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Ceramic cells for high pressure NMR spectroscopy of proteins

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ABSTRACT

Application of high pressure to biological macromolecules can be used to find new structural states with a smaller specific volume of the system. High pressure NMR spectroscopy is a most promising analytical tool for the study of these states at atomic resolution. High pressure quartz cells are difficult to handle, high quality sapphire high pressure cells are difficult to obtain commercially. In this work, we describe the use of high pressure ceramic cells produced from yttrium stabilized ZrO_2 that are capable of resisting pressures up to 200 MPa. Since the new cells should also be usable in the easily damageable cryoprobes a completely new autoclave for these cells has been constructed, including an improved method for pressure transmission, an integrated safety jacket, a displacement body, and a fast self-closing emergency valve.

1. Introduction

With high pressure NMR spectroscopy it is possible to obtain novel information about protein biochemistry and biophysics at atomic resolution that is difficult or impossible to obtain with other methods [1–3]. By observing the pressure response of proteins one obtains not only data on the local and global mechanical and dynamical properties of the macromolecule [4] but can also shift conformational equilibria and stabilize folding and functional intermediates [5–8]. In addition, physicochemical processes such as pressure induced denaturation, depolymerization, and dissociation can be studied in detail [7,9,10].

In general there exist two different approaches to perform high pressure NMR spectroscopy. Either the whole probe head including the sample tube [11–13] or only the sample tube itself (originally a glass capillary) [14–16] can be pressurized. Both methods, known respectively as the Jonas and the Yamada method, have advantages and disadvantages [3]. The advantage of the Yamada method is that it can be applied in commercially available NMR probe heads and also allows performing on-line pressure changes in a few minutes [32] or even fast pressure jumps in the millisecond range [17] to gain information on time dependent folding processes in biomolecules. One drawback is that glass capillaries, typically manufactured of borosilicate or quartz glass, tend to be fragile and require experienced handling. Another approach uses sapphire cells [18–20], but low availability of high quality sapphire cells leads to the necessity of searching for new materials for the production of high pressure cells.

High pressure sample tubes produced from alumina-toughened zirconia (AZO) by injection molding (Daedalus Innovations, Philadelphia) were used for offline applications studying encapsulated proteins in low viscosity fluids [29,30] but recently were also offered for on-line experiments.

In this work special ceramic cells (outer diameter 4 mm, inner diameter 1.1 or 2 mm) and a suitable high pressure autoclave for transmitting the pressure have been designed that simultaneously satisfies important safety regards. The manufacturing of the ceramic cell has been executed by HiPer Ceramics GmbH (Germany). The ceramic is composed of ZrO_2 partially stabilized by Y_2O_3 (PSZ). The addition of stabilizing Y_2O_3 to the monoclinic ZrO_2 affects the microstructure (cubic phase with a fraction of monoclinic and tetragonal exclusions). The resulting enhanced mechanical stability [21] is confirmed with a high tensile strength of about 1000 N/mm^2. Table 1 summarizes the most important parameters of commonly used materials for high pressure cells.

The maximum pressure p_{max} that capillaries can withstand in routine long-time usage can be calculated according to Sherman and Stadtmuller [22] by

$$p_{max} = \tau \ln \frac{d_o^2}{d_i^2} \quad (1)$$

with the sheer strength τ and the outer and inner diameters d_o and d_i of the capillary. The sheer strength τ can be approximated from the tensile strength σ as $\tau = \sigma/2$.

The average maximum pressure p^*_{max} that can be applied repeatedly to the capillary in routine application is considerably smaller

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Table 1
Physical and mechanical properties of the materials used for high pressure NMR capillaries.[a].

	Ceramic			Borosilicate glass	Quartz glass	Sapphire	
Manufacturer	HiPer		Daedalus	Schott	Qsil	Saphikon	
Tensile strength σ (N/mm^2)	1000			7	50	140	
Expansion coefficient (10^{-6} K^{-1})	11.4			3	0.5	8.8	7.9
χ_{magn} (10^{-9} m^3 kg^{-1})	−1.4 (ZrO_2)			−0.86	−0.49	−0.21	−0.25
d_{outer} (mm)	4	4	5	4	4	3.18	
d_{inner} (mm)	1.1	2	3	0.8–1	0.8–1	1.73	
d_{outer}/d_{inner}	3.64	2	1.67	4–5	4–5	1.84	
$p_{max,theor.}$ (MPa)	1290	693	513	9.7–11.3	69.6–80.5	85.2	
$p^*_{max,theor.}$ (MPa)	745	400	296	5.6–6.5	40.2–46.5	49.2	
$p^*_{max,real}$ (MPa)	200[c]	200[d]	250[b]	200[c]	200[c]	200[c]	

