Physiological buffers for NMR spectroscopy

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Summary

Two 'physiological' buffers for NMR spectroscopy of biological samples are described. They reflect intracellular and extracellular fluids as far as possible, are easy to prepare, and do not lead to any additional ^1H NMR resonances.

A principal advantage of structure determination by NMR spectroscopy compared to X-ray crystallography is the possibility to perform the experiments under conditions which can approximate to a large degree the properties of the natural, physiological solvent. Surprisingly, most NMR spectroscopists do not make use of this evident advantage of the NMR method, but select solution conditions which are by no means physiological. Examples are the use of distilled water only, which results in an abnormally low ionic strength, or the addition of sodium chloride which is inappropriate for intracellular proteins. The use of optimized buffers may be especially important when structures of polypeptides are studied that have been prepared under denaturing conditions (e.g. by chemical polypeptide synthesis) and where no functional assay exists to decide if the native structure has been adopted. Extreme experimental conditions, such as very low pH, low ionic strength and high temperature (leading to small exchange rates and line widths), are sometimes preferred for structural determinations of stable proteins. In such cases, after complete assignment and structure determination, it may be important to exclude structural changes when the protein is subjected to more physiological conditions. Here, we present two easy-to-prepare buffers, A and B, which reflect physiological conditions as closely as possible without causing any disturbing ^1H resonances.

Physiological buffer A is suited for intracellular proteins. It approximates the intracellular composition of frog muscle (Godt and Maughan, 1988). This composition was used since no complete compilation for mammalian cells could be found in the literature and since a similar ionic composition can be expected for the cytoplasm of unspecialized cells of almost all species. The buffer contains 47.0 mM K_3PO_4, 9.0 mM $KHCO_3$, 2.4 mM $MgHPO_4$, 0.3 mM K_2SO_4, 2.2 mM KCl, 4.5 mM Na_2HPO_4, and 67.2 mM CD_3COOD. The pH is adjusted to 7.2 with KOH. Note that the pH of this buffer (as of most buffers) is somewhat dependent on the concentration and decreases on dilution. Therefore, when concentrated stock solutions of the buffer are prepared, it is important to adjust the pH in such a way that it is 7.2 in the final dilution. This buffer is cheap, easy to prepare and does not have any signals in ^1H NMR spectra besides the H_2O signal. For measurements of ^{15}N- and/or ^{13}C-enriched proteins the buffer is suitable as well, since it does not contain any nitrogen, and since the concentration of ^{13}C in the buffer is low because of its low natural abundance of 1.1%. In addition, with the almost exclusively used indirect detection methods for heteronuclei, perdeuterated acetate will not give rise to a detectable signal. As in any buffer system, the ionic components in the proposed buffer result in an increased damping of the electric component of the HF field and to some heating of the sample. However, modern spectrometers and probeheads are designed for aqueous solutions with moderate ionic strengths. Under such conditions (e.g. our physiological buffers), tuning of the probeheads does not repre-

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TABLE 1
COMPOSITION OF BUFFERS A AND B COMPARED TO
INTRACELLULAR AND EXTRACELLULAR FLUIDS

Component	Physiological buffer A[a] (mM)	Cytoplasmic fluid[b] (mM)	Physiological buffer B[a] (mM)	Human blood serum[c] (mM)
K+	160.7	141	5.0	3.5–5.0
Free K+	147.9	140	5.0	
Na+	9.0	9.0	144.1	135–145
Free Na+	8.3	8.3	143.5	
Mg^{2+}	2.4	9.1	0.85	0.78–1.03
Free Mg^{2+}	1.0	0.8	0.80	
Ammonium compounds	–	6.5	–	
Phosphate compounds[d]	–	75.5	–	1.9–3.2
Phosphate	53.9	1.4	2.2	0.8–1.1
HPO_4^{2-}	27.9	0.7	1.3	
$H_2PO_4^-$	11.1	0.3	0.3	
$KHPO_4^-$	12.8	0.4	0.0	
$MgHPO_4$	1.4	0.0	0.05	
$MgH_2PO_4^+$	0.05	0.0	0.0	
SO_4^{2-}	0.3	0.3	0.4	
Total carbonate	9.0	9	25.0	
CO_2/H_2CO_3	1.0	1	1.8	1.8
HCO_3^-	7.9	8	22.8	24[e]
CO_3^{2-}	0.08	0	0.4	
Cl^-	2.2	2.2	101.3	97–110
Carboxylate compounds[f]	–	86	–	21[e]
Total acetate	67.2	–	21.1	–
CH_3COO^-	67.0	–	21.0	–
CH_3COOH	0.2	–	0.1	–
Eq	158	158	150	145–156
I	188	215	153	146–159

