

## PRESSURE EFFECTS ON BIOMOLECULES

**K. Heremans**

*Department of Chemistry, Katholieke Universiteit Leuven, Belgium*

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### Contents

1. Introduction
  2. Pressure effects compared with temperature effects
    - 2.1 The Le Chatelier and Braun principle
    - 2.2 Compressibility, thermal expansivity, heat capacity
      - 2.2.1 Definitions
      - 2.2.2 Statistical thermodynamics: Molecular fluctuations
    - 2.3 Phase changes in single component systems: Clausius-Clapeyron
    - 2.4 Solutions
      - 2.4.1 Molecular interpretation of compressibility and thermal expansivity
      - 2.4.2 Molecular interpretation of reaction volumes: Electrostriction
  3. Role of water and solvent composition
    - 3.1 Cavities in biopolymers
    - 3.2 Hydration of biopolymers
    - 3.3 Water activity and osmotic pressure
    - 3.4 Pressure effect on glass transitions
  4. Modeling the pressure-temperature behavior of Biomolecules
    - 4.1 Thermodynamic modeling
    - 4.2 Computer modeling
  5. Lipids and biomembranes
  6. Nucleic acids
  7. Polysaccharides
  8. Proteins
    - 8.1 Ligand-binding
    - 8.2 Protein-protein interactions
    - 8.3 Protein unfolding and intermolecular aggregation
      - 8.3.1 Protein unfolding: Conformational intermediates
      - 8.3.2 Stability phase diagram and intermolecular interactions
    - 8.4 Gel formation
    - 8.5 Mixtures of proteins and polysaccharides
  9. From molecules to cells.
  10. Conclusion: the specificity of pressure effects
- Acknowledgments  
Glossary  
Bibliography  
Biographical Sketch

## Summary

Pressure effects on proteins are among the most interesting when compared to lipids, nucleic acids and polysaccharides with the exception of starch. It is assumed that this is the consequence of the non periodic character of the structure of proteins and the specific interactions with water. The stability diagram that gives the conditions of temperature and pressure for the biological activity can be understood on the basis of the fact that the volume change for the unfolding is temperature as well as pressure dependent. Such a phase diagram, based on a two-state model, suggests that the heat-, pressure- and cold-unfolded state of the protein do not differ from each other qualitatively. More detailed studies however, indicate different structural changes for heat, cold and pressure unfolding. It is suggested that the shape of the phase diagram reflects the mechanism of unfolding and the aggregation of the unfolded protein. The results are relevant for the understanding of the occurrence of life under extreme conditions as shown by the fact that the inactivation of microorganisms, viruses and enzymes show stability diagrams similar to those observed for proteins.

## 1. Introduction

Whereas the effects of temperature on living matter are part of the very old stock of knowledge of mankind, the discovery that pressure affects life goes back to the mid 19<sup>th</sup> century. In addition, over a period of about a century the emphasis in our understanding of the effect of pressure on organisms has shifted from the question of how organisms can survive under such conditions to the recent discovery that some organisms actually like these extreme conditions.

The chapters in this volume show the wide varieties of life adapted to the extreme conditions of temperature, pressure, cold, salinity, pH, etc. Under such extreme conditions one encounters many, though not exclusively, prokaryotes, many of which belong to the archaea, an evolutionary line of microorganisms quite distinct from the other prokaryotes. Whether the presence of a nucleus and other organelles in eukaryotes reflects the sensitivity of a complex system in comparison with the simplicity of the prokaryotes, remains to be investigated. The biotechnological developments that have resulted from the research in this field are still expanding.

If we concentrate on the behavior of molecules then the observation of “a fact of possible biological interest” by P.W. Bridgman in 1914 stands out as a landmark. He observed that “if the white of an egg is subjected to hydrostatic pressure at room temperature, it becomes coagulated, presenting an appearance much like that of a hard boiled egg”. Being interested in phase transitions of compounds as a function of pressure and temperature, he also notes that “the effect of temperature, which is not large, seems to be such that the ease of coagulation increases at low temperatures, contrary to what one might expect”.

With a strategy developed to search for new pressure-induced transitions, Bridgman observed new phases of water among them the so-called “hot ice”. He reports: “It is well known that under ordinary conditions water is abnormal in many respects. The effect of high pressure is to wipe out this abnormality...the modification of ice stable at

high pressures giving indications of being the last form, corresponding to the completely normal liquid".