[a] The data are taken from the datasheets of the manufacturers [24–26], the magnetic susceptibilities of borosilicate and quartz glass were taken from [27,28]. Due to its single crystalline structure, the coefficient of expansion and magnetic susceptibility of sapphire are tensorial quantities (the left side, values parallel, right side perpendicular to the principal axis of the system).
[b] Aluminia toughened ZrO_2 (AZO), the cells were only tested up to 200 MPa by us, thus the maximum obtainable pressure resistance was taken from the web-page of the manufacturer.
[c] Typical maximum pressures capillaries that are used in our laboratory in repeated experiments. However, a few capillaries withstand much higher pressures when tested (see text).
[d] Cells were tested up to 200 MPa.

than the maximum pressure p_{max} reached in a single experiment before the capillary is destroyed. It can be approximated by using the apparent sheer strength $\tau^* = 3^{-1/2}\,\tau$ [22], leading to the final expression for the average maximum pressure p^*_{max} that a capillary can resist

$$p^*_{max} = \frac{\sigma}{\sqrt{3}} \ln \frac{d_o}{d_i} \quad (2)$$

There are thus two factors that determine which pressure p_{max} a capillary should withstand theoretically, the ratio of the outer to the inner diameter and the sheer strength. The corresponding values are summarized in Table 1. It is obvious that ceramic theoretically has by far the best properties, and in principle for reasonable inner and outer diameters (e.g. 4 and 1.1 mm) maximum pressures can be reached that are above the pressure range required for protein studies. In fact the tensile strength of ceramic is close to that of titan (1000–1200 N/mm^2). The average maximum pressures that can be theoretically reached for the other materials are below the pressure of 200 MPa where they are really used in high pressure experiments. Even borosilicate with a theoretical p_{max} of about 10 MPa can be used (and is used in our laboratory) at 200 MPa. The reason why this is possible is that special treatments of the capillaries as tempering improve the material properties significantly. In addition, only a few of the capillaries tested usually survive a pressure of 200 MPa. On the other hand, in our laboratory a sapphire cell with the dimensions given in Table 1 survived 320 MPa and Akasaka's group used quartz cells with inner diameters of 1 mm and outer diameters of 3.5 mm up to 400 MPa [23].

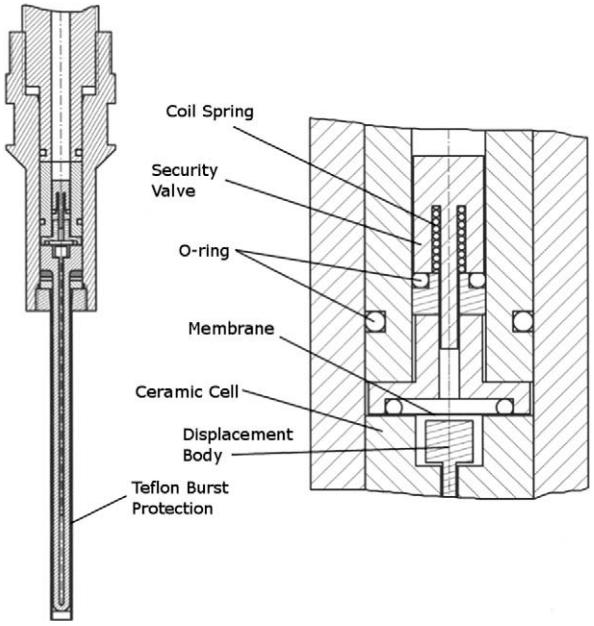

Fig. 1. Design of the ceramic high pressure system. Schematic representation of the system including the autoclave (produced of TiAl6V4, Hempel Special Metals GmbH, Germany) and the ceramic cell (produced of Y_2O_3 partially stabilized ZrO_2 (PSZ), HiPer Ceramics GmbH, Germany) that is located inside the Teflon burst protection tube (left). Blow up of the pressure transmission part showing the polyethylene membrane for separating sample and pressure fluid, the displacement body and the safety valve (right).

2. Results and discussion

A high pressure system usable in high field NMR spectroscopy has to fulfill several conditions: (i) The pressure cell has to withstand a given target pressure (in our case 200 MPa) with an inner diameter as large as possible and an outer diameter small enough to fit in a 5 mm probe head; (ii) the system has to be handled easily; (iii) the cell should allow the recording of high resolution NMR spectra; and (iv) it should be safe when the cell itself bursts inside the probe head. The new system consisting of a ceramic cell, the titan autoclave and a Teflon safety jacket (Fig. 1) meets the above requirements.

