Journal of Biomolecular NMR, 10 (1997) 279–282
KLUWER/ESCOM

J-Bio NMR 498

The button test: A small scale method using microdialysis cells for assessing protein solubility at concentrations suitable for NMR

Stefan Bagby, Kit I. Tong, Dingjiang Liu, Jean-René Alattia and Mitsuhiko Ikura*

Division of Molecular and Structural Biology, Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, 610 University Avenue, Toronto, ON, Canada M5G 2M9

Received 28 August 1997
Accepted 12 September 1997

Keywords: Microdialysis cell; NMR sample preparation; Protein solubility; Solution condition screen; TFIIB

Summary

A simple method has been developed for screening solution conditions to determine conditions under which a protein is soluble at the high concentrations typically used for NMR spectroscopy. The method employs microdialysis cells or 'buttons'. The low sample volume (5 μl) required for each microdialysis button permits testing of a wide range of solution conditions and temperatures with high protein concentrations, using a small amount of protein. Following precipitation of several NMR samples of the C-terminal core domain of human TFIIB, the microdialysis button screen facilitated identification of conditions in which precipitation of the TFIIB core domain was eliminated. The microdialysis button method for screening solution conditions is generally applicable and has been used to permit rapid identification of suitable NMR sample solution conditions for proteins involved in transcription and cell adhesion.

With the necessary spectroscopic technology and methodology now in place to allow detailed NMR studies of 30 kDa-plus polypeptides and complexes (Shan et al., 1996; Constantine et al., 1997; Garrett et al., 1997), one of the major obstacles to protein structure determination by NMR spectroscopy lies in finding solution conditions under which a protein is soluble at high concentrations (typically 1 mM or higher; Schein, 1990; Wagner, 1993). Advances in this area of protein NMR spectroscopy would help to speed up the structure determination process, thus facilitating further functional characterization and increasing the potential contribution of NMR to biomacromolecular studies, for example in the development of new therapeutics (Shuker et al., 1996).

In preliminary studies of the 23 kDa C-terminal core domain (TFIIBc) of human general transcription factor TFIIB, a protein essential for transcription by RNA polymerase II, it was soon apparent that TFIIBc readily precipitates at the protein concentrations required for high-resolution NMR studies. To help overcome this problem, we developed a simple method for screening solution conditions, which we term the button test. The button test employs microdialysis cells or 'buttons' (Fig. 1), which are more conventionally used in protein crystallization trials (McPherson, 1989). The low sample volume (5 μl) required for each microdialysis button permits testing of protein solubility at high protein concentrations under a large number of conditions. Such a screen can be extremely useful in the initial stages of NMR study of a protein, particularly in cases where solubility difficulties are expected, based on initial characterization and/or previously published results. The button test facilitated identification of conditions under which precipitation of TFIIBc was eliminated, allowing extensive NMR analysis of this general transcription factor (Bagby et al., 1995).

Initially, we used NMR sample solution conditions like those employed for transcription assays, for example 35 mM HEPES-KOH (pH 8.0), 60 mM KCl and 6% (v/v) glycerol. Under these conditions, TFIIBc consistently precipitated within two days of beginning acquisition of NMR data at 25 °C. A similar sample behaviour was observed upon changing to more conventional NMR sample conditions of 10 mM sodium phosphate, pH 7.0, 100 mM KCl.

*To whom correspondence should be addressed.

