Supplementary material

Stability and biological activity of *E. coli* derived soluble and precipitated bone morphogenetic protein-2

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Enhanced precipitation of rhBMP-2 by divalent anions

Divalent anions appear to enhance precipitation of rhBMP-2. Here, we address the question if enhanced precipitation is due to the presence of the divalent anion or if it's an ion concentration effect.

Material and Methods

Experiments were carried out in transparent pureGrade 96 well plates (BRAND, Germany) using the following stock solutions: 500 mM acetate (pH 4,5), 200 mM oxalate (pH 4,5) and 200 mM NaCl. Addition of the stock solutions occurred in the following order: i) 20 µl of acetate stock solution, ii) appropriate volumes of oxalate and NaCl stock solutions and iii) ddH_2O to reach a volume of 190 µl in each well. Finally, 10 µl of rhBMP-2 stock solution (1 mg/ml) were added to each well and the plates allowed incubating at 8°C overnight. Prior to measurement, the 96 well plates were shaken for 10 seconds at medium speed and the absorbance at 340 nm was measured using a Multiskan GO spectrophotometer (Thermo Fisher Scientific, USA). All analyses were carried out at least in triplicate.

Results

The results clearly show that oxalate at concentrations as low as 5 mM (in 50 mM acetate, pH 4.5) caused precipitation of rhBMP-2 while NaCl concentrations above 40 mM were required to precipitate rhBMP-2 in 50 mM acetate, pH 4.5 (Figure 1S) corroborating the conclusion that divalent anion enhanced precipitation is a more specific and not only an anion concentration effect (see also main manuscript where these precipitation data are shown and discussed as function of the ionic strength).

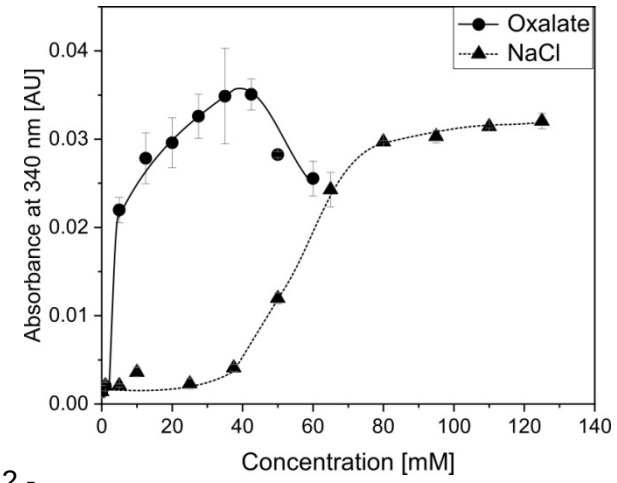

Figure 1S Oxalate and NaCl dependent precipitation of rhBMP-2. Precipitation of rhBMP-2 as function of the oxalate and NaCl concentration in 50 mM acetate (pH 4.5). The data are presented as mean ± SD, n = 3.

Resolubilisation of rhBMP-2 precipitates

rhBMP-2 forms precipitates in various buffer systems. Here, we address the question if formed precipitates can be resolubilised when the precipitates are transferred into a buffer in which rhBMP-2 was shown to be soluble (50 mM MES, pH 5).

Material and Methods

40 µl of rhBMP-2 stock solution (500 µg/ml rhBMP-2 in 50 mM MES, pH 5) was diluted 1:20 in 760 µl malonate (pH 5), MES (pH 7), phosphate (pH 7), MOPS (pH 7.5), TRIS (pH 8), CHES (pH 9) and CAPS (pH 10.5) buffers (each 50 mM). As a soluble (negative) control 40 µl of the rhBMP-2 stock solution was diluted 1:20 in 760 µl 50 mM MES buffer (pH 5). The mixture was allowed to incubate overnight at 8°C. Afterwards, the samples were centrifuged at 17.000 x g for 15 minutes. 790 µl of the supernatant were removed and the same volume of 50 mM MES buffer (pH 5) was transferred to the precipitates (or the remaining volume of the negative control). This mixture was vortexed and allowed to incubate for about 24 hours at 8°C. Afterwards, the mixture was centrifuged at 17.000 x g for 15 minutes. As a positive control, rhBMP-2 with a concentration of 25 µg/ml in 50 mM MES buffer (pH 5) was treated the same way except that no supernatant was removed and no exchange with fresh MES buffer (pH 5) was carried out. After centrifugation, 700 µl supernatant of each sample were transferred to a new centrifuge tube and the protein concentration was determined by an adapted sensitive Bradford assay [1] using BSA (#23209, Thermo Fisher, USA) in 50 mM MES buffer (pH 5) as standard. The determined concentration of the positive control (supernatant of 25 µg/ml rhBMP-2 in 50 mM MES buffer, pH 5) was set as 100 % recovery. All experiments were carried out in triplicates.