During the last century research was conducted on the inactivation of enzymes, viruses, antigens and antibodies, microorganisms, tissues and cells. One topic of particular interest is the pressure-induced denaturation of proteins. Here we see the first report on the pressure and temperature effects on ovalbumin and hemoglobin with the data reported as a stability diagram. At low temperatures and high pressures, negative activation energies are reported and interpreted as the pressure-induced penetration of water into the protein as the first step in the denaturation process. Recent high pressure computer simulations support this proposal. It is now possible to make a unified phenomenological description of the cold, heat and pressure denaturation of proteins.

This contribution concentrates on the structure properties rather than on the structure function relationship of biomolecules such as proteins, lipids, nucleic acids and polysaccharides. The results are relevant for the understanding of the occurrence of life under extreme conditions.

## **2. Pressure effects compared with temperature effects**

### **2.5 The Le Chatelier and Braun principle**

Pressure and temperature are macroscopic variables that define the properties of systems at equilibrium. Has thermodynamics any relevance to pressure effects? Yes, because when Lord Kelvin proposed the term, he was mainly concerned with the dynamic effects of heat.

From a thermodynamic point of view pressure is the potential for volume changes. This definition reflects the analogy with temperature as the potential for heat flux and the chemical potential (also called "chemical pressure") as the potential for the flux of matter.

The physico-chemical viewpoint of using extreme conditions is to explore the effect of temperature and pressure on the conformation, the dynamics and the reactions of biomolecules. The unique properties of biomolecules are determined by the delicate balance between internal interactions in the biomolecules which compete with interactions with the solvent. The primary source of the dynamical behaviour of biomolecules is the free volume of the system and this may be expected to decrease with increasing pressure. As temperature effects act via an increased kinetic energy as well as free volume, it follows that the study of the combined effect of temperature and pressure is a prerequisite for a full understanding of the dynamic behavior of biomolecules. By intuition, pressure effects should be more easier to interpret than temperature effects. This follows from the Le Chatelier and Braun principle (1888) which states, in its most general form, that a system, when subjected to a perturbation, responds in a way that tends to eliminate its effects. A quantitative expression for the Le Chatelier-Braun principle was given by van 't Hoff for the effect of temperature in 1884 and for the effect of pressure by Planck in 1887.

If  $K$  is a quantity characteristic of an equilibrium, then the influence of temperature and

pressure can be written as:

$$\left(\frac{\partial \ln K}{\partial T}\right)_p = \frac{\Delta H}{RT^2} \quad (1)$$

$$\left(\frac{\partial \ln K}{\partial P}\right)_T = -\frac{\Delta V}{RT} \quad (2)$$

These formula summarize the basic thermodynamic concepts in our description of pressure and temperature effects on equilibria. Note the positive sign for the temperature dependence and the negative sign for the pressures effects. For an endothermic process, the equilibrium shifts towards the products upon an increase in temperature, for a positive volume change, an increase in pressure shifts the equilibrium to the reactants.

Similar equations describe the effects on the kinetics of reactions. The concept of activation energy,  $E_a$ , was introduced by Arrhenius in 1889 and the activation volume,  $V_a$  ( $\Delta V^\ddagger$ ), in 1935 by Evans and Polanyi. An important point with relation to pressure effects on reaction velocities, is that the reaction, depending on the mechanism, may be either accelerated or retarded by high pressure, i.e.  $V_a$  may be negative or positive. Negative activation energies are meaningless from a physical point of view. The fact that they are nevertheless observed indicates a mechanism whereby a fast strongly exothermic equilibrium process precedes a slow ratelimiting process. This is observed in the temperature dependence of the pressure-induced denaturation of proteins. This point is discussed in section 4.1.

## 2.6 Compressibility, thermal expansivity, heat capacity

Before considering the heat capacity, the thermal expansivity and the compressibility of proteins and other biomolecules it is useful to consider pure substances. We will do so by considering the effect of pressure and temperature on the volume of a system, the isothermal compressibility ( $\kappa_T$ ) and the isopiestic thermal expansivity at constant pressure ( $\gamma_P$ ), but we will also consider the more familiar heat capacity at constant pressure ( $C_P$ ).