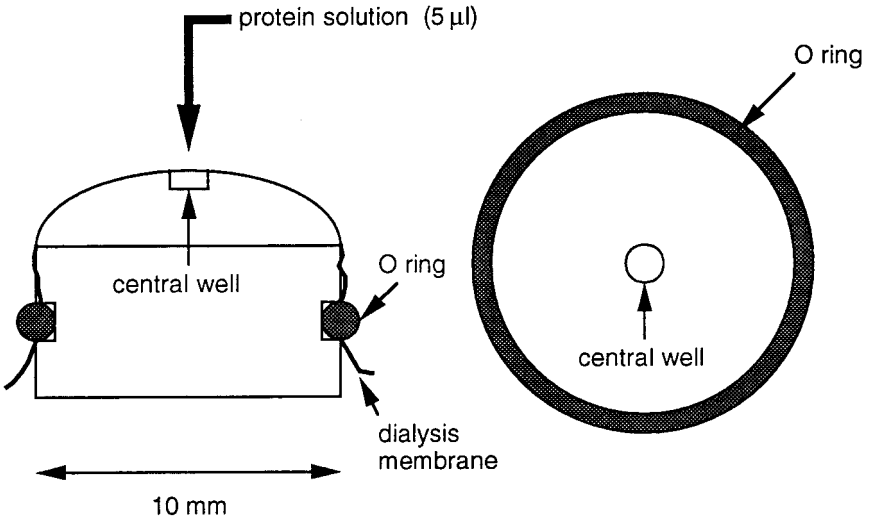

Fig. 1. A microdialysis cell shown in vertical cross section (left), and viewed from directly above (right). The various parts of the microdialysis cell are labelled, and its approximate diameter is indicated.

Prompted by these difficulties to use a new method for NMR sample solution condition screening, we tried the microdialysis button, a tool used by crystallographers to screen conditions for protein crystallization (McPherson, 1989). Forty different solution conditions were screened, using a total of 4.6 mg of TFIIBc at a concentration of approximately 1 mM, assessing the effects of salt concentration, pH, and buffer system. (As well as these three variables, the effects of various protein co-solutes might be assessed, for example polyols and sugars, detergents, and reducing agents (Schein, 1990).) All test solutions contained 0.1 mM EDTA, 5 mM DTT and 50 µM NaN_3. Test solutions contained either no buffer, 10 mM sodium phosphate or 10 mM Tris-HCl. KCl concentrations of 0, 50 and 100 mM were tested. The protocol used to set up the button test is described in detail in Table 1.

Certain test samples showed extensive precipitation, clearly visible to the naked eye just one day into the microdialysis cell trials (Fig. 2). Other test solutions were still clear after four weeks, and others showed discolouration and/or evidence of fibre formation when viewed using a microscope. In the case of TFIIBc, the results of the button test were clear: this polypeptide is more soluble in lower pH environments without salt. Hence, for

TABLE 1
PROCEDURE FOR SETTING UP THE BUTTON TEST

(1) The protein sample was first exchanged into a buffer system of lower ionic strength than the test buffer systems, such that an osmotic gradient was formed from the test buffer side to the sample side. In our screening of solution conditions with the carboxy-terminal core domain (TFIIBc) of human TFIIB, for example, we first exchanged the protein into distilled, deionised water containing 7.5 mM dithiothreitol (DTT). The protein was subsequently concentrated to 1 mM or higher in order to simulate the conditions in an NMR sample. An initial screen using protein at lower concentration might be informative for particularly troublesome systems.
(2) A standard piece of dialysis membrane (for example, Spectra/Por molecular porous membrane from Spectrum, 6000–8000 molecular weight cutoff) was prepared according to the manufacturer's instructions.
(3) The wet dialysis membrane was cut into 1 in. squares and these pieces were kept moist by placing them between wet Kimwipes.
(4) Using a micropipette tip (for example, Bio-Rad tips for protein electrophoresis, catalogue number 223-9915), 5 µl of concentrated protein sample was pipetted into the central well on the top surface of the microdialysis cell (Fig. 1). A circular motion was used to avoid air bubble formation in the protein solution.
(5) The membrane was secured with a rubber O-ring which fits in the groove running around the circumference of the microdialysis cell.
(6) The microdialysis cell was submerged in 5 ml of test solution contained, for example, within a scintillation vial. In the case of the complex between TBP core domain and residues 11–77 of $TAF_{II}230$, the test solutions were precooled to 4 °C and then kept at 4 °C for 2–4 h after submersion of the microdialysis cell to allow equilibration before transferring to a higher temperature environment.
(7) For the TFIIBc screen, the test samples were placed in a temperature-controlled environment at 25 °C. Each day, the clarity of the samples was monitored using the naked eye and/or a standard dissecting microscope. The effect of temperature on solubility can also be investigated by placing test samples with the same solution conditions at different temperatures.
(8) In order to confirm that any precipitation, discolouration or fibre formation is dependent on the presence of protein, microdialysis cells were also set up containing buffer solutions with no protein.
(9) Microdialysis cells can be obtained from Cambridge Repetition Engineers[a]. The same company also makes O-ring applicators which facilitate the process of securing the dialysis membrane.

[a] Cambridge Repetition Engineers, Green's Road, Cambridge CB4 3EQ, U.K. (Telephone +44-(0)-1223-64655; FAX +44-(0)-1223-467328).

