Research Square

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Enhanced Ca2+ Influx in Mechanically Distorted Erythrocytes: Measurements with 19F Nuclear Magnetic Resonance Spectroscopy

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Research Article

Keywords: 5FBAPTA, 13C NMR, 19F NMR, calcium transport, Piezo1, mechanosensitive Ca2+ flux, red blood cell, yoda1

Posted Date: November 24th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-107038/v1

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Version of Record: A version of this preprint was published at Scientific Reports on February 12th, 2021.
See the published version at https://doi.org/10.1038/s41598-021-83044-z.

Abstract

We present the first direct nuclear magnetic resonance (NMR) evidence of enhanced entry of Ca2+ ions into human erythrocytes (red blood cells; RBCs) when these cells are mechanically distorted. For this we loaded the RBCs with the fluorinated Ca2+ chelator, 1,2-bis(2-amino-5fluorophenoxy)ethane-N,N,N ' ,N ' -tetraacetic acid (5FBAPTA), and recorded 19F NMR spectra. The RBCs were suspended in gelatin gel in a special stretching/compression apparatus. The 5FBAPTA was loaded into the cells as the tetraacetoxymethyl ester; and 13C NMR spectroscopy with [1,6-13C]D-glucose as substrate showed active glycolysis albeit at a reduced rate in cell suspensions and gels. The enhancement of Ca2+ influx is concluded to be via the mechanosensitive cation channel Piezo1. The increased rate of influx brought about by the activator of Piezo1, 2-[5-[[(2,6-dichlorophenyl)methyl]thio]-1,3,4-thiadiazol-2-yl]-pyrazine (Yoda1) supported this conclusion; while the specificity of the cation-sensing by 5FBAPTA was confirmed by using the Ca2+ ionophore, A23187.

Full Text

This preprint is available for download as a PDF.

Figures

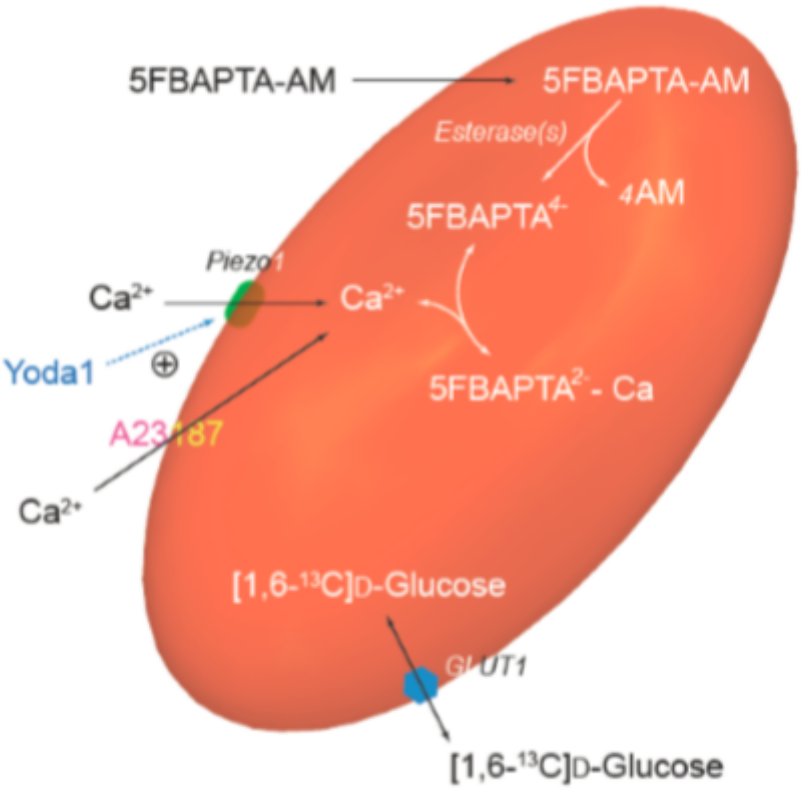

Figure 1

RBC experimental system used in this study. The graphics is that of an RBC under strain in a stretched gel (see below); it is loaded with the Ca2+-sensing chelator 5FBAPTA that yields separate 19F NMR signals from the free and Ca2+-complexed forms. Ca2+ enters via the mechanosensitive cation channel Piezo1; this can be activated (+ symbol) by the small-molecule compound Yoda1. Ca2+ entry into the RBC can also be mediated by the Ca2+-selective ionophore A23187. [1,6-13C]D-glucose enters via the glucose transporter GLUT1; it was used in conjunction with 13C NMR spectroscopy to measure glycolytic flux under various experimental conditions. The model of the distorted RBC is based on Cartesian translation in Mathematica [27] with the shape defined by the parametric equations given in [28].

