ymerization reaction described here must occur on the surface of solid, liquid, or even gaseous methane, the yield being about 0.01 percent per day in the top layer. Any other source of ionizing radiation would add to this. We suspect that this phenomenon is general and applies to other gases as well. It has wide implications for the origin of the large molecules necessary for the origin and support of life as well as for an understanding of the composition of meteoritic and planetary matter.

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Affinity and Phosphorylation Constants for the Inhibition of Esterases by Organophosphates

Abstract. *Equations based on the assumption of a reversible first step in the reaction between organophosphate inhibitors and esterases are proposed for the bimolecular rate constant which now includes an affinity constant and a phosphorylation constant. The treatment applies when the inhibition reaction follows first-order kinetics.*

The bimolecular rate constant k_i is generally considered to be the most reliable criterion by which to measure the inhibitory power of an organophosphate for an esterase. The equation for evaluating k_i is based on the assumption that the organophosphate I and the esterase E react directly to form the irreversibly phosphorylated active center (EI_a) (*1*). This clearly implies that k_i is simply a phosphorylation constant. Perhaps because of this implication, studies which have attempted to relate the structure of inhibitors to their inhibitory power have tended to interpret structure largely in terms of phosphorylation potential. Correlation between phosphorylation potential, as expressed for example by the Hammett constant (*2*), and inhibitory power has in general been only moderately good (*3*, *4*), and at times there has been no correlation (*5*).

Inhibitory power is also expressed by (I_{50}), which is an empirical value, but which may be considered as a "loosely" defined function of k_i (*4*).

In contrast to the assumptions on which k_i is based, it is widely accepted that the reaction between the organophosphate and the esterase probably involves a reversible step which precedes phosphorylation and results in formation of an enzyme-inhibitor complex $(EI)_r$ (*6*).

The inhibition reaction is then,

$$\begin{matrix} E & + & I & \underset{k_2}{\overset{k_1}{\rightleftharpoons}} & (EI)_r & \xrightarrow{k_p} & (EI)_q \\ e & & i & & r & & q \end{matrix} \qquad (1)$$

where e and i are the initial concentrations of E and I, and r and q are the concentrations of $(EI)_r$ and $(EI)_q$, respectively. The individual rate constants are k_1, k_2, and k_p.

The reversible step will depend on the affinity of the inhibiting compound for the active site and is governed by the affinity constant K_a where $K_a = k_2/k_1$. Irreversible phosphorylation is governed by the phosphorylation constant k_p.

According to this interpretation, inhibitory power will depend on the affinity of the organophosphate for the active site of the esterase as well as on the rate of phosphorylation. But present procedures (*1*, *7*, *8*) ignore the possibility of a reversible step and the consequent affinity constant. What then is the relationship between k_i as it has been determined and K_a and k_p? Furthermore, it seems legitimate to consider whether the experimental procedures which have been used for determining k_i are adequate within the context of a reversible step.

One possible solution is given in the following treatment. The rate at which $(EI)_r$ is formed will be proportional to the concentration of free enzyme $(e - r - q)$ and to the inhibitor concentration. For real values of i and assuming $k_2 > 0$, it is evident that only a fraction of the enzyme remaining uninhibited at any instant during the reaction, that is $(e - q)$, will be in the form of $(EI)_r$. If i is substantially greater than e, it will remain essentially constant over the course of the reaction.

