High hydrostatic pressure is a powerful tool for studying the structure and function of macrobiomolecules. In recent years significant advances have been made in the field of high pressure biology with improvements in the apparatus and experimental methods for high pressure study. Many researchers from various countries including the US, Europe, Japan, Brazil and China are engaged in the field of high pressure biology. There are also international conferences in this field held in Japan and Europe regularly, which help to improve research communications in high pressure biology. At present the potential of applying high hydrostatic pressure in biotechnology is attracting increasing attention. In this review we will focus on recent applications and its potential use.

Utilities relating to biopolymer structures and functions

Pressure-assisted isolation of proteins

Current procedures for extraction of membrane proteins usually involve the use of detergents. However, the replacement of the natural environment lipid by detergent molecules in most cases induce irreversible protein denaturation. High pressure may be a novel method for purification and characterization of membrane proteins without detergent. As known, high pressure leads to higher viscosity, lower lipid fluidity in biomembranes, and thus weaker protein-lipid interactions. The susceptibility for shedding under high hydrostatic pressure is different for each membrane protein and is probably related to the interactions with its immediate lipid or protein neighbors. Usually a few kilobar pressure may help to extract proteins from membrane, and more importantly, certain proteins as well as their endogeneous lipid can be shed off into the supernatant, thus avoiding irreversible denaturation. This technique has been successfully applied to extract delta 6-desaturase enzyme, lung surfactant protein SP-C, protein kinase C, blood-group antigens from human erythrocytes and immunogenic proteins from tumor cells, etc.

Enhancing the dissociation or association of antigen-antibody complexes

The high binding affinity between antigens and antibodies helps to produce antigens or antibodies with high purity. However, the application of this method in industry is hampered by the separating conditions (low pH, high ionic strength, etc.) that often lead to the inactivation of antigens or...
antibodies. Studies show that most interactions between antibody and antigen are sensitive to high pressure, and dissociation of the complex may occur at pressure above 1000 bar, while antibody and monomeric antigens are not denatured at pressure up to 4000 bar. Therefore proper pressure may be applied to separate the complexes. As shown by enzyme-linked immunoassay, albumin and β-galactosidase with high yield, high purity and high activity can be obtained with assistance of high pressure.

High pressure also has the potential of application in clinical assays. For example, in the detection of endogenous antibodies with standard antigens, inaccurate quantifications or in some cases false negatives may result from the binding of endogenous antigens to antibodies. High pressure may induce the disruption of the endogenous immune complexes and the release of antibodies, which greatly increase the binding of standard antigens to endogenous antibodies, consequently improving the sensitivity and specificity of clinical assays.

It is interesting that high pressure can also enhance the binding of HIV-1 24 to anti-HIV-1, the mechanism of which is still unclear. This phenomenon could also find practical applications shortly and also reduce the amount of reagents required in immunoassays.

**Enzyme reactions under high pressure**

Kunugi and other researchers have studied the pressure dependence of several enzyme reactions, and found that pressure affects the activities of enzymes in considerably different ways, depending on the nature of the scissile bond of the substrate and the type of substrate used. High pressure can alter the reaction rate or specificity of enzymes, which has been applied to improve the peptide yield in proteolytic enzyme catalyzed reactions. For instance, in the thermolysin-catalyzed peptide formation reaction from Cbz-Asp and Phe-OMe, a nearly six-fold increase in the peptide yield was observed under high pressure.

Hydrolysis of proteins by protease is the conventional way to obtain useful polypeptides. However, the native conformations of protein are usually stable and insensitive to the protease hydrolysis. High pressure may be used as a sort of denaturation-inducing agent to control the hydrolysis of proteins. Kunugi and coworkers found that one protein in a mixture of proteins can be selectively hydrolysed by protease under increasing pressure, which was mainly brought about by the unfolding of the substrate proteins at high pressure and the exposure of inner peptide bonds to protease. So the specific or nonspecific hydrolysis depends on the stability of proteins at high pressure. Although high pressure may also change the structure of proteases, they usually remain relatively highly activite as shown in previous studies. Kunugi et al. have applied this principle in limited proteolysis of proteins, and they reported that bovine serum albumin was highly-uniqeuly hydrolyzed by thermolysin at a specific site under high pressure.

High pressure can also be used as a denaturant in chemical modifications. High pressure may induce limited protein unfolding and control the number of modified residues, because most proteins can partially unfold at certain high pressures, and regain their native conformations and activities upon the release of pressure. Kunugi used pressure instead of chemical denaturants in the ferrocenation and methylation of proteins, and proved it to be a useful and convenient way to obtain functionally active proteins.

**Enhancement of sol-get transitions in macromolecules**

Sol-gel transitions exist in protein and polysaccharide solutions at certain conditions. Because water plays different roles in the mechanism of gel formation, some gels are formed with positive molar standard volume change (ovalbumin, soy protein, etc.), while the others are with negative (agarose and gelatin). According to Le Chatelier’s principle, increasing pressure is in favor of the movement of reactions to a smaller volume. Thus, the volume change makes the sol-gel transitions in macromolecules sensitive to pressure, which enables the production of food gels by high pressure. Study showed that the pressurized gels often have higher strength and softness but lower viscosity compared to gels prepared under high temperature.