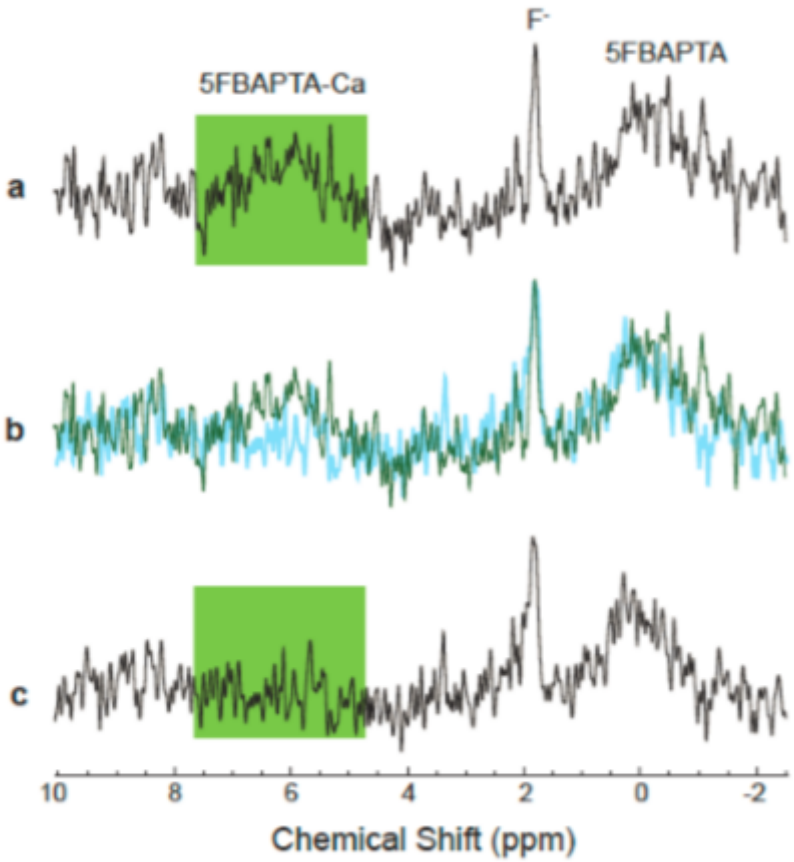

Figure 2

19F NMR (376.46 MHz) spectra of RBCs that had been loaded with 5FBAPTA and compressed by 75% in gelatin gel (35% w/v) at 4°C for 17 h, and then maintained at 20°C; for the sample that gave spectrum a, the duration of compression at 20°C was 32.75 h; and for the relaxed control sample b it was 34.25 h. The green highlighting around ~6 ppm identifies the Ca2+ complex of 5FBAPTA inside the RBCs; the broad peak arbitrarily set to a chemical shift of 0.000 ppm is from free 5FBAPTA; and the sharper peak at ~1.9 ppm was assigned to free F- from the gelatin. b shows the superposition of a (green) and c (blue). Sample details: 3.5 mL of RBCs (Ht = 0.80) incubated in 300 mM sucrose and 15 mM glucose, with 60 µL of 200 mM 5FBAPTA-AM in DMSO giving a total concentration of 4 mmol (L RBC)-1. Two mL of these cells (Ht = 0.82) were added to 1.75 g of bovine gelatin (Gelita) in 5 mL of the NaOH/saline solution as

described in Materials and Methods, giving a packing density (taking into account the partial specific volume of gelatin = 0.71 [29]) of 0.20. NMR settings: BBO probe; 90° RF pulse duration, 35 µs; 1024 FIDs per spectrum; inter-transient delay 5 s contributing to a total time of spectral accumulation of 1.5 h; spectral width was 10 kHz (26.56 ppm); and FIDs were modified with 20 Hz line broadening by exponential multiplication. The spectra were processed in TopSpin 4.0 and transferred to Adobe Illustrator for producing the graphics.