Results

The results clearly show that rhBMP-2 precipitates can be resolubilised, independent in which buffer system the precipitation was performed (Fig. 2S). The overall recovery of soluble rhBMP-2 after precipitation and resolubilisation ranged from 85 – 100 % compared to the non-precipitated sample (= 100 %, positive control). The negative control of rhBMP-2 in 50 mM MES buffer (pH 5) treated from the beginning in the same way as the other samples only revealed 5 % of the original concentration.

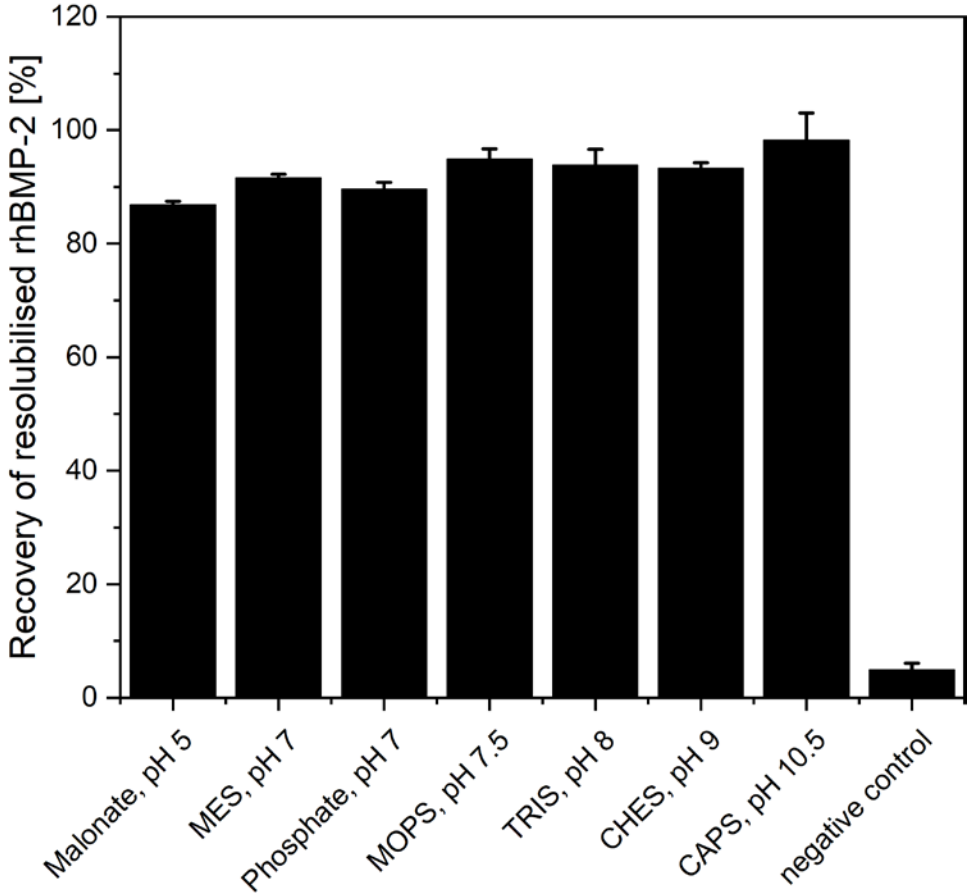

Figure 2S Recovery of soluble rhBMP-2 after resolubilisation of rhBMP-2 precipitates. rhBMP-2 was precipitated in indicated buffers, pelleted by centrifugation and resolubilized in the same volume of 50 mM MES buffer (pH 5). The recovery of soluble rhBMP-2 from rhBMP-2 precipitates is given in comparison to non-precipitated rhBMP-2 in MES buffer (pH 5) (= 100 %). The negative control is rhBMP-2 in 50 mM MES buffer (pH 5) treated in the same way as the precipitated samples (for details see results). The data are presented as mean ± SD, n = 3.

References

[1] C.D. Georgiou, K. Grintzalis, G. Zervoudakis, I. Papapostolou, Mechanism of Coomassie brilliant blue G-250 binding to proteins: a hydrophobic assay for nanogram quantities of proteins, Anal. Bioanal. Chem. 391 (2008) 391-403.