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The effect of tritiated water on the alkaline phosphatase inactivation after ultrasound and gamma-rays irradiation

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S1. Experimental details

1. Alkaline phosphatase

Alkaline phosphatase (lyophilised) was provided by Sigma Aldrich, CAS registry number: 9001-78-9. Its enzymatic activity and Michaelis constant was assayed via fixed-time method. 1 mL of solution A (1 M diethanolamine, 0.5 mM magnesium chloride, 5 mM p-nitrophenylphospate) was mixed with 20 μL of ALP solution (0.2 $g \cdot L^{-1}$). After 2 minutes at 36 °C reaction was stopped by addition of excess NaOH solution (0.5 M). Reaction product content was determined using UV-Vis spectrophotometry (UV-1280, Shimadzu) at 405 nm.

2. Michaelis constant determination

The Michaelis constant was determined from the Lineweaver-Burk plot. After 20 days of storage of ALP solutions at 10°C Michaelis constant values were 2.8±0.6 mM for native ALP, 3.5±0.9 mM for sample after ultrasonic treatment, and 3.1±0.5 mM for sample after γ-irradiation.

3. Tritiated water

Tritiated water HTO was obtained using isotope exchange of H_2O with tritium gas T_2. H_2O was put on vessel walls in a thin layer and frozen with liquid nitrogen. A vacuum was created and the water layer was treated with tritium gas atomised via 2000 K tungsten wire, heated electrically [S1]. Resulting tritiated water was thawed and collected with additional water, then purified with activated charcoal (Russ. *SKT-3*, by *ZAO EKhZ*). Two HTO samples with 0.34 and 1.3 GBq/ml activity were obtained.

4. Ultrasonication

Ultrasonication was carried out in a thermostated (24 °C) cell through ultrasound conductive medium, with intensity of 2 W/cm^2, frequency of 0.88 MHz. 0.2 g/L, 1 g/L ALP solutions were treated, sample volume being 10 mL (Fig. S1).

5. γ-Irradiation

γ-Irradiation was carried out with the set of γ-emitting ^{137}Cs sources located uniformly around the sample. Resulting dose rate was 2.3 Gy/min. Samples 10-20 mL in volume were irradiated at room temperature in glass vials.

6. Inactivation rate

Initial inactivation rate was determined as a derivative of experimental dependency of enzyme activity on time at $t = 0$. To accomplish this, the dependence was approximated by the function $A = A_\infty + b \cdot \exp(-kt)$, where A is enzymatic activity, A_∞ – remaining constant activity, b – activity decrease $b = (A_0 - A_\infty)$, A_0 – initial activity, t – time, k – inactivation rate constant. The initial rate of inactivation in this case is determined as follows $r_0 = k \cdot b$. Approximation parameters and initial inactivation rates are shown in Table S1.

7. Hydrogen peroxide quantification

H_2O_2 content was determined with potassium iodide and molybdenum-based catalyst [S2]. Solution A (0.05 M NaOH, 0.40 M KI, 0.2 mM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) was mixed with equal volume of 0.10 M potassium biphthalate solution, the resulting solution then mixed with analysed sample in equal portions. Peroxide content was calculated as $[H_2O_2]$, mM $= 40.0 \cdot A$, where A is absorbance at 350 nm.

8. Cavitation intensity

Cavitation intensity was assayed with IC-3MS (BSUIR, Minsk) cavitometer (Fig. S1). The apparatus is based on analysis of the spectrum of cavitation noise in the frequency range 5 kHz-10 MHz [S3]. The apparatus is multifunctional and is intended for the following measurements:

- Total bubble activity;

- Transient bubble activity;
- Subharmonic intensity;
- Integral hydrophone output;
- Fundamental frequency of the driving field.

Table S1. Approximation parameters and initial inactivation rates obtained from experimental dependences of the relative activity of the enzyme on the time after ultrasound and gamma irradiation exposure.

Exposure	A_∞	b	k, day^{-1}	R^2	r_0
US	0.151 ± 0.026	0.627 ± 0.036	0.108 ± 0.016	0.98402	0.068 ± 0.011
γ-irradiation	0.437 ± 0.015	0.438 ± 0.017	0.136 ± 0.017	0.99468	0.060 ± 0.008

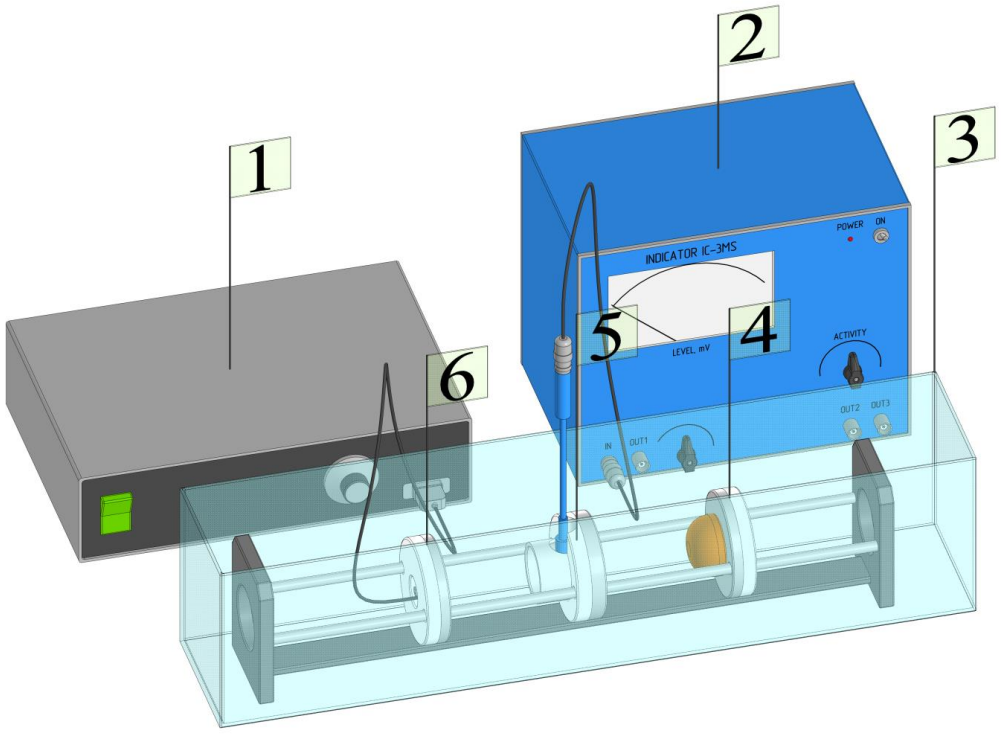

Figure S1. Ultrasonication setup. 1 – ultrasonic generator, 2 – cavitometer, 3 – thermostated vessel with ultrasound conductive medium, 4 – acoustic absorber, 5 – ultrasonic cell and hydrophone, 6 – piezoceramic ultrasound transducer.

References

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