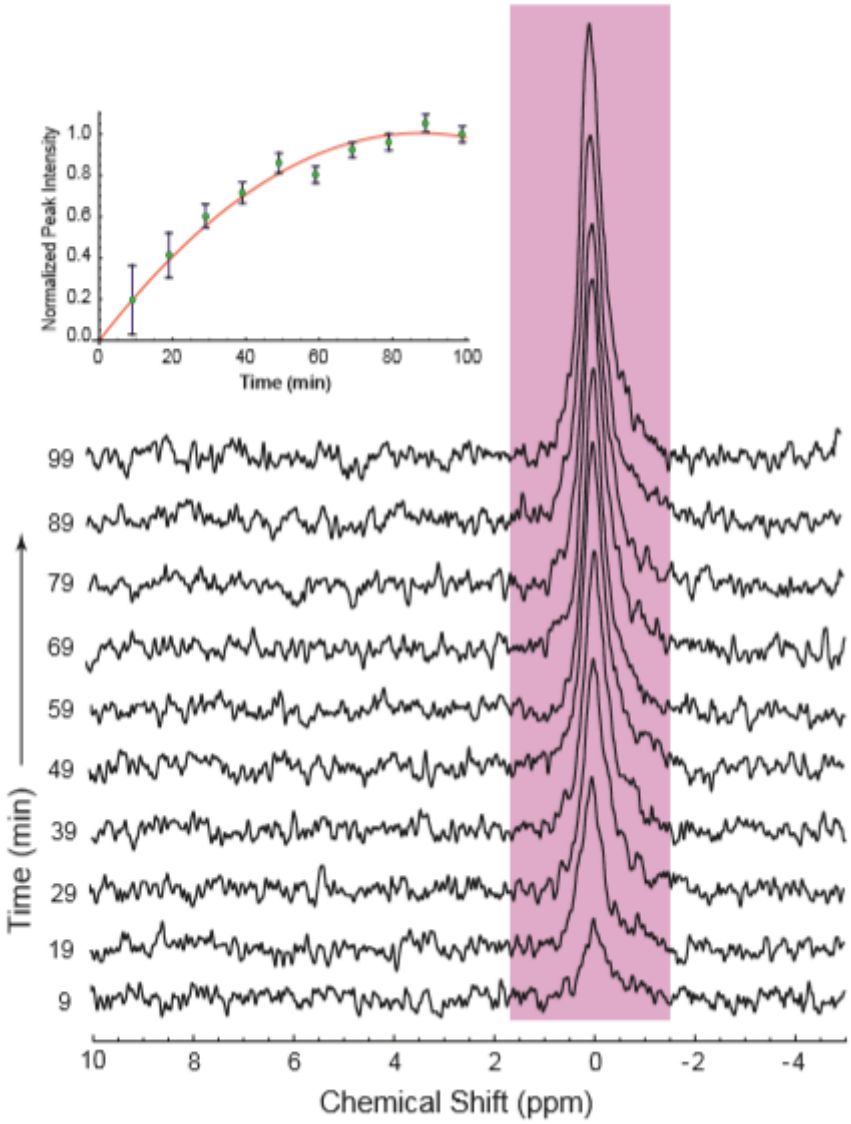

Figure 3

19F NMR spectral time course of loading 5FBAPTA into RBCs in suspension at 37°C. The pink area highlights the resonance of free 5FBAPTA that was assigned a chemical shift d = 0.0. Acquisition of the first spectrum started at 4 min after mixing 10 µL of 200 mM 5FBAPTA-AM in DMSO with the 0.5 mL RBC suspension of Ht = 0.65. NMR: 5-mm 19F probe; 90° RF pulse duration, 8 µs; 128 FIDs per spectrum; intertransient delay 4.61 s, adding to a total accumulation time of the summed FIDs of 10 min; spectral width was 10 kHz (26.56 ppm); and 20 Hz line broadening was applied by exponential multiplication of

the FIDs. The inset shows the 5FBAPTA peak intensities (green dots) from each spectrum over the 99-min time course; the time indicated is at the centre of FID accumulation. The solid red line is an empirical quadratic fit to the data [(0.023 ± 0.001) t - (0.00013 ± 0.00001) t2] used to obtain the initial slope at t = 0 and to guide the eye. The data-fitting was carried out with Mathematica's NonlinearModelFit [27]. The errors bars are reciprocals of the S/N derived automatically in TopSpin 4.0.

