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Freezing-induced metabolic changes in the liver and hindlimb muscle of the moor frog *Rana arvalis* and the Siberian salamander *Salamandrella keyserlingii*

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Motivation and Aim: Among amphibians, only a few species can survive prolonged (several weeks) freezing. Berman et al. [1] demonstrated that adult Siberian salamanders *Salamandrella keyserlingii* may survive prolonged freezing at up to 50 °C, while the moor frog *Rana arvalis* is capable of surviving freezing at –16 °C [2]. Little is known on the freezing response of these Eurasian species. We performed a metabolomics analysis of the liver and hindlimb muscles of the frozen and control Siberian salamanders and moor frogs in order (1) to quantify the major metabolites present in the tissues; (2) to determine the low molecular cryoprotectants used by these species; (3) to elucidate the changes in energy-related molecules; and (4) estimate the degree of cellular stress.

Methods and Algorithms: For each species, five adult animals were exposed to 7 days of freezing (–5 °C for *R. arvalis*, –8 °C for *S. keyserlingii*), and the control sample of the same size was kept at +1 °C. Liver and hindlimb muscles of the studied animals were extracted as quickly as possible, frozen in liquid nitrogen and used for metabolite extraction. Identification and quantification of metabolites was performed using 1H NMR and mass spectrometry analysis.

Results: We determined concentrations of approximately 60 substances in the studied organs. The only low molecular weight cryoprotectant in *S. keyserlingii* was glycerol (earlier found in [3]); unlike all other studied amphibians, the Siberian salamander does not use glucose as a cryoprotectant [4]. *R. arvalis* used both glycerol and glucose, which was unexpected, since the representatives of the genus *Rana* were believed to employ glucose only. We found high levels glycolysis products in both species, mostly lactate and alanine. Low amounts of ethanol and 2,3-butanediol were also detected; the latter is an interesting substance also detected in significant quantities Rana amurensis in response to anoxia [5]. The moor frog accumulated succinate, typical for ischemic conditions in vertebrates. However, this was not the case for the Siberian salamander, which suggests alternative strategies for ischemia adaptation. Freezing resulted in profound DNA damage, indicated by high concentration of nucleotide degradation products in both organs.

Conclusion: Our findings indicate that freeze-tolerant amphibians differ in the changes of