CORRESPONDENCE

KINETIC MODELING OF BLOOD PLATELET SHAPE CHANGES

To the Editor,

In a recent article in Blood, Hantgan has drawn certain conclusions about platelet shape change kinetics that deserve comment. Previous work indicates that the shape changes induced by stimulation with ADP can be effectively modeled by a simple A -> B -> C series reaction involving two first-order rate constants, k1 and k2 (hereafter called the ABC model), which can be written symbolically as

\[ \text{disc} \rightarrow \text{"sphere"} \rightarrow \text{smaller spiny sphere} \]

A prior analysis of turbidimetric data in terms of the specific physical and kinetic laws that describe this model led to the conclusion that the rate constants were identical. Such behavior is characteristic of a stochastic (Poisson) process, and if true, may imply the existence of a rate-limiting step common to both "sphering" (formally the A -> B reaction) and to the extension of long, filiform pseudopods at the expense of a concomitant decrease in body volume (formally the B -> C reaction).

Supposedly applying the same ABC model to ADP-induced changes in right-angle light scattering by platelet suspensions, Hantgan, on the other hand, concluded that the two rate constants were different and therefore that the mechanism was not the same as previously suggested. This conclusion and others that follow from it appear to be based on a misconception as to what constitutes the ABC model, as follows. According to the model, the intensity I(θ, t) scattered at angle θ and time t by a mixture of three morphologically distinct platelet forms A, B, and C is given by the sum of the products of their individual scattered intensities a(θ), b(θ), and c(θ), and their respective time-dependent concentrations A(t), B(t), and C(t) (Equation 1):

\[ I(θ, t) = a(θ)A(t) + b(θ)B(t) + c(θ)C(t) \]

Inserting the integrated rate equations that describe the concentrations in terms of the rate constants yields an explicit expression for the change in scattered intensity containing the five independent constants \( a, b, c, k_1, \) and \( k_2 \), the values of which are to be determined. This can be cast into a two-exponential form with constant coefficients (Equation 2):

\[ I(θ, t) = P_0 + P_1 \exp\left(-k_1t\right) + P_2 \exp\left(-k_2t\right) \]

where specifically (Equation 3):

\[ P_0 = A_0 c \]
\[ P_1 = A_0 [a - c + k_1(b - c)/(k_2 - k_1)] \]
\[ P_2 = A_0 k_1(c - b)/(k_2 - k_1) \]

Note that fitting data to the model by means of least squares requires use of the expanded or full equation resulting from the insertion of Equations 3 into Equation 2; otherwise the partial derivatives \( dI/dθ, dI/db, dc/dc, dI/dk_1, \) and \( dI/dk_2 \) needed in the analysis procedure will be incorrectly evaluated (the composite constants \( P_i \) are not independent, and by themselves have no explicit meaning in terms of the desired parameters).

While the aforementioned constitutes a rigorous description of the ABC model, it is often true that "several sums of exponentials may be consistent with the same set of data, and the question of uniqueness of representation is left open in many applications. In particular, an arbitrary two-exponential decay function such as that used by Hantgan can have the same general form as Equation 2 and may even fit the data well, but the parameters which best values are to be found (here \( P_1 \) and \( k_2 \)) have quite different meanings: \( P_1 \) and \( k_2 \) are in fact independent constants (arbitrary amplitudes) that refer to separate single-exponential decay processes that begin at the same time and proceed in parallel, and \( P_1 - P_2 - P_0 \). No reference to the component intensities \( a, b, \) and \( c \) is inherent in or otherwise implied by these definitions, the rate constants appearing in the arbitrary two-exponential model are not related to the rate constants defined by the ABC model, and the procedure used virtually guarantees that two different rate constants will found for asymmetric progress curves. Hantgen's Equation 2 thus does not represent an A -> B -> C reaction scheme as claimed, but is instead analogous to the independent parallel reaction scheme A -> B, C -> D. This model—which without further qualification implies the existence of two initial platelet forms and two products—has been tested on the turbidimetric data of reference 3 (using the necessary but physically unjustified assumption that \( A_0 = B_0 \)), and not surprisingly, the rate constants turn out to be almost exactly the same as those obtained by Hantgan using the arbitrary two-exponential fit.

A key question is whether the right-angle scattering data agree in form and time dependence with the earlier turbidimetric data, and it is instructive to compare the progress curve predicted by Hantgan's equation and rate constants with the progress curve generated by a stochastic ABC model having arbitrarily adjusted intensity parameters and a single-rate constant \( k_1 = k_2 = 0.156 \text{s}^{-1} \), the value derived from the turbidimetric data under comparable conditions. The two curves are remarkably similar, so much so that a careful reanalysis of Hantgan's scattering data in terms of the actual ABC model may lead to results that agree with those found by turbidimetry instead of apparently contrasting with them. Perhaps it will eventually turn out that the platelet shape change is not a stochastic process after all, but that appears to be something that remains to be decisively established.