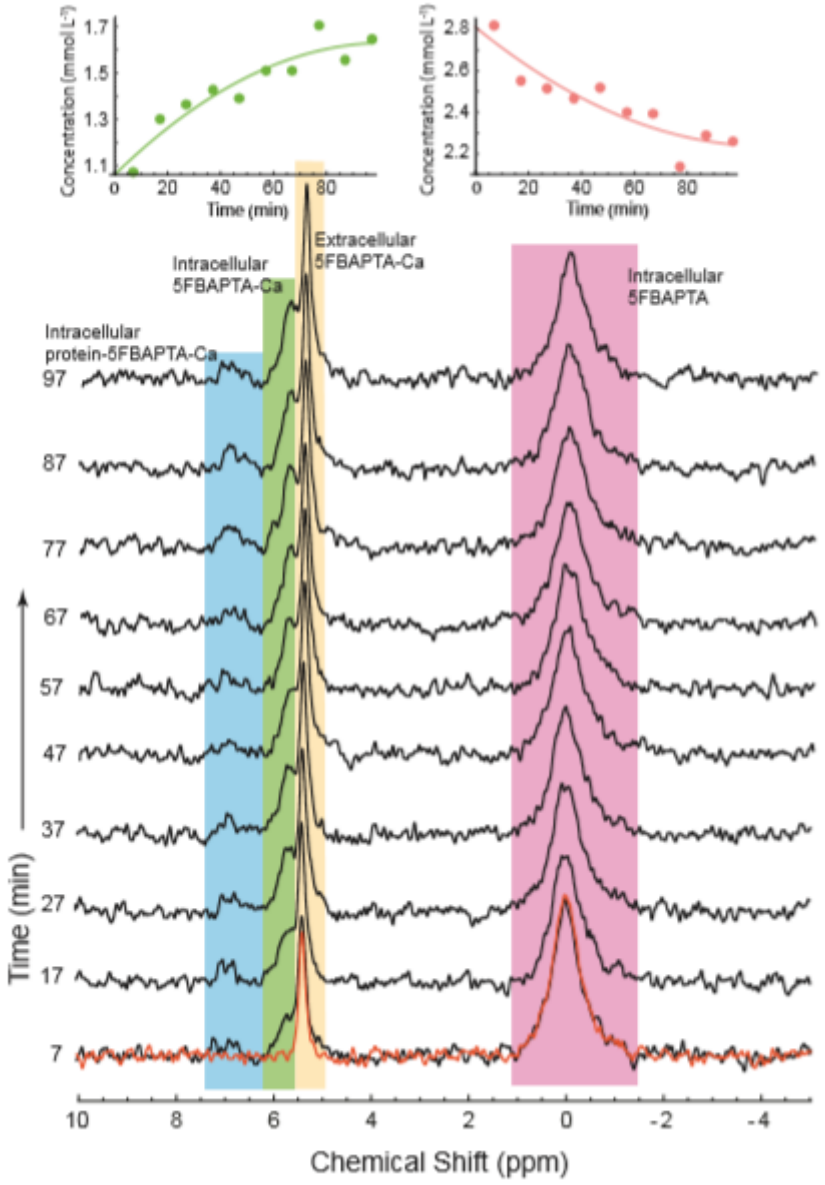

Figure 4

19F NMR spectra of RBCs loaded with 5FBAPTA and treated with Yoda1 in the presence of Ca2+, at 37°C. The peak from free 5FBAPTA inside the RBCs (initially 4 mmol [L RBC]-1) is highlighted in pink; yellow highlights the peak from the extracellular 5FBAPTA-calcium complex; green highlights the peak from the intracellular 5FBAPTA-calcium complex; and blue highlights the peak from the intracellular protein-5FBAPTAcalcium complex. The sample was 0.5 mL RBCs (Ht = 0.73) in 154 mM NaCl and 10 mM D-glucose. The spectra were recorded every 10 min; the superimposed red spectrum at 7 min is from the 6th spectrum of a 1 h time course recorded with the RBCs in the presence of 2 µL 1 M CaCl2 (corresponding

to 4.0 mM Ca2+ concentration averaged over the sample). Then, Yoda1 was added as 1 µL of 14 mM in DMSO; this value combined with a knowledge of the Ht gave a concentration of 38 µmol [L RBC]-1.

