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Minireview

# Linear and non-linear pressure dependence of enzyme catalytic parameters

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**Abstract**

The pressure dependence of enzyme catalytic parameters allows volume changes associated with substrate binding and activation volumes for the chemical steps to be determined. Because catalytic constants are composite parameters, elementary volume change contributions can be calculated from the pressure differentiation of kinetic constants. Linear and non-linear pressure-dependence of single-step enzyme reactions and steady-state catalytic parameters can be observed. Non-linearity can be interpreted either in terms of interdependence between the pressure and other environmental parameters (i.e., temperature, solvent composition, pH), pressure-induced enzyme unfolding, compressibility changes and pressure-induced rate limiting changes. These different situations are illustrated with several examples.
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*Keywords:* Hydrostatic pressure; Volume change; Activation volume; Compressibility; Rate-determining step; Inactivation; Unfolding; Dissociation

## 1. Introduction

Pressure has long been known to alter the rate constants of chemical and biological reactions. The pressure dependence of an equilibrium constant ($K$) and a rate constant ($k$) can be described by Eqs. (1) and (2).

$$\left(\frac{\partial \mathrm{Ln}K}{\partial P}\right)_T = -\frac{\Delta V}{RT} \quad (1)$$

$$\left(\frac{\partial \mathrm{Ln}k}{\partial P}\right)_T = -\frac{\Delta V^{\neq}}{RT} \quad (2)$$

In these equations $\Delta V$ and $\Delta V^{\neq}$ are the reaction volume and the activation volume, respectively; $R$ is the gas constant (82 ml atm $K^{-1}$ $mol^{-1}$) and $T$ is the absolute temperature. Thus, volume changes and activation volumes are related to free energy changes and activation free energy changes by the relations $(\partial \Delta G/\partial P)_T = \Delta V$ and $(\partial \Delta G^{\neq}/\partial P)_T = \Delta V^{\neq}$.

The pioneer works of Laidler [1], Johnson and Eyring [2] provided theoretical treatment for the influence of pressure on simple enzyme systems. The overall effect of pressure on enzyme reactions depends on the sign and magnitude of volume changes accompanying the binding and elementary chemical steps, substrate concentration and environmental conditions (temperature, solvent, etc). There are several recent books on high-pressure biochemistry and biophysics [3–7]. In addition, extensive reviews on theory and experimental of pressure effects on enzymes are available [8–12].

Values of volume changes and activation volumes provide unique information for determining molecular mechanism of enzyme-catalyzed reactions. However, interpretation of the size and magnitude of the volume changes and activation volumes is not straightforward because the experimental values of $\Delta V$ (or $\Delta V^{\neq}$) are the sum of three

*Abbreviations:* BChE, butyrylcholinesterase; HPSF, high-pressure stopped-flow

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contributions (Eq. (3)): (1) the intrinsic contribution ($\Delta V_{intr}$), i.e., interactions between chemical groups due to formation and breakage of bonds; for example, electrostatic interactions in an environment of low dielectric constant are accompanied by positive volume changes [8,11]. (2) A solvational contribution ($\Delta V_{solv}$), including solvation changes of the system's various components and changes in the water structure around interacting groups; for example, formation of hydrophobic interactions are accompanied by positive volume change due to release of free water molecules previously structured around non-interacting hydrophobic groups. (3) An enzyme conformational term ($\Delta V_{conf}$) associated to the conformational change accompanying binding or chemical steps. This latter contribution includes possible change in cavity size and/or disruption of voids due to substrate-induced conformational changes in flexible core segments close to the cavities:

$$\Delta V = \Sigma \Delta V_{intr} + \Delta V_{solv} + \Delta V_{conf.} \quad (3)$$

Solvent effects in chemical and enzyme reactions have been carefully studied, and it has long been known that there is a prominent contribution of solvation-related terms to activation volumes. Water molecules stabilize polar transition states. Electrostriction of solvent around charged groups is accompanied by large negative volume changes. For example, ionogenic reactions like ester hydrolysis are favored by pressure. The contribution of solvation to $\Delta V^{\neq}_{kcat}$ can be quantified in the case of reactions involving small molecules [13]. For example, the activation volume ($\Delta V^{\neq}$) at 25 °C for chemical hydrolysis of positively charged aromatic esters is expected to be −5 to −10 ml/mol. Given that $\Delta V^{\neq}_{intr}$ for breakage of an ester bond is +10 ml/mol, it follows that $\Delta V_{solv}$ is −5 to −10 ml/mol. In enzyme reactions, the solvational term is often the dominant contribution to net volume change or activation volume. Studies on the effect of osmotic pressure on protein conformation and enzyme kinetics [14,15], and cosolvent denaturation of proteins have emphasized the importance of preferential interactions of cosolvent on the solvational term [16]. By contrast, the conformational contribution to $\Delta V$ is often negligible, except in the case of oligomeric enzymes where cooperativity may be accompanied by large alterations in protein geometry and architecture.

## 2. Kinetic measurements under hydrostatic pressure

### 2.1. High-pressure equipments

The pressure dependence of enzyme-catalyzed reactions can be investigated using high-pressure optical cells equipped with sapphire windows for UV/visible or fluorescence measurements under pressure [17]. This method is convenient for recording steady-state velocity, but owing to the time needed for sealing the cell and thermal equilibration, information is lost within the first minutes of the reaction time-course. The use of a stopped-flow apparatus designed for operation under high hydrostatic pressure at controlled temperature (HPSF, from sub-zero temperature, −40 °C in hydro-organic solvents, to +40 °C) allow to investigate pre-steady state kinetics and steady-state kinetics over a time-scale of several minutes [18–20]. Fast reactions (dead time less than 2 ms) can be studied using a similar apparatus [21]. The stopped-flow device is accommodated into a high-pressure optical vessel; this vessel is connected through a fiber optic cable to a spectrophotometer or a fluorimeter. Measurements of reaction velocity are routinely carried out from atmospheric pressure ($P_0$=1 atm=1.013 bar=1.013 $10^5$ Pa) to $P$=200 MPa at varying temperatures. Silicon oil and highly purified water are generally the hydraulic fluids. Pressure is generated using either a screw pump or by a pneumatic pump, and is monitored on manganin electronic gauges. The steady-state catalytic parameters, $K_m$ and $k_{cat}$, can be determined at each pressure by non-linear computer fitting of the Michaelis–Menten equation using available kinetic soft wares.

### 2.2. Experimental approaches for transient reaction studies: single-step reactions

In carrying out their biological functions, enzymes go through a number of subtle conformational changes that are related to their dynamic structural flexibility. These processes are very rapid and therefore difficult to study.

Several approaches can be implemented for studying single-step reactions. Fast reaction methods (such as stopped-flow) or methods allowing to reduce the reaction velocity, e.g., by carrying out experiments at low temperatures, can be used [22,23]. This artifice can be associated to a fast reaction method to improve the detection of "apparent" velocity. However, the addition of organic solvents (antifreezes) to the aqueous solution is necessary to maintain the media liquid at temperatures below 0 °C. By the use of this approach, it can be possible to determine the different steps and identify the various intermediates in a reaction pathway.

To complete dynamic information about enzyme systems obtained both with temperature variations and kinetic studies (specially at low temperatures which permit exploration of wide temperature ranges, roughly from −50 to +50 °C), other thermodynamic parameters, e.g., pressure, chemical potentials, can be used.

## 3. Single step and transient enzyme reactions

### 3.1. General considerations

Enzymes fluctuate between different conformational states. Fluctuations can be either limited to local conformational changes or affect large domains. According to

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