Computer Analysis of Feulgen Hydrolysis Kinetics

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Summary. A least squares fit of Feulgen hydrolysis time curves to the Bateman function is performed using an especially adapted parameter transformation together with a standard conjugate gradients iteration procedure. The method has been applied to a large number of measured data, and the use and limits of the computer evaluation are discussed.

Introduction

The time course of the appearance and loss of aldehyde groups stainable with Schiff's reagent during Feulgen hydrolysis is both of practical and of theoretical interest. On the practical side, a fast but reliable hydrolysis scheme is needed to yield maximum stainability. Conditions to reach maximum staining have recently been discussed for cells fixed in alcoholic fixatives (Kjellstrand, 1977). There are meanwhile rather reliable ways for reaching maximum staining under all kinds of conditions, and the hydrolysis scheme is the least problem in reaching quantitatively reproducible maximum staining.

The theoretical aspect of interest in Feulgen hydrolysis kinetics concerns cytological factors affecting the shape of the hydrolysis curve. Considering the strong influence of physical factors such as acid strength and hydrolysis temperature on the shape of the hydrolysis curve, cytological factors such as species- or tissue-specific nuclear proteins can be detected only after the physical factors have been quantitatively accounted for. Such a quantitative analysis ideally depends on a complete mathematical analysis of the shape of the hydrolysis curve.

Three parameters are required minimally to model the processes underlying chromatin hydrolysis. These are a constant, \( y_0 \), representing the maximum amount of potentially stainable groups present initially, a rate constant \( k_1 \), measuring the rate of depurination relative to the amount of purine-bearing sugars present, and a second rate constant, \( k_2 \), measuring the loss of DNA by depolymerization.
Using these three parameters, the time dependence, \( y(t) \), of stainable groups present, i.e. the Feulgen hydrolysis curve as measured, can be described by the following equation (Böhm and Seibert, 1966):

\[
y(t) = \frac{y_0 k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t}).
\]

A quantitative analysis of the parameters of Feulgen hydrolysis therefore depends on finding those values for the three parameters which most closely fit the measured data. Approximate values for the three parameters can be obtained graphically by estimating \( k_1 \) and \( y_0 \) from the initial slope of the hydrolysis curve and the maximum of the measured values (Bachmann, 1968, 1970) and \( k_2 \) from the tail-end of the measured curve beyond the point of inflexion. For some purposes such values are sufficiently accurate. A quantitatively reliable determination of the influence of cytological factors on the two rate constants, however, depends on obtaining best estimates and on obtaining some statistical measure of the reliability of such estimates. For this a systematic refinement of initial estimates of the parameters is needed which requires a computer for execution. Böhm and Seibert (1966) were the first to publish a computer method for obtaining Feulgen hydrolysis parameters. We have used this and similar methods to fit hydrolysis curves by Eq (1). Very quickly it became apparent that there is never a unique solution of the parameter fitting problem, and in some cases the numerical procedure even produces an apparently good fit with parameters far beyond any biologically meaningful range.

In the following we report on the mathematical reasons for this problem and present an improved method for calculating the hydrolysis parameters. Applying this method to a very large number of hydrolysis curves measured over the years in our laboratory, we have reached some clear conclusions about the kinds of data which justify computer analysis and the reliability of the parameters obtained.

**Numerical Analysis**

Mathematically, the problem of parameter fitting is equivalent to finding the global minimum \((y_0, k_1, k_2)\) of the error function

\[
V(y_0, k_1, k_2) = \sum_{i=1}^{N} [y_i - \bar{y}(t_i; y_0, k_1, k_2)]^2
\]

(least squares fit), where \( y_i \) is the value measured at time \( t_i \) \((i = 1, \ldots, N)\), and

\[
\bar{y}(t_i; y_0, k_1, k_2) = \frac{y_0 k_1}{k_2 - k_1} (e^{-k_1 t_i} - e^{-k_2 t_i})
\]

the value of the estimate function \( \bar{y}(t) \) at the same time, depending on the parameters.

This resembles very closely the standard problem of “exponential approximation”, i.e. approximating a set of measured data by a sum of exponential functions

\[
\bar{y}(t) = \sum_{j=1}^{m} a_j e^{-\lambda_j t}, \quad a_j, \lambda_j \geq 0,
\]