Ceramic high pressure cells with an outer diameter of 4 mm and an inner diameter of 1.1 mm were tested using a maximum pressure of 210 MPa outside the spectrometer before use. According to Eq. (2) granting a safe working pressure for long-time usage a p_{max} of more than 310 MPa would have to be tested what is outside of the range accessible in the presently available ceramic cells. However, in case of bursting the safety provisions (see below) would protect the probe head from damage. The pressure is transmitted by a flexible polyethylene membrane that has been integrated to separate the valuable biological sample from the pressure fluid (in our case methyl cyclohexane). The polyethylene membrane is resistant to methyl cyclohexane and was proved to be mechanically stable in our setup. Because samples are in most cases very valuable a displacement body has been implemented into the ceramic cell to reduce the sample volume from around

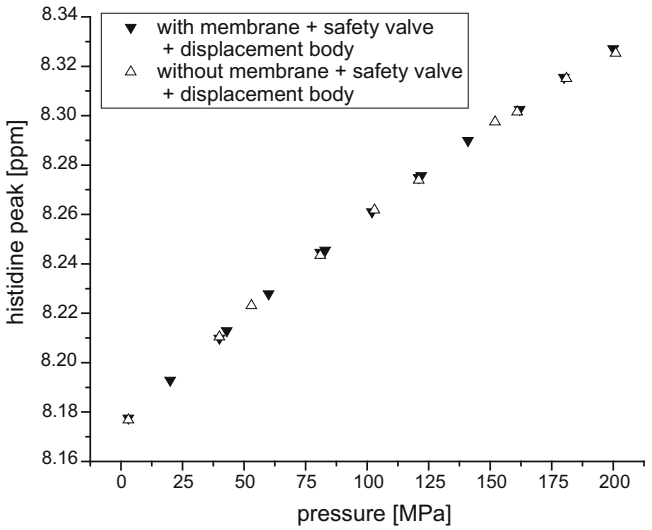

Fig. 2. Performance of the pressure transmission system. The sample consists of 100 mM histidine in a 100 mM D_2O phosphate buffer pH 6.5, containing 1 mM DSS, 1 mM EDTA and 1 mM NaN_3. Measurements were performed at 500 MHz and 298 K. The pressure dependence of the $H^{\varepsilon 1}$-resonance of histidine is plotted as a function of the pressure measured in the transfer line in the absence and presence of the polyethylene membrane, the safety valve, and the displacement body.

350 to 150 μL. The membrane has to resist the mechanical stress but has also to be sufficiently elastic to guarantee that the pressure of the on-line system is completely transmitted.

The general form of the ceramic cell is similar to that described earlier for sapphire cells [19] but contains a cavity that supports the displacement body and allows the pressure transmission by a membrane. The autoclave itself was developed on the basis of an autoclave described by us earlier [20] but has also similarities to that used by Urbauer et al. [19] that is also commercially available (Daedalus Innovations). However, our autoclave is devised also to reduce the length of the ceramic cell as much as possible. Teflon burst protections are also used earlier in the quartz cell system [1,2,5,16].

This is shown in Fig. 2 where the pressure dependence of the chemical shifts of histidine $H^{\varepsilon 1}$ protons is used to measure the pressure directly inside the ceramic cell. The static pressure is produced by a pressure bench and transmitted by methyl cyclohexane in high strength pipes to the autoclave. The pressure of the pressurizing fluid is measured by a Burdon manometer, the pressure inside the cell is measured by the histidine $H^{\varepsilon 1}$ chemical shift that changes nearly linearly with pressure. The measurements have been performed in the absence and presence of the displacement body, the safety valve, and polyethylene membrane. The pressure was varied between ambient pressure (0.1 MPa) and 200 MPa. The observed chemical shift changes are identical within the limits of error (Fig. 2); the possible deviation of pressure is less than 0.4 MPa and therefore negligible for practical applications.

When the material of the pressure cell has a magnetic susceptibility different to the solvent, magnetic field inhomogeneities are created at the border between inner cell surface and solvent. Therefore an ideal material would be susceptibility matched, that would have the same magnetic susceptibility χ as the solvent (in biology water with $\chi(H_2O) = -9.05 \times 10^{-9}\ m^3\ kg^{-1}$). Ceramic has the smallest difference of magnetic susceptibilities to water $\Delta\chi$ from all materials listed in Table 1, although $\Delta\chi$ deviates still considerably from zero. As a consequence of the relatively large $\Delta\chi$ the inner surface of the ceramic cell has to be very smooth for allowing a good shom of the sample. This has been done by carefully polishing the inner surface with diamond powder. After polishing the obtainable line width of the methyl signal of DSS dropped from 25 to 2.05 Hz (Fig. 3a) leaving still an additional line broadening of approximately 1 Hz from the ceramic tube. However, under these conditions high quality HSQC spectra from proteins can be recorded from proteins (Fig. 3b) even at high fields.