**Living organisms under high pressure**

**Microorganisms**

Many microorganisms can exist under a hydrostatic pressure of hundreds of bars (<600 bar) with reduced rate, altered structure as well as different physiological features. Since high pressure has a significant effect on the protein synthesis of microorganisms, it will be very useful to separate baraphilic bacteria for the high expression of proteins induced by high pressure. There has been some preliminary study in this field in our laboratory.

High pressure can also kill microorganisms like high temperature. Usually at pressures above 600 bar the number of living microorganisms decreases greatly with increasing pressure. By now the pressure variability of many microorganisms have been studied, which is useful in the application of high pressure in food processing. The study in our laboratory showed that incubation at 25°C, 2300
bar for 30 minutes may completely kill E. coli, and most bacteria were inactivated in the first 10 minutes. E. coli growing at 37°C has the maximum high pressure endurance. Usually several kilobars may reduce the number of microorganisms in food by several orders of magnitude, while spore are often much more resistant to high pressure and could be removed with a repeated cycle of high and low pressures.

**Viruses**

Viruses is one of the most important infectious pathogens. Studies show that high pressure treatment greatly decreases the infectious activity of some viruses, such as VSV, HIV, SIV, influenza virus, etc. We obtained similar results. Since the high pressure may retain most of the activity of major proteins in plasma (except for VIII) at the same time kill the contaminating viruses, inactivation of viruses by high pressure may find important applications in the preparation of plasma products.

**Cells**

Like microorganisms cells may also change considerably in structure, morphology and characteristics due to high pressure. Yamaguchi *et al.* have observed the inhibition of ability to proliferate of Ehrlich ascites tumor cells caused by high hydrostatic pressure. In addition, suitable pressure treatment resulted in augmentation of immunogenicity of some tumor cells and normal T lymphocytes. Subjection of EL-4-leukemia cells to hydrostatic pressure of 1200–1500 bar increased their weak basal immunogenicity to a potent practical level. Injection of such pressure-treated EL-4 cell vaccine into mice significantly delayed tumor development and increased their survival time after subsequent challenge with untreated EL-4 cells. This may be attributed to the modulation in the projection of surface antigens upon pressure treatment. T lymphocytes, effector of autoimmune diseases, can be transformed into effective immunogens by high pressure treatment. Such vaccine not only generates a specific immune response but also induces remission in rats with established adjuvant arthritis.

**High pressure in food processing and preservation**

Traditional food processing methods involve the use of high temperature. However, a different kind of food processing method using high pressure has been in study in Japan and some European countries since the beginning of 1990’s. The principle of this method is that high pressure (several hundred to several thousand atmospheric pressures) may induce the formation of gels and granules of starch, protein coagulation, lipid phase transitions and other changes as effectively as heating. At the same time the sterilization of the pathogenic microorganisms inside the food is completed as mentioned above. Compared with traditional thermal processing, high pressure treatment can extend the preservation of food by killing the inner pathogens, while retaining the food color, taste and nutrients, because pressure below 10 kilobar does not destroy the covalent structures of vitamins and other critical small molecules. In addition, proteins and other biomolecules denatured by high pressure are much more readily taken in by the human body, therefore high pressure is especially suitable for the processing of juice, fruit jams, fresh seafood, etc., which can be destroyed by high temperature. Our study showed that several kinds of food are obviously different in color, taste and flavor after high pressure treatment.

Great advances have been made in the study of high pressure food processing in recent years. In Japan, the US and some European countries, high pressure processed food has already been on sale, which is superior to the traditional food in many aspects and is popular with customers. High pressure processing is suitable to all types of food which contain water. Unlike heat, pressure can reach everywhere inside the food in a very short time, which requires less energy compared to thermal processing.

**Other applications of high pressure**

**Enhancing the detection of living cells**

Flow cytometry combined with fluorescent staining is a powerful tool to analyze heterogeneous microbial populations. However, the fluorescence intensity of labeled cells varies considerably among strains, resulting in inaccurate detection. A pretreatment with nonlethal levels of high pressure (a few hundred of bars) can enhance CF or CDCF (both are common living cell dyes) staining in the absence of all proliferation or significant loss of variability, as is necessary for precise determination of the number of variable cells in a sample. Thus application of moderate high pressure could potentially be a new procedure for detection of living cells.

**A new histological fixative**

Hydrostatic pressure can also be used as a new method of histological fixative to specific organs. High pressure fixation is uniform, fast and can be handled safely. The specimens processed with pressure are firm, elastic and pale, somewhat resembling the formalin fixed ones. Fine structures in the heart and intestine may be well preserved by pressure. In addition, high pressure fixation can also retain the biological activity of alkaline phosphatase, which is rarely preserved in the formalin fixed kidney.