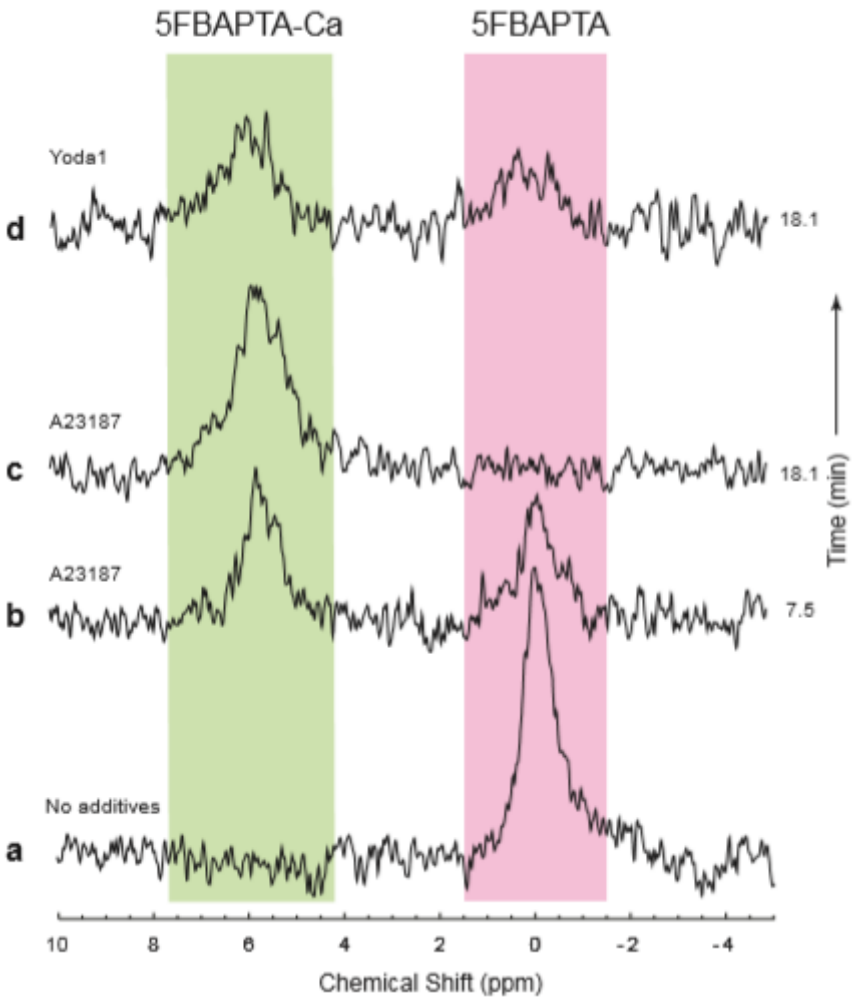

Figure 5

19F NMR spectra showing A23187 (and Yoda1 in comparion) stimulated uptake of Ca2+ in RBCs loaded with 5FBAPTA (4 mmol [L RBC]-1), at 37°C. The pink box highlights the resonance corresponding to intracellular free 5FBAPTA assigned the chemical shift d = 0.0 ppm; and the green box highlights the peak from the calcium complex that was centred at ~5.8 ppm. The sample of 0.5 mL RBCs (Ht = 0.62) was constituted in 154 mM NaCl, 10 mM glucose. a, The RBCs had been loaded with 4 mM 5FBAPTA as described in Methods. Then, for b and c, added with brisk mixing (by five-fold rapid inversion and re-inversion of the NMR tube) were: 5 µL 1 M CaCl2 making the concentration 10 mM averaged over the volume of the sample; and 0.5 µL 20 mM A23187 in DMSO giving a concentration of 32 µmol (L RBC)-1. For d, 5 µL 1 M CaCl2 and 1.0 µL 14 mM Yoda1 in DMSO giving a concentration of 45 µmol (L RBC)-1 were mixed into the 0.5 mL susupension. The times listed on the right of the spectra indicate the mid-point of spectral accumulation after a 2 min lag between mixing the sample and starting FID accumulation. NMR settings: 5-mm 19F probe; 90° pulse duration, 7.5 µs; intertransient delay, d1 = 5 s;

128 FIDs of 1024 complex points each; spectral width 10 kHz (26.545 ppm); total time per spectrum, 10 min 47 s; a 20 Hz line broadening was applied by an exponential multiplication of the summed FIDs.

