Unreliability of rate constants derived from a linear transformation of kinetic data, with special reference to cholesterol equilibration between phospholipid vesicles

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In the time-dependent transfer of a lipid from a donor to an acceptor vesicle population a_t is the amount transferred to the acceptor vesicles at time t , a_∞ is the equilibrium transfer value and a_0 is the value at zero time. In order to plot kinetic data (a_t) as $\ln[(a_\infty - a_0)/(a_\infty - a_t)]$ against time and to fit these with a linear regression, it is necessary to know the equilibrium value, a_∞ , or to choose one. Here it is shown that even if a very large error is made in the choice of a_∞ , the resulting plot can still be acceptably linear and the correlation coefficient of the regression acceptably high. When a_∞ is overestimated the rate constant derived from the slope of such a plot is underestimated. In extreme cases a 10-fold error can occur.

INTRODUCTION AND THEORY

The time-dependent transfer of a lipid from a donor to an acceptor vesicle population usually approximates to a first-order reaction and can be described by the simple exponential:

$$a_t = a_\infty - (a_\infty - a_0) \exp(-kt) \quad (1)$$

where a_t is the amount transferred to the acceptor vesicles at time t , a_∞ is the equilibrium transfer value, a_0 the value at zero time, and k is the overall rate constant. In an iterative non-linear curve-fitting procedure the values of a_∞ , a_0 and k are simultaneously optimized to give the best fit to the data. The parameters derived from the fit can then be fairly compared with the theoretical or expected values.

Even if no computer is available, k can still be derived from the slope of a plot of $\ln(\Delta a)$ against t , where, starting from any value of a_t , Δa is the amount subsequently transferred during any fixed time interval (Δt). a_∞ can then be calculated from eqn. (2):

$$a_\infty = (a_n - a_1) / \{\exp(-kt_1) - \exp[-k(t_n + \Delta t)]\} \quad (2)$$

where a_1 and a_n are the first and last measurements and t_1 and t_n the times at which they were made. The above (Guggenheim, 1926) and other similar (Christie Smith, 1926) procedures for deriving k and a_∞ for a reaction that has not gone to completion have been reviewed by Pardue (1989).

Linear transformation of kinetic data

Although non-linear curve-fitting routines are now widely available, rate constants and half-times are still frequently determined by the traditional, but much less desirable, method of linear regression (Backer & Dawidowicz, 1981; McLean & Phillips, 1981, 1982, 1984; Fugler *et al.*, 1985; Mütsch *et al.*, 1986; Lund-Katz *et al.*, 1988; Thomas & Poznansky, 1988). To do this, the kinetic data are transformed by using a logarithmic rearrangement of eqn. (1) (McKay, 1938):

$$\ln[(a_\infty - a_0)/(a_\infty - a_t)] = kt \quad (3)$$

and the rate constant is derived from the slope of a plot of $\ln[(a_\infty - a_0)/(a_\infty - a_t)]$ against time. This approach, which requires that the equilibrium value, a_∞ , be known, encounters a problem when time constraints or degradation prevent the reaction being followed to completion. Although, as noted above, Guggenheim (1926) has shown that k can be determined without knowing a_∞ ,

the more usual approach is to substitute a theoretical or expected value of a_∞ into eqn. (3), in order to derive k . Two recent examples of this are studies on the effect that the phospholipid composition of the donor vesicles has on the rate of cholesterol transfer to egg phosphatidylcholine acceptor vesicles (Thomas & Poznansky, 1988; Lund-Katz *et al.*, 1988). In these studies, cholesterol transfer from the sphingomyelin-containing donor vesicles did not approach the awaited equilibrium value. In order to transform the kinetic data and make the linear regression, it was assumed that cholesterol partitions equally between sphingomyelin and phosphatidylcholine and therefore that a_∞ depended solely on the relative concentrations of the donor and acceptor vesicles. This assumption is neither experimentally (see the Discussion) nor theoretically justifiable. A change in any one of the on–off rates will cause both the overall rate constant and a_∞ to change.

Dependence of a_∞ on the individual rate constants

Assuming that a given lipid equilibrates between donor and acceptor vesicles via the aqueous phase, then at t_∞ , where the net flux of the lipid on and off the two vesicle populations is zero, it follows that:

$$d_\infty \cdot k_{-1} = w_\infty \cdot k_{+1}$$

or

$$d_\infty = w_\infty \cdot k_{+1} / k_{-1} \quad (4)$$

$$a_\infty \cdot k_{-2} = w_\infty \cdot k_{+2}$$

or

$$a_\infty = w_\infty \cdot k_{+2} / k_{-2} \quad (5)$$

(where d_∞ is the concentration of the lipid in the donor vesicles and w_∞ that in water at t_∞). From eqns. (4) and (5) we get:

$$a_\infty / d_\infty = (k_{+2} / k_{-2}) / (k_{+1} / k_{-1}) \quad (6)$$

From eqn. (6), which is also valid for the transfer of the lipid via collisional contact, it can be seen that a change in any one of the four rate constants will result in a change in the equilibrium value, a_∞ . Only in the special case where the on–off rate constants change in parallel, so that k_{+1} / k_{-1} and k_{+2} / k_{-2} remain constant, can the overall rate constant change and the value of a_∞ remain unchanged.

Linear and non-linear curve-fitting to model data

To assess to what extent a bad estimate of a_∞ could affect the interpretation of kinetic data, five families of data sets with the

Table 1. Parameters derived from non-linear and linear fits

Five families of data sets were generated from the equation $a_t = a_\infty / \exp(kt)$ using values of a_∞ from 20 to 100 and a value for the rate of 0.347 h^{-1} ($t_{1/2} = 2 \text{ h}$), each datum was given a random error between $+3$ and -3 . Each of the five families of data sets was fitted as in Fig. 1, and the means $\pm$ s.d. for the parameters are given below.

Equilibrium value used*	As in Fig. 1(a) (non-linear fit)			As in Fig. 1(b) (linear fit with correct equilibrium value)		As in Fig. 1(c) (linear fit assuming 100 as the equilibrium value)	
	Equilibrium value found	Rate constant found (h^{-1})	r^2	Rate constant found (h^{-1})	r^2	Rate constant found (h^{-1})	r^2
20	20 ± 4	0.40 ± 0.15	0.977 ± 0.011	0.33 ± 0.09	0.794 ± 0.067	0.03 ± 0	0.754 ± 0.069
40	40 ± 4	0.36 ± 0.05	0.994 ± 0.002	0.35 ± 0.07	0.949 ± 0.019	0.07 ± 0.01	0.956 ± 0.009
60	59 ± 2	0.36 ± 0.02	0.996 ± 0.001	0.34 ± 0.02	0.981 ± 0.005	0.12 ± 0	0.976 ± 0.019
80	78 ± 2	0.37 ± 0.02	0.999 ± 0	0.34 ± 0.03	0.995 ± 0.001	0.20 ± 0.01	0.974 ± 0.005
100	98 ± 3	0.35 ± 0.02	0.999 ± 0	0.33 ± 0.01	0.992 ± 0.002	0.32 ± 0.01	0.992 ± 0.002

* Value used to generate the data.

same overall rate constant (0.347 h^{-1} , i.e. $t_{1/2} = 2 \text{ h}$) and a_∞ values of between 20 and 100 were generated, for a 6 h time period (i.e. 87.5% completion), from the equation:

$$a_t = a_\infty / \exp(kt) \quad (7)$$

To make the generated data a little more realistic, a uniformly distributed random error ranging between $+3$ and -3 was added. One such family of data sets is plotted in Fig. 1(a) as a_t against time and has been fitted by a non-linear curve-fitting procedure. The same data are plotted in Figs. 1(b) and 1(c) as $\ln[a_\infty/(a_\infty - a_t)]$ against time; for Fig. 1(b) the a_∞ values used to generate the data have been used in the transformation, and for Fig. 1(c) a value of 100 was used. The data sets in both these Figures have been fitted by linear regression.

A visual inspection of the linear regressions in Fig. 1(c) shows that, despite the incorrect value used for a_∞ , the fits to the data sets are apparently quite acceptable. The error of ± 3 effectively hides the curvature found in plots using data without this added error (results not shown). Average values of the parameters derived from fits to the five families of data sets are given in Table 1. The r^2 values resulting from the non-linear exponential fit are superior to those from either of the linear fits. However, the r^2 values resulting from the linear regressions are similar whether or not the correct a_∞ value is used.

DISCUSSION

The cholesterol content of subcellular organelle membranes is not simply related to either their total phospholipid (Colbeau *et al.*, 1971) or their total lipid (Paton, 1970) content. *In vitro* the equilibrium distribution of cholesterol between two populations of phospholipid vesicles (Wattennberg & Silbert, 1983; Yeagle & Young, 1986) and between phospholipid vesicles and red blood cells depends on the phospholipid composition of the vesicles

Fig. 1. Plots of model kinetic data against time for the transfer of a lipid between donor and acceptor vesicle populations

Data were generated from the equation $a_t = a_\infty / \exp(kt)$ using values for the equilibrium distribution (a_∞) of the lipid of 20 (●), 40 (○), 60 (◆), 80 (□) and 100 (■), and a value for the rate constant (k) of 0.347 h^{-1} ($t_{1/2} = 2 \text{ h}$); these data were then given a random error between $+3$ and -3 . In (a) the plotted data have been fitted by the above equation. In (b) and (c), the same data are plotted as $\ln[a_\infty/(a_\infty - a_t)]$ against time; in (b) the correct values of a_∞ were used and in (c) 100 was used. In (b) and (c), the data sets were fitted by linear regression.

(Lange *et al.*, 1979). Despite this, it is often assumed, in order to make a linear transformation of the data from kinetic experiments, that at equilibrium cholesterol partitions equally between all types of phospholipid. If this assumption is made when the phospholipid in the donor vesicles has a higher cholesterol affinity than that in the acceptor vesicles (e.g. sphingomyelin as against egg phosphatidylcholine), then the rate of cholesterol transfer will be underestimated. In an extreme case, even if the reaction is followed for three half-lives (i.e. 87.5% completion), a 10-fold underestimate of the rate constant is possible (Table 1).

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