### 2.6.1 Definitions

The relative change in volume of a system with pressure, at constant temperature, is defined as the isothermal compressibility:

$$\kappa_T = -\left(\frac{\partial \ln V}{\partial P}\right)_T \quad (3)$$

The relative change in volume of a system with temperature, at constant pressure, is defined as the isopiestic thermal expansivity:

$$\gamma_P = \left( \frac{\partial \ln V}{\partial T} \right)_P \quad (4)$$

The change in heat content of a system (enthalpy, H) with temperature, at constant pressure, is defined as the isopiestic heat capacity:

$$C_P = - \left( \frac{\partial H}{\partial T} \right)_P \quad (5)$$

The change in heat content with pressure, at constant temperature, is related to the isopiestic thermal expansivity via the thermodynamic Maxwell relations.

Water and hexane are of particular interest since they represent typical examples of polar and nonpolar materials respectively. The compressibility of water is considerably smaller than that of most organic liquids. The volume of water is reduced by 10% at 300 MPa and by 15 % at 600 MPa. The volume of hexane is reduced by 20% at 300 MPa and by 25% at 600 MPa. The contribution from the strong intermolecular hydrogen bonding may be seen from the differences between water and glycerol or ethanol, benzene and hexane as given in Table 1. The distinction is less pronounced for the heat capacity.

	thermal expansivity $10^{-6}/\text{K}$	compressibility 1/100 GPa	heat capacity J/mol K
Water	210	46	75
Ice I	150	12	38
Glycerol	500	21.4	224
Ethanol	1120	109	112
Benzene	1220	96	136
Hexane	1380	166	195

Table 1: Isopiestic thermal expansivity ( $\gamma_P$ ), isothermal compressibility ( $\kappa_T$ ) and heat capacity ( $C_P$ ) of some liquids and ice I.

Data are at 25°C and 0.1 MPa.

Except for water, the temperature and pressure dependence of these parameters is less documented in the literature. Such data would be extremely valuable for the precise modeling of the effect of temperature and pressure on biomaterials. For water, the temperature dependence of the thermal expansivity shows a minimum at 4°C and the temperature dependence of the compressibility shows a minimum at 45°C. The minima disappear at 300 MPa.

If one performs the compression under adiabatic conditions, i.e. when no heat exchange with the medium takes place, then the following relation gives the temperature increase for a given pressure increase

$$\left(\frac{\delta T}{\delta P}\right)_S = \left(\frac{T}{C_p}\right)\left(\frac{\delta V}{\delta T}\right)_P = \frac{\gamma_P T}{\rho C_p} \quad (6)$$

In this equation,  $\gamma_P$  is the thermal expansivity,  $\rho$  the density and  $C_p$  the heat capacity of the system. The equation predicts for water a temperature increase of 2 K/100 MPa at 25°C and no temperature increase at 4°C. For hexane the temperature increases by 40 K/100 MPa at 18°C. It is clear that a pressure drop results in a temperature decrease of the same order of magnitude. At higher temperatures adiabatic heating gives larger temperature increases.

Pressure has a minor effect on the viscosity of pure water up to about 600 MPa. For the same pressure range, the viscosity of hexane increases tenfold.

### 2.6.2 Statistical thermodynamics: Molecular fluctuations

The compressibility is a thermodynamic quantity of interest not only from a static but also a dynamic point of view. Its relevance to the biological function can be understood through the statistical mechanical relation between the isothermal compressibility  $\kappa_T$ , and the volume fluctuations of biomolecules:

$$\langle V - \langle V \rangle \rangle^2 = \kappa_T k_B T V \quad (7)$$

where the expression between brackets indicates an average for the isothermal-isopiestic ensemble.  $k_B$  is the Boltzmann constant. Because of the small size of most biomolecules, the volume fluctuations are relatively large.

In a thermodynamic system with constant  $T$  and  $P$ , the isopiestic heat capacity can be regarded as a measure of the entropy fluctuations of the system:

$$\langle S - \langle S \rangle \rangle^2 = k_B C_p \quad (8)$$

A physical picture of entropy fluctuations means changing the conformation between ordered and less ordered structures. This can occur by hindered internal rotations, low frequency conformational fluctuations and high frequency bond stretching and bending modes.