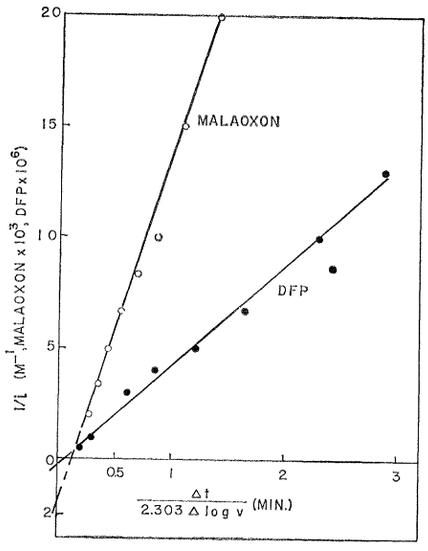

Fig. 1. Plot of the reciprocal of the inhibition rate ($\Delta t/2.303\ \Delta \log v$) against the reciprocal of the inhibitor concentration ($1/i$) according to Eq. 10 for the reactions of DFP and malaoxon with human serum cholinesterase at 37°C, *p*H 7.6. The unit of the ($1/i$) scale is $10^6\ M^{-1}$ for DFP and $10^3\ M^{-1}$ for malaoxon. The slopes gives k_i, the "bimolecular reaction constant," and the intercepts of the extrapolated lines on the ordinate and abscissa give $(-1/K_a)$ and $(1/k_p)$. The K_a and k_p values for malaoxon were $7.7 \times 10^{-4}\ M$ and 11 min^{-1}, respectively. Corresponding values for DFP could not be reliably determined, although estimates were made.

The reaction between E and I follows first-order kinetics over significant ranges of inhibitor and esterase concentrations when i is constant (*7*, *9*). The fraction of the esterase in the form of reversible enzyme-inhibitor complex must then bear a constant relationship to the total available esterase. That is, the ratio $[r/(e - q)]$ must be constant. The reversible reaction will then approximate a steady state at any instant during the reaction from which,

$$k_1 (e - r - q) i = k_2 r, \qquad (2)$$

and

$$r = \frac{(e - q) i}{i + K_a} \qquad (3)$$

The rate of irreversible inhibition is

$$dq/dt = k_p\, r \qquad (4)$$

Substituting Eq. 3 into Eq. 4 gives

$$dq/dt = \frac{i}{(i + K_a)}\, k_p\, (e - q) \qquad (5)$$

According to Eq. 5 the reaction is first-order with respect to e when i is constant.

Integrating Eq. 5 between the limits

q_1 and q_2 and t_1 and t_2 and observing that $\log (e - q_1)/(e - q_2) = \log (v_1/v_2) = (\Delta \log v)$ for the interval (Δt) (*1, 8, 9*), gives

$$\frac{1}{i} = \frac{\Delta t}{2.303\ \Delta \log v}\frac{k_p}{K_a} - \frac{1}{K_a} \qquad (6)$$

The ratio k_p/K_a has the dimensions of the bimolecular rate constant [min^{-1} (mole/liter)$^{-1}$, or $\text{min}^{-1}\ M^{-1}$]. If the value of K_a from Eq. 3 is substituted into Eq. 5 and we place

$$k_i = k_p/K_a \qquad (7)$$

then

$$dq/dt = k_i\ (e - q - r)i \qquad (8)$$

The expression for the rate of inhibition proposed earlier by Aldridge (*1*) is

$$dq/dt = k_i\ (e - q)i \qquad (9)$$

where direct interaction of E and I to form $(EI)_q$ is assumed.

The dimensions of the k_i terms in Eqs. 8 and 9 are the same, but the two constants have different meanings. The k_i in Eq. 9 is evidently a simple rate constant. The k_i of Eq. 8 has a more complex meaning as given by Eq. 7 and includes an equilibrium as well as a rate constant, and the term *rate* should therefore not be applied. Since both k_i terms govern the same reaction sequence and the term *bimolecular rate constant* is widely accepted in this context, it is suggested that the k_i of Eq. 8 be called the *bimolecular reaction constant.*

Substitution of Eq. 7 into Eq. 6 gives

$$\frac{1}{i} = \frac{\Delta t}{2.303\ \Delta \log v}k_i - \frac{1}{K_a} \qquad (10)$$

Equation 10 was derived on the assumption of a reversible step and on the observation that the inhibition reaction is first-order when i is constant. But i can be varied for different experimental reactions and yet remain constant over the course of any individual reaction. Equation 10 can then be applied experimentally since various values of $(\Delta t/2.303\ \Delta \log v)$ can be determined for corresponding values of $(1/i)$. Values of $(\Delta t/2.303\ \Delta \log v)$ are obtained from a plot of $\log v$ against t at constant i. According to Eq. 10, the plot of $(1/i)$ against $(\Delta t/2.303\ \Delta \log v)$ will be linear. The slope will be k_i, the intercept on the $(1/i)$ axis will be $(-1/K_a)$, and the intercept on the $(\Delta t/2.303\ \Delta \log v)$ axis will be $(1/k_p)$.

