RECORDING OF FAST BIOCHEMICAL REACTIONS USING A LOGARITHMIC TIME SWEEP

EUGENE HAMORI AND TOSHIHIRO HIRAI, *Department of Biochemistry, Tulane Medical Center, New Orleans, Louisiana 70112 U. S. A.*

Kinetic data are conventionally obtained by recording concentration changes on a linear time scale. Our work is concerned with the application of a time base (oscilloscope time sweep) which moves not linearly but logarithmically with the elapsed time of observation.

When using such a time base the units of the horizontal scale (x) are related to the elapsed time of the observation (t) as $x = \ln(t)$. Since in this type of recording the fast initial changes of the reaction are spread out by the rapidly moving time coordinate and the slower changes are compressed by the decelerating time base, practically the entire time-course of the reaction can be accommodated on a small record with an even apportionment of the abscissa to the time domains involved (nanoseconds, microseconds, seconds, etc.). In a logarithmic recording a first-order reaction represented by the function $y = \exp(-kt)$ will be transformed to a sigmoidal curve $y = \exp[-k\exp(x)]$ shown in Fig. 1. If the data are stored in a digitized form, the derivative of this curve can be readily computed, $y' = -k\exp[x - k\exp(x)]$, an asymmetrical bell-shaped curve whose peak is at $x = -\ln(k)$ (Fig. 2). Thus the numerical value of the rate constant can be simply read off from the peak position of the curve. The rate equation of a second-order reaction, $1/y = 1/y_0 + kt$, would appear as $y = y_0/[y_0 k\exp(x) + 1]$ in the x-y system. The derivative of this curve, $y' = -y_0^2 k\exp(x)/[y_0 k\exp(x) + 1]^2$, is a symmetrical bell-shaped curve whose peak is at $x = -\ln(y_0 k)$ (Fig. 3). Thus logarithmic recording allows an immediate visual distinction between the first- and second-order reactions and the establishment of the rate constants without additional replotting. (A zeroth order reaction, $y = kt$, will appear as an exponential curve, $y = k[\exp(x)]$, on the logarithmic record, easily distinguishable from the traces of the first- or second-order reactions.) Complex reactions will appear as multiple-step sigmoidal curves. The derivatives of these will have several peaks related to changes occurring at various time domains. If the complex reaction is the result of a minor perturbation of an equilibrium (as in a relaxation experiment), the peak positions of the curve will indicate the individual relaxation

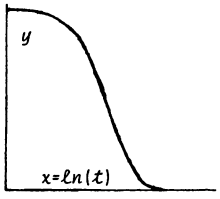

FIGURE 1

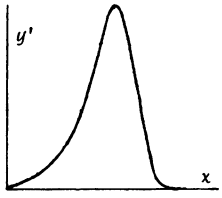

FIGURE 2

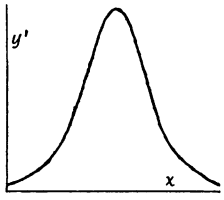

FIGURE 3

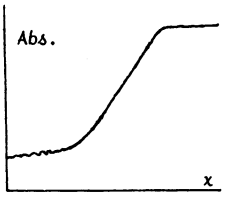

FIGURE 4

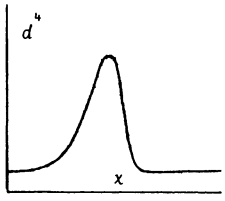

FIGURE 5

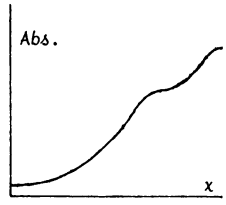

FIGURE 6

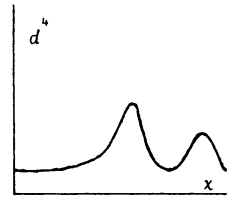

FIGURE 7

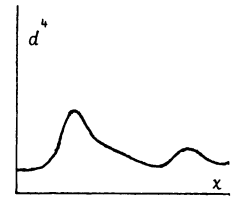

FIGURE 8

times involved, provided they are sufficiently separated in time (if not, a shifting of the peak positions will occur; however, this can be corrected by a simple iterative computer program).

Our instrumentation consisted of a Durrum stopped-flow apparatus (Durrum Instrument Corp., Sunnyvale, Calif.) and a Tektronix digital processing oscilloscope (DPO) coupled to a programmable desk calculator (TEK 31 Tektronix, Inc., Beaverton, Ore.). The logarithmic time base was generated by feeding an adjustable ramp function into a four-decade logarithmic amplifier (Solid State Electronics Corp., Sepulveda, Calif., 3076) and connecting its output to the external-volts output of the DPO's time base (7B70). The direct record of the reaction captured by the DPO had to be smoothed first in the calculator to eliminate the excessive noise which hindered the subsequent differentiation of the curve. Fig. 4 is the record of the pseudo-first-order reaction between Fe^{+++} and SCN^{-} before curve smoothing. Fig. 5 is the derivate of the smoothed version of the same curve raised to the fourth power. Figs. 6 and 7 are corresponding curves for the reaction between chymotrypsin and *p*-nitrophenyl acetate. Fig. 8 represents the amylose/iodine reaction. The latter figures indicate the presence of two distinct processes in these complex reactions, respectively.

Our work suggests that the logarithmic recording of kinetic results combined with the processing of the data in a DPO can be a very useful tool in the fast-reaction kinetic studies of biochemical reactions.

This work was supported by National Institutes of Health grant GM 20008.

FAR-ULTRAVIOLET STOPPED-FLOW CIRCULAR DICHROISM

JERRY LUCHINS, *Department of Biological Sciences, Columbia University, New York 10027 U. S. A.*

To follow directly the secondary structure changes involved in rapid protein-folding processes, we have designed a stopped-flow circular dichroism (CD) instrument capable of millisecond-range time resolution in the far-ultraviolet region. A stabilized Xe light source, piezo-optical birefringence modulator and phase-sensitive, heterodyning