[a] The concentrations of all ionic species c_i, the ionic equivalent (Eq = ½ $\Sigma |z_i| c_i$) and the ionic strength (I = ½ $\Sigma z_i^2 c_i$) were calculated by a numerical solution of the corresponding equations with the equilibrium constants (Godt and Maughan, 1988; Smith and Martell, 1976): $[Mg^{2+}]$ $[HPO_4^{2-}]$ / $[MgHPO_4]$ = 20 mM; $[Mg^{2+}]$ $[H_2PO_4^-]$ / $[MgH_2PO_4^+]$ = 200 mM; $[K^+]$ $[HPO_4^{2-}]$ / $[KHPO_4^-]$ = 324 mM; $[Na^+]$ $[HPO_4^{2-}]$ / $[NaHPO_4^-]$ = 324 mM; $[H^+]$ $[PO_4^{3-}]$ / $[HPO_4^{2-}]$ = 1.82 × 10⁻¹¹ M; $[H^+]$ $[HPO_4^{2-}]$ / $[H_2PO_4^-]$ = 1.59 × 10⁻⁷ M; $[H^+]$ $[CO_3^{2-}]$ / $[HCO_3^-]$ = 6.31 × 10⁻¹⁰ M; $[H^+]$ $[HCO_3^-]$ / $[H_2CO_3 + CO_2]$ = 5.01 × 10⁻⁷ M.
[b] From Godt and Maughan (1988).
[c] From Seidel and Schmidt-Gayk (1980).
[d] Phosphate compounds are phospholipids in the blood serum, nucleotides and other phosphorylated metabolites in the cytoplasm. The value given is the concentration of the negative charges.
[e] From Lehninger (1982).
[f] Carboxylate compounds are proteins and metabolites with carboxylate groups. The value given is the concentration of the negative charges.

sent a real problem. Unavoidable heating effects during broadband decoupling can easily be compensated at ionic strengths higher than physiological, by temperature-compensated pulse sequences (Wang and Bax, 1993).

The concentrations of all the relevant ionic species are given in Table 1. In our buffers, inorganic phosphate is

taken as substitute for all phosphate compounds in the cell, and acetate for all protein and metabolite carboxyl groups. This is necessary to obtain physiological ionic strength. Most of the Mg^{2+} ions in the cell are complexed by nucleotides and nucleic acids, so that the actual concentration of free Mg^{2+} is much lower than the magnesium content of the cells. The concentration of magnesium compounds was reduced from 13.5 to 2.0 mM to obtain the physiological concentration of free Mg^{2+} of approximately 1.0 mM. The concentration of K^+ was increased correspondingly. Physiological buffer A has an ionic strength of 188 mM and an ionic equivalent of 158 mM, both corresponding to physiological values. Since the buffer contains acetate and phosphate, there is a wide pH range (pK_a = 4.7 and 7.0, respectively) with a good buffer capacity for titration experiments. For measurements in D_2O, the deuterated acetic acid should be added after lyophilization of the other compounds. In this way the pH of the solution is about 13 before lyophilization, so that the losses of carbonate during lyophilization are small.

Physiological buffer B is suited for extracellular proteins. It has been designed to simulate the ion conditions of human blood plasma (Seidel and Schmidt-Gayk, 1980) (Table 1). It contains 2.2 mM Na_2HPO_4, 0.4 mM Na_2SO_4, 94.6 mM NaCl, 5.0 mM KCl, 0.85 mM $MgCl_2$, 17.0 mM Na_2CO_3, 8.0 mM $NaHCO_3$, and 21.1 mM CD_3COOD. The pH is adjusted to 7.4 with NaOH. The buffer has an ionic strength of 153 mM and an ionic equivalent of 150 mM.

As mentioned above, there is no unique buffer which simulates perfectly the physiological ionic environment of a protein inside or outside the cell. However, the above buffers represent good compromises between the different requirements, namely an approximation of the physiological conditions, simple preparation, and the absence of strong artifact signals in ¹H NMR spectra. It is clear that sometimes the composition of these buffers must be varied by addition of specific ionic or nonionic compounds. For a protein with exposed sulfhydryl groups, a reducing agent such as dithioerythrol (DTE) has to be added, or when an enzyme needs a specific substrate or cofactor this has to be added to the solution in an appropriate concentration.

References

Godt, R.E. and Maughan, D.W. (1988) Am. J. Physiol., 254, C591–604.
Lehninger, A. (1982) Principles of Biochemistry, Worth Publishers Inc., New York, NY.
Seidel, D. and Schmidt-Gayk, H. (1980) In Innere Medizin, 5th ed. (Ed, Schettler, G.) Thieme, Stuttgart, pp. 548–562.
Smith, G.L. and Martell, A.E. (1976) Critical Stability Constants: Inorganic Complexes, Vol. 4, Plenum Press, New York, NY.
Wang, A.C. and Bax, A. (1993) J. Biomol. NMR, 3, 715–720.