bar{j}(t)$. We supplement their work with Fig. 3, which illustrates superposition, at large values of t only, by means of the more sensitive function $\Delta(t)$. In this example, with $s = 0.7$, the initial state (Q_i) is varied while the final state is held fixed ($Q_f = 0.1$). Unlike the other figures in this paper, except Fig. 7, a single time scale ($t_{1/2}$ value) is used (that for $Q_i = 10$) for all curves. Curves for $Q_i \neq 10$ have been translated to the right to achieve superposition with the $Q_i = 10$ curve at large times. Conclusion: despite different starting points, the final ap-

FIG. 7. 100Δ as a function of $t/t_{1/2}$ ($Q_i = 10$) for fixed s and Q_f but various Q_i , with superposition at large t . Compare Fig. 3.

proach to the same final equilibrium state is made along the same nonequilibrium "track" (1). This is a consequence of the domination of the system behavior, at large t , by a single eigenvalue of the kinetic matrix from Eq. 2.

Fig. 4, with $s = 0.5$, illustrates the fact that the early peak in $\Delta(t)$, which occurs when $Q_i > 1$ and $Q_f < 1$, is particularly dependent upon Q_i and disappears as $Q_i \rightarrow 1$ (each curve in Fig. 4 has its own $t_{1/2}$ value, as usual). The same phenomenon can be seen in Fig. 3 ($s = 0.7$). On the other hand, the second peak is particularly dependent upon Q_f and disappears (Fig. 5, $s = 0.7$) as $Q_f \rightarrow 1$ (also when $Q_i > 1$ and $Q_f < 1$). Incidentally, in Fig. 4, the peak of $\Delta(t)$ increases with Q_i and $\Delta(\text{peak}) \rightarrow 0.14$ as $Q_i \rightarrow \infty$.

We turn now to some examples with $s > 1$ (anticooperativity). When $s = 1$, the p_j^e distribution (Eq. 1) is binomial (approximately Gaussian). When $s < 1$, the distribution is flattened or even ($s \rightarrow 0$) double-peaked (at p_0^e and p_4^e). When $s > 1$, the single peak is sharpened and the center of the distribution (p_1^e, p_2^e, p_3^e) is favored by s factors (Eq. 1). A consequence of this quite different situation when $s > 1$ is that the Δ values are generally much smaller, as are also the $\sigma^2 - \sigma_e^2$ values. The former effect is seen in Fig. 6, the analogue of Fig. 1 (in which $s < 1$). That is, s is varied, with $Q_i = 2.0$ and $Q_f = 0.1$. Though $\Delta(t)$ is smaller, it behaves in a rather more complicated way.

Fig. 7 corresponds to Fig. 3 ($s = 0.7$). In Fig. 7, we have $s = 1.5$, $Q_f = 0.1$, and Q_i is varied. All curves are on the same time scale ($t_{1/2}$ for $Q_i = 10$), and those for $Q_i \neq 10$ have been shifted to the right to give superposition at large t . The fact

that these are very small values of Δ (compared to the case when $s < 1$) means, of course, that the system is almost in internal equilibrium, despite the gyrations of Δ in Fig. 7. This can be verified by a test of superposition using $\bar{j}(t)$, as in ref. 1. The $\bar{j}$ superposition here is found to be virtually exact, though this was not the case for intermediate $s < 1$ in ref. 1. Incidentally, the $\bar{j}(t)$ curves here are not exponential in form [whereas exponential behavior accompanied superposition when $s < 1(1)$].

Fig. 7 shows also (as in Figs. 3 and 4) the disappearance of the early peak in $\Delta(t)$ as $Q_i \rightarrow 1$. On the other hand, as in Fig. 5, only the early peak remains if we let $Q_f \rightarrow 1$ keeping, say, $Q_i = 2.0$. (In both cases, $Q_i > 1$, $Q_f < 1$.)

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1. Hill, T. L. & Chen, Y. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 1711-1715.
2. Hill, T. L. & Chen, Y. (1971) *Proc. Nat. Acad. Sci. USA* **68**, 2488-2492.
3. Hill, T. L. & Plesner, I. W. (1964) *J. Chem. Phys.* **41**, 1359-1367.
4. Hill, T. L. & Plesner, I. W. (1964) *J. Chem. Phys.* **43**, 267-285.
5. Gordon, R. (1968) *J. Chem. Phys.* **49**, 570-580.
6. Glauber, R. J. (1963) *J. Math. Phys.* **4**, 294-307.
7. Silberberg, A. & Simha, R. (1968) *Biopolymers* **6**, 479-490.
8. Orwell, R. A. & Stockmayer, W. H. (1969) *Advan. Chem. Phys.* **15**, 305-324.