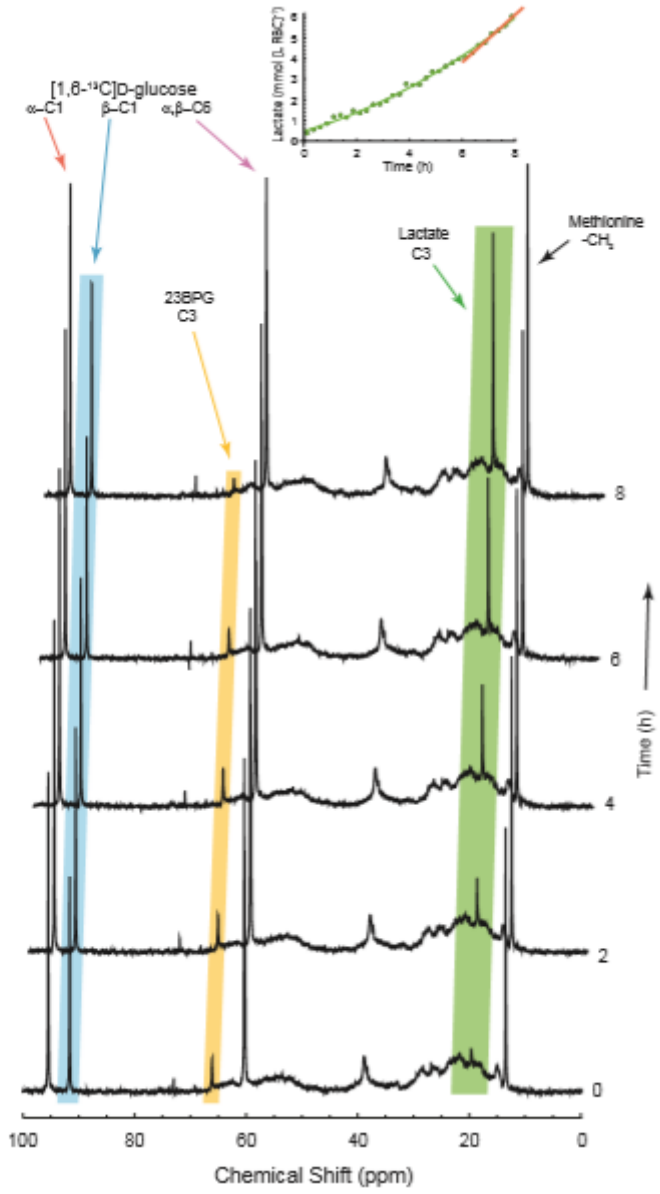

Figure 6

13C NMR (100.46 MHz) spectral time course of glycolysis in RBCs loaded with 5FBAPTA, at ~34°C. 3 mL RBCs (Ht = 0.75) loaded with 5FBAPTA-AM were supplemented with [1,6-13C]D-glucose and 13CH3-Lmethionine (final concentrations averaged over the sample volume were 8 mM and 7 mM, respectively). The times indicated on the right are relative to an arbitrary start time when the sample was removed from storage at 4°C, after preparation 1 h before. Every eighth spectrum is shown. The inset shows the concentration of lactate corrected to account for Ht. A quadratic (0.489 + 0.368 t + 0.0398 t2; solid green) was regressed (using Mathematica's NonlinearModelFit) onto the full time course data set (green dots) that included the transient stage of the progress curve (see Discussion for why this routinely occurred), and the straight line ([-3.10 ± 0.84] + [1.15 ± 0.11] t; solid orange) was regressed onto the integrals from the more linear last 2 h (8 points). 020406080100