Since we usually cannot work in a pressure range where the high pressure tube is in the safety range for long time duty as defined by Eq. (2) it is absolutely required to have a safety system that prevents damage of the probe head when the ceramic sell bursts. The safety system devised here (Fig. 1) consists of the Teflon hose and an emergency valve. The Teflon burst protection should hinder the pressuring fluid of leaking into the NMR probe head if the ceramic cell bursts. In addition, accelerated fragments of the destroyed cell should be stopped by the burst protection. The emergency valve has to be able to disconnect the ceramic cell rapidly from the pressure fluid line, since otherwise the pressurized liquid would disrupt the thin-walled Teflon tube. The emergency

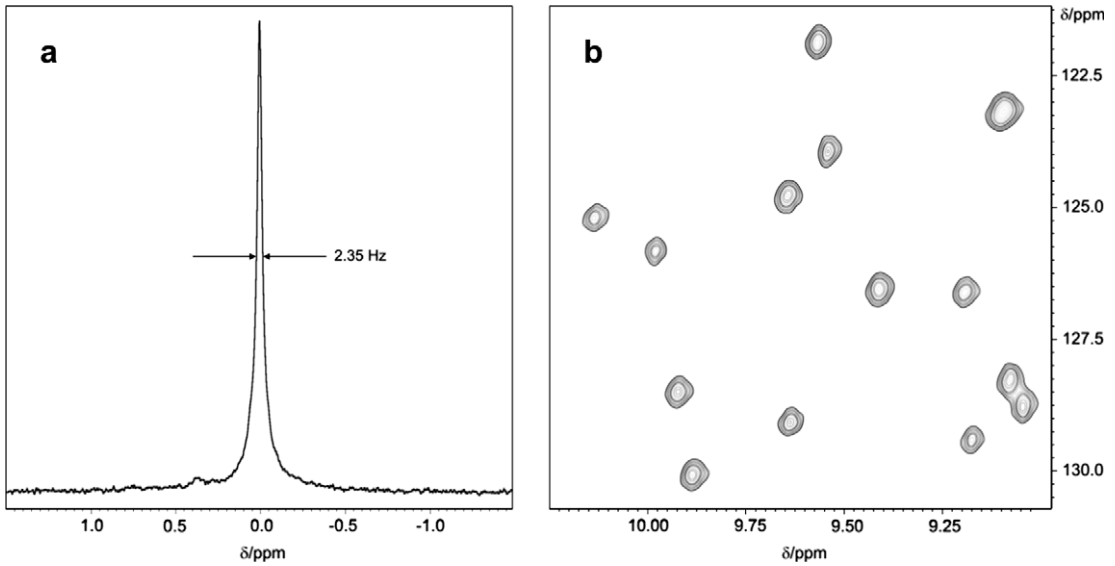

Fig. 3. Magnetic field homogeneity obtainable in ceramic cells. NMR spectra obtained after polishing the inner surface of the ceramic cell. (a) ^1H spectrum of 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) measured at 600 MHz, showing the methyl resonance of DSS. A line broadening of 0.3 Hz was applied to the time-domain data. (b) Selected region of the ^1H–^{15}N HSQC spectrum of *Plasmodium falciparum* thioredoxin (*Pf*Trx) [31] measured at 800 MHz.

valve consists of a cap, a rod, an O-ring, a bearing, and a coil spring of bronze. The cap closes the valve when large pressure differences suck the rod into the bore of the bearing, the spring prevents closing of the valve during the process of filling or during normal operation where only very small pressure gradients occur.

The implemented safety system was tested carefully outside the magnet. Several ceramic cells were disrupted by high pressure but the burst protection was never damaged severely. The self-closing safety valve was tested with a special setup: high pressure has been applied to a special steel tube with the same geometry as the ceramic cell. The steel tube had a valve at the bottom that could be opened rapidly to release pressure. In a series of experiments in the pressure range from 20 to 200 MPa the closing of the safety valve was observed. The average loss of pressure until the safety valve closed was approximately 3–5 MPa. Only very small fluid volumes in the μL scale could leave the system before the valve closed. The final (not intended) test of the system was the burst of a ceramic cell inside the cryoprobe at 200 MPa that occurred without any damage to the probe head.

According to Eq. (2) a high pressure cell that is expected not to break for an "indefinite" time during pressure experiments at a p^*_{max} of 200 MPa has to withstand in a test experiment a maximum pressure p_{max} of 346 MPa. Only when appropriate safety precautions are introduced as we have described here, one can risk the use ceramic cells at 200 MPa with significantly smaller p_{max} values as we do here. Pressure cells with an outer diameter of 5 mm that are commercially available (Daedalus Innovations) do not allow for a safety lining when a 5 mm probe is used.

In conclusion, we have developed a high pressure cell system that is easy to handle (much easier than quartz capillaries) and can be safely used in cryoprobes.

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