There is one last point. It seems premature to enter into a detailed discussion as to whether the transient "sphere" has short, bleblike pseudopods and/or is somewhat flattened instead of being truly spherical, particularly when the optical measurements clearly dem-

onstrate that the shape change is a continuous transformation

\[ P_0 \rightarrow \ldots \rightarrow P_1 \rightarrow P_N \]

and that \( N \) is a very large number. The intermediate “sphere” inferred by the simple ABC representation of the actual reaction is nothing more—or less—than that particular stage of the ongoing morphologic alterations that, because its optical properties are significantly different from either the initial disc or the final product, happens to produce a maximum optical effect. Note also that, unlike turbidimetric data, there is no known theoretical basis for assigning platelet shapes or sizes on the basis of single-angle scattering data. The question of the continuously changing morphology can probably be answered by painstaking time-resolved microscopy similar to that carried out by Milton and Frojmovic and using even more sophisticated selection criteria; but it is not likely that microscopic examination of platelet samples fixed before, once during, and sometime after stimulation will shed much light on the shape of the transient(s). This is especially true when samples are taken at the optical maximum, which does not correspond to the time at which the concentration of the transient is equal, and even there one expects to find an approximately equal mixture of all three platelet forms.

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REFERENCES


To the Editor.

Although Dr Deranleau’s case is somewhat overstated, he has raised some issues that merit a detailed response. The sequential kinetic model that was employed is of exactly the same form as Deranleau’s, and the choice of the curve-fitting routine was well suited to the task of extracting the rate constants from the data. Finally, although the light-scattering kinetic data obtained at saturating concentrations of ADP and in the absence of Ca++ can be described adequately by a two-exponential model with either equal or equal rate constants, the former approach (which I employed) is supported by the unique dependence of the two-rate constants on the ADP and Ca++ concentrations, as well as by considerable morphologic data.

Similarity of models. Starting with Equation 2 in Deranleau’s letter, one can derive an expression for the fractional change in scattering intensity, \([I(t) - I(0)]/I(0)\), by straightforward algebraic manipulations. First, the initial scattering intensity, \(I(0) = A_{00}\), is subtracted from both sides of the equation, and the resultant expression normalized by dividing by \(A_{00}\). For convenience, we can define \(P_0 = (c - a)/a\), \(P_1 = P_1/a\) and \(P_2 = P_2/a\), and, noting that \(P_0 \neq P_1 \neq P_2\), rearrange the equation with this result (Equation 1):

\[
[I(t) - I(0)]/I(0) = P_0 - P_1 + P_1 \exp(-k_1 t) + P_2 \exp(-k_2 t)
\]

One further rearrangement leads to an equation of exactly the same form as my original Equation 2 (note that in Reference 1, I termed these rate constants \(k_1\) and \(k_2\) to distinguish them from the parameters that describe the rapid, reversible ADP-binding event (Equation 2):

\[
[I(t) - I(0)]/I(0) = P_1 \exp(-k_1 t) - 1 + P_2(1 - \exp(-k_2 t))
\]

I believe that this treatment verifies that we both have employed the same sequential model, and that Deranleau’s assertion that I misunderstood the model is invalid.

Validity of the curve-fitting procedure. Although the preexponential terms in Equation 2 are themselves functions of the scattering intensities of the initial discoid platelet, the intermediate forms, and the final spheroechinocyte, as well as the rate constants, the approach was to concentrate on determination of only the rate constants. This procedure enabled me to simplify the curve-fitting problem by employing a four-parameter fit (rather than the five-term fit used by Deranleau). The mathematical validity of this procedure is stated by Magar: “for those who are interested in relaxation times only, the explicit expressions for the \(A_{00}\) are best left alone.” Note that here \(A_{00}\) are the preexponential parameters analogous to \(P_1\) and \(P_2\) in Equation 2. It is common practice for investigators employing either relaxation methods or conventional kinetic techniques to take this approach.

In response to Deranleau’s criticism, I reexamined five of my data sets, obtained in the presence of a saturating ADP concentration, in the absence of Ca++, with his full five-term model. I found that with the 75-100 data points per set available, I was unable to uniquely determine all five parameters with any confidence. Therefore, I employed an alternative approach. Are the two rate constants equal? Deranleau employs Equation 2, which becomes indeterminate when \(k_1 = k_2\), to determine that, in fact, \(k_1 = k_2\). In order to avoid this mathematical dilemma, I used an equation that applies to that special case when the sequential model is valid, but the two rate constants are indeed equal. Here again, I have started with an expression and rearranged it to express the fractional change in scattering intensity (Equation 3):

\[
[I(t) - I(0)]/I(0) = (c - a)/a + (1/a)\{\frac{1}{a - c} + (b - c)k \exp(-kt)\}
\]

\[ k = k_1 + k_2 \approx k_1 \approx k_2 \]

Correspondence
Kinetic modeling of blood platelet shape changes [letter]

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