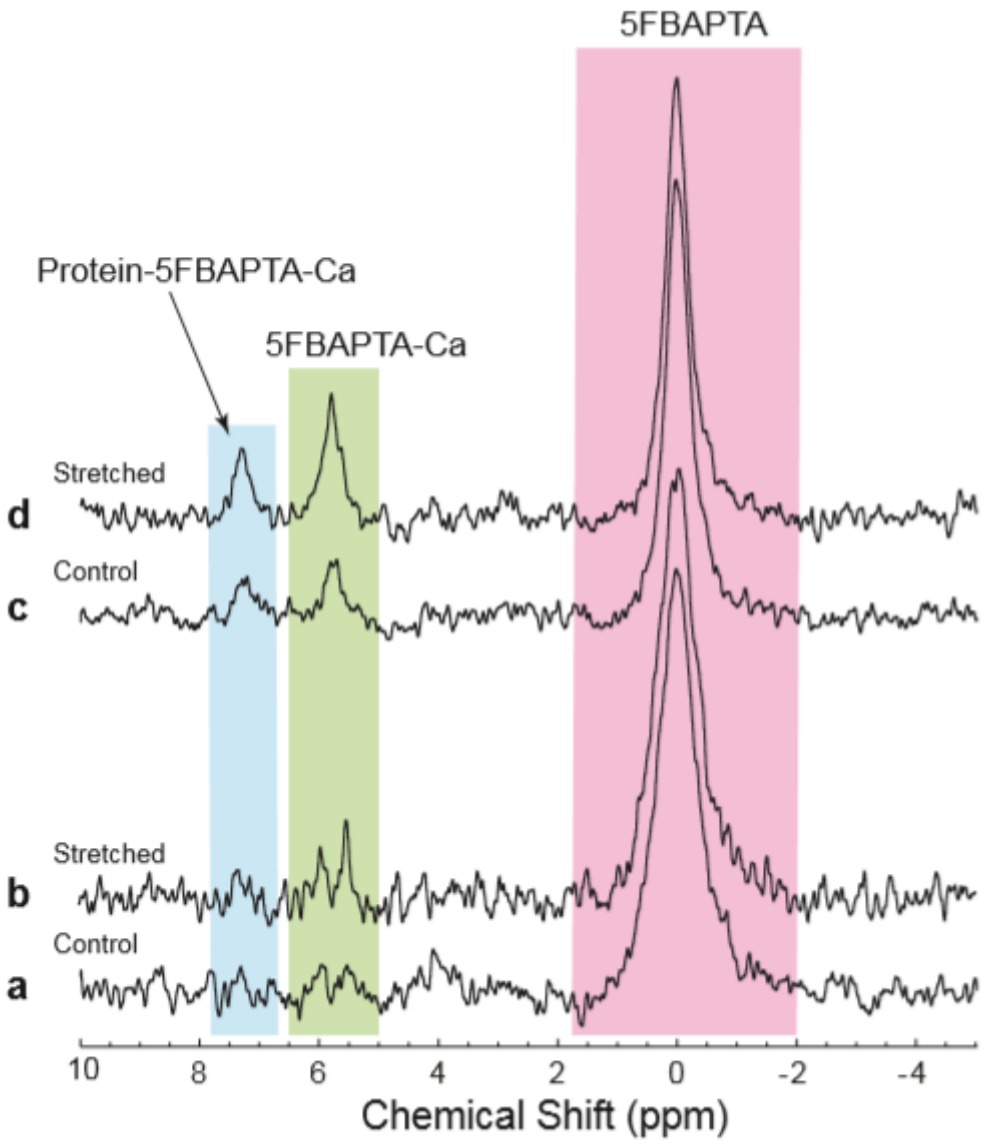

Figure 7

Net Ca2+ entry into RBCs after being stretched 75% in gel for 42 h at 20°C. The 19F NMR spectra were recorded as described in Fig. 5 after extracting the RBCs from the gel, using the protocol of Fig. S1. The pink highlighting denotes free intracellular 5FBAPTA, green the Ca2+ complex of 5FBAPTA, and blue the ternary complex between Ca2+ and protein inside the cells. a, Control (relaxed) RBC-gel; b, the stretched sample; c, sample a frozen and thawed in liquid nitrogen to lyse the RBCs; d, sample b treated as for c. NMR settings: 5-mm 19F probe; total time per spectrum, 10 min; other parameters as for Fig. 5.

Supplementary Files

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- SIEnhancedCa5FBAPTAFINAL.pdf