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A simple method for the measurement of the hemolytic power of crude snake venoms

Marco A. Gómez-Leiva
Departamento de Bioquímica, Universidad de Costa Rica.
Liliana Pazos-Sanou
Departamento de Fisiología, Universidad de Costa Rica.

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Resumen: La acción citolítica de sustancias simples y de agentes biológicos complejos puede ser descrita por ecuaciones tipo Hill. Se propone la medición del poder hemolítico de venenos de serpientes en términos de la disminución de la acción lítica en una dilución 1:10. La ventaja de este procedimiento es que el poder citolítico es una función de las propiedades bioquímicas del sistema, más que de la concentración de los principios activos. Se muestran los poderes hemolíticos de algunos venenos de serpientes sobre eritrocitos humanos 0 Rh (+).

The quantitative analysis of the cell-lytic power of a pure substance or a complex biological agent is a troublesome task. For example, hemolytic activities are measured by means of different parameters like the so-called "Hemolysis Index 50" (Bernheimer *et al.*, 1980) or as "...the concentration of hemolysin necessary to produce complete hemolysis within 10 minutes" (Mebs, and Gebauer, 1980). Unfortunately, neither example is of a clear biochemical significance, since they depend only on the concentration of the lytic substance rather than on the intrinsic biochemical potency. Comparison between the lytic power of two substances measured by different parameters is also very difficult.

A simple way to deal with this problem is to define the lytic power of a biochemical agent in terms of the decrease in its action upon a ten-fold dilution:

$$\frac{dF}{d\log [V]}$$

where F is the fraction of lysis observed after equilibrium has been reached and [V] is the concentration of the agent in mg/L. This idea has been tested on the hemolytic power of the venoms of a group of snakes on 0 Rh (+) human erythrocytes in the presence of egg-yolk phosphatidylcholine.

The activities of the venoms as a function of their concentrations were determined by incubating for 1 hour* 0.10 mL of a 1% *per* volume egg-yolk lecithin suspension; 2.9 mL of buffered 0.15 M NaCl (pH = 7.2; 0.01 M phosphates) and 0.10 mL of a 2.5% suspension of washed 0 Rh(+) erythrocytes at 37°C followed by the addition of 1.0 mL of the venom solution. The concentrations ranged from 0.01 μg/mL to 1000 μg/mL. The extent of cell lysis was then determined by measuring the hemoglobin liberated spectrophotometrically at 540 nm.

The results obey an empirical Hill-type equation (Table 1), similar to that observed for hemoglobin oxygenation:

$$F = \frac{A\,[V]^n}{1 + A\,[V]^n} \qquad [1]$$

where n and A are the well-known parameters which measure the degree of cooperativity and the affinity between the erythrocyte membrane and the active components of a particular venom. This mathematical behavior is the same as that observed for the hemolytic action of complement (Eisen, 1980). Equation [1] can be converted to:

*Kinetic determinations made by Pazos-Sanou and Mata-Segreda in this laboratory indicate that 1 hour is sufficient time to attain equilibrium.

TABLE 1

Parameters for equation [1] and hemolytic powers of some snake venoms

	A	n	Hemolytic power
Bothrops asper	0.6	0.8	0.45
Bothrops godmani	0.2	0.8	0.26
Bothrops schlegellii (Green type)	0.4	0.6	0.28
Bothrops schlegelii (Yellow type)	0.04	0.8	0.071
Lachesis muta	0.6	0.4	0.19
Crotalus scutulatus	0.07	0.2	0.024
Crotalus durissus	0.06	0.1	0.013
Crotalus d. terrificus	0.1	0.2	0.047
Naja naja	0.5	0.2	0.12
Naja n. atra	0.6	0.2	0.21
Micrurus nigrocinctus n.	2.5	0.5	0.25
Bungarus multicinctus	0.4	0.2	0.070

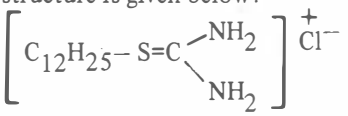
$$\log \frac{F}{1-F} = \log A + n \log [V] \qquad [2]$$

and the parameters easily obtained from plots of log (F/1−F) vs log[V], where the slope equals n and A is evaluated from the intercept.

From equation [1] one obtains:

$$DF/\log [V] = 2.3\ n\ A\ [V]^n / (1 + A[V]^n)^2 \qquad [3]$$

and finally we define the lytic power as dF/dlog [V] arbitrarily evaluated at [V] = 1 mg/L. Table 1 shows the results obtained. As a point of reference, an analogous experiment was carried out by using the synthetic detergent S-dodecylthiouronium chloride (Pazos, *et al.*, 1978) whose structure is given below:



For this compound $A = (4 \pm 1) \times 10^{-5}$, n = 2.7 ± 0.3 and the lytic power equals $(1.1 \pm 0.2) \times 10^{-4}$. The detergent operates through a mechanism that involves positive cooperativity (n > 1). When it is added, the bilayer structure in the membrane is progressively disrupted in such a way that an excess of the cationic amphiphile converts the system to one of globular detergent micelles in which small amounts of phospholipids are incorporated (Tanford, 1973). On the contrary, the venoms show negative cooperativity (n < 1), but an attempt to describe a possible mechanism by which these agents disrupt the bilayer ensamble is beyond the scope of this communication, since other processes besides the phospholipase A_2-catalyzed production of the tensoactive lisolecithin are involved (Fletcher, *et al.*, 1981).

The simplicity of this procedure enables one to determine the cell-lytic power of either pure substances or complex biological agents such as snake venoms in terms of the empirical parameters which define the system at hand, rather than in terms of the concentration at which those substances are present, making comparison of different cases meaningful.

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