Fig. 2. Three microdialysis cells showing the effect of salt concentration on the solubility of the carboxy-terminal core domain of human TFIIB, which comprises residues 1–3 followed by 111–316 of TFIIB (full-length human TFIIB has 316 residues). The protein in the central well of each of these microdialysis cells was dialysed against one of three solutions containing 10 mM Tris-HCl, pH 7.0, 0.1 mM EDTA, 5 mM DTT and 50 μM NaN_3 with different KCl concentrations: 0 (left microdialysis cell), 50 mM (central cell) and 100 mM (right cell). The sample without KCl began to show some evidence of fibre formation two weeks after starting the screen, but still no precipitation was visible to the naked eye. In contrast, the third sample, containing 100 mM KCl, precipitated just one day after starting the screen. The middle sample became turbid two days into the trial and showed extensive precipitation by the third day.

our TFIIBc NMR samples, we did not add salt and used 10 mM sodium phosphate as buffer, with the pH adjusted to 5.8. Interestingly, these conditions are very different from those used for transcription assays (see above). Gel mobility shift assays to monitor formation of complexes involving DNA, TBP, TFIIA and TFIIBc showed that TFIIBc retains normal activity under the solution conditions used for TFIIBc NMR samples. Also, the ^{1}H-^{15}N HSQC spectra of TFIIBc under transcription assay solution conditions and under the solution conditions used for NMR analysis were essentially identical. The observation that TFIIBc is more soluble in salt-free conditions may be due to a significant hydrophobic contribution to the surface of this polypeptide (Bagby et al., 1995).

Protein solubility and stability can be limiting factors in NMR studies, particularly for the larger polypeptides (30 kDa or more) that are now tractable using multidimensional heteronuclear methods. The button test is an efficient, small scale way of tackling this problem. With the help of this condition screening procedure, we were able to record numerous multidimensional NMR spectra for the purpose of characterizing the three-dimensional solution structure (Bagby et al., 1995), backbone ^{15}N dynamics and interactions of TFIIBc.

The button test has since been applied to domains from the cadherin family of cell adhesion receptors and to two complexes involved in regulation of RNA polymerase II transcription initiation: TATA binding protein (TBP) core domain bound to a fragment (residues 11–77) from the largest subunit of TFIID, and TFIIB bound to the activation domain of VP16. These screens demonstrated the utility of the button test in optimizing NMR sample solution conditions.

Acknowledgements

We thank Kathy Johns for first suggesting the use of microdialysis cells and for loaning us more than 40 microdialysis cells, and Sungjoon Kim, Edio Maldonado and Danny Reinberg for providing numerous batches of TFIIBc. This work was supported by grants to M.I. from the Medical Research Council of Canada (MRCC) and the National Cancer Institute of Canada. S.B., D.L. and J.-R.A. acknowledge the MRCC, Human Frontier Science

Project and Ontario Cancer Institute/Amgen for the award of postdoctoral fellowships. M.I. is a Howard Hughes Medical Institute International Research Scholar.

References

Bagby, S., Kim, S., Maldonado, E., Tong, K.I., Reinberg, D. and Ikura, M. (1995) *Cell*, **82**, 857–867.

Constantine, K.L., Mueller, L., Goldfarb, V., Wittekind, M., Metzler, W.J., Yanchunas Jr., J., Robertson, J.G., Malley, M.F., Friedrichs, M.S. and Farmer II, B.T. (1997) *J. Mol. Biol.*, **267**, 1223–1246.

Garrett, D.S., Seok, Y.-J., Liao, D.-I., Peterkofsky, A., Gronenborn, A.M. and Clore, G.M. (1997) *Biochemistry*, **36**, 2517–2530.

McPherson, A. (1989) *Preparation and Analysis of Protein Crystals*, Robert E. Krieger, Malabar, FL, U.S.A.

Schein, C.H. (1990) *Biotechnology*, **8**, 308–317.

Shan, X., Gardner, K.H., Muhandiram, D.R., Rao, N.S., Arrowsmith, C.H. and Kay, L.E. (1996) *J. Am. Chem. Soc.*, **118**, 6570–6579.

Shuker, S.B., Hajduk, P.J., Meadows, R.P. and Fesik, S.W. (1996) *Science*, **274**, 1531–1534.

Wagner, G. (1993) *J. Biomol. NMR*, **3**, 375–385.