Intersection of the axes in the predicted quadrants by the extrapolated line would support the assumption of a reversible intermediate, as well as providing a means of evaluating k_p and K_a. If no reversible step occurred, the line would pass through the origin, but for this to be conclusive, the supporting experiment would have to include values of i and t^{-1} of an order which could reasonably be assigned to K_a and k_p.

Preliminary experiments were carried out using diisopropyl phosphorofluoridate (DFP) and malaoxon, (*O, O*-dimethyl S [1,2-dicarboethoxyethyl] phosphorothiolate) as inhibitors and a commercial preparation of human serum cholinesterase (*10*). Inhibitor and enzyme solutions were mixed and allowed to react from 0.5 to 3 minutes at *p*H 7.6, 37°C. Inhibition was stopped and the residual activity was measured by adding the mixture to a solution of acetylcholine substrate. The procedure has been described in detail (*9*).

The results were plotted according to Eq. 10 and are shown in Fig. 1. Both plots are reasonably linear. Extrapolation of the line for malaoxon gave intercepts from which significant values of k_p and K_a were calculated, but the intercepts of the line for DFP were too close to the origin to be of real significance. The results with malaoxon supported the proposed treatment. Because of experimental limitations those with DFP were inconclusive.

A value for k_i can be calculated for each point in Fig. 1 either by the equation of Aldridge (*1*), where

$$k_i = \frac{2.303\ \Delta \log v}{\Delta t}\frac{1}{i} \qquad (11)$$

or by rearranging Eq. 10, from which

$$k_i = \frac{2.303\ \Delta \log v}{\Delta t}\left(\frac{1}{i} + \frac{1}{K_a}\right) \qquad (12)$$

When calculated by Eq. 11, k_i values for malaoxon inhibition increased progressively from $7.7 \pm 0.4 \times 10^3$ to $1.36 \pm 0.12 \times 10^4 M^{-1}\ \text{min}^{-1}$ as i increased over the range, 5×10^{-4} to $5 \times 10^{-5}\ M$. The average k_i was $1.17 \pm 0.25 \times 10^4\ M^{-1}\ \text{min}^{-1}$. When calculated by Eq. 12, from the value of K_a obtained graphically ($7.7 \times 10^{-4}\ M$), the average was $1.42 \pm 0.11 \times 10^4\ M^{-1}\ \text{min}^{-1}$ and k_i did not vary significantly with change in i.

A similar analysis for DFP gave an average k_i of $4.18 \pm 0.71 \times 10^6$ and a lowest value of $2.96 \pm 0.34 \times 10^6\ M^{-1}\ \text{min}^{-1}$ at the highest DFP concentration ($2 \times 10^{-6}\ M$). These differences were barely significant, but together with the intercept values they suggested K_a and k_p values in the order of $1 \times 10^{-5}\ M$ and $30\ \text{min}^{-1}$.

Determination of k_i based on Eq. 11 is then valid only when K_a is several times i, and the present results suggest that k_i should be determined over a range of inhibitor concentrations. In addition, the present treatment or indeed that of Aldridge (*1*) should only be applied if the plots of $\log v$ against t are reasonably linear.

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Distribution of Narrow-Width Magnetic Anomalies in Antarctica

Abstract. *Data for aeromagnetic profiles obtained in Antarctica during the 1963–64 austral summer were used together with earlier results to construct a map showing the areal distribution of narrow-width magnetic anomalies. Numerous anomalies are associated with known volcanic mountains in western Antarctica. A large area of few anomalies is probably a result of an extension of the thick metasedimentary section observed in the Ellsworth Mountains. Portions of the Trans-Antarctic Mountains have associated anomalies which are probably caused by late Cenozoic volcanic rocks.*

Two field investigators (*1*) from the University of Wisconsin flew approximately 48,000 km of aeromagnetic profiles in Antarctica from November 1963 through January 1964. They used