Like the compressibility and heat capacity, the thermal expansivity can also be related to the fluctuations of the system, although this relation is not as widely known. The thermal expansivity is proportional to the cross-correlation of the volume and entropy fluctuations:

$$\langle SV - \langle S \rangle \langle V \rangle \rangle = k_B T V \gamma_P \quad (9)$$

This agrees with the intuition, that the thermal expansivity is a coupling between the thermal ( $T$ ,  $S$ ) and the mechanical ( $P$ ,  $V$ ) parameters.

## 2.7 Phase changes in single component systems: Clausius-Clapeyron

Before discussing the effects of temperature and pressure on dilute solutions of biological macromolecules, it is instructive to consider the effects on pure phases and phase transitions. The change in Gibbs free energy ( $G$ ) of the system with pressure and temperature is given by the following expressions:

$$\frac{\delta G}{\delta T} = -S \quad \frac{\delta G}{\delta P} = V \quad (10)$$

These relations allow us to compare the energy input in a given amount of water by applying a pressure of 1 GPa with a temperature increase to 100°C. It turns out that the energy input on heating is about twice that on compressing. It is of interest to note that for hexane the situation is almost exactly the opposite. More generally, a temperature increase of a system involves changes in energy and density whereas pressure affects primarily the density. This makes studies at constant density and variable energy important from a theoretical point of view.

The above equations can easily be applied to phase transitions of pure substances.  $\Delta H$  When  $\Delta G$  refers to the difference in free energy between the two phases, we get:

$$\frac{\delta \Delta G}{\delta T} = -\Delta S \quad \frac{\delta \Delta G}{\delta P} = \Delta V \quad (11)$$

The second derivative with respect to temperature and pressure respectively gives the Maxwell equations.

$$\left( \frac{\delta \Delta V}{\delta T} \right)_P = - \left( \frac{\delta \Delta S}{\delta P} \right)_T \quad (12)$$

These useful equations express the relation between the temperature effect on the volume changes and the pressure effect on the entropy changes.

Changes in the free energy difference ( $\Delta G$ ) with temperature and pressure are given by:

$$d(\Delta G) = (\Delta V) dP - (\Delta S) dT \quad (13)$$

Integration of this equation may be performed under the condition that  $\Delta V$  and  $\Delta S$  are either temperature or pressure dependent or not. When  $\Delta V$  and  $\Delta S$  are pressure and temperature dependent reentrant phase behavior may be found as observed for the effect of pressure on the nematic-smectic A transition of certain liquid crystals and polymers. When both quantities are pressure and temperature independent the phase boundaries are straight lines and a simple Clausius-Clapeyron equation is obtained. More details are given in the section on proteins 4.1.

The Clausius-Clapeyron equation gives the effect of pressure on the melting temperature

( $T_m$ ) of compounds

$$\frac{dT_m}{dP} = \frac{T_m \Delta V}{\Delta H} \quad (14)$$

Since the volume ( $\Delta V$ ) and enthalpy change ( $\Delta H$ ) on melting are in general positive one can expect an increase in melting temperature with increasing pressure. For many organic compounds  $dT_m/dP$  is ca. 15 K/100 MPa. As a first approximation, the effect of a pressure increase by 100 MPa would then correspond to the physical effect of a decrease in temperature by 15 K. A notable exception to this general rule is water. At room temperature, a pressure of about 1 GPa is needed to obtain the phase of ice (VI) which differs from the normal ice by its higher density. An illustration of the Clausius-Clapeyron equation may be found in the effect of pressure on the temperatures of the transitions in phospholipids as discussed in section 5.

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### Biographical Sketch

**Karel Heremans** (1940) studied chemistry at the Katholieke Universiteit Leuven after Greek-Latin Humanities in Antwerp. He obtained his PhD under the supervision of Paul Putzeys with a thesis on the molecular light scattering of proteins. During a postdoctoral period he studied protein-protein interactions with light scattering under high pressure. He worked on the effect of pressure on phase transitions in lipids, on the activity of lipid-bound enzymes, on nucleic acids, ribosomes and microtubuli assembly and on reactions of proteins in general.

At present he is professor of physical biochemistry at the Faculty of Sciences where his research concentrates on the stability of biopolymers as a function of pressure and temperature. These studies are performed with infrared spectroscopy and the diamond anvil cell. The primary focus is now on the relation between protein unfolding and intermolecular aggregation as model systems for molecular diseases.

He is past president of the Belgian Biophysical Society and present Chairman of the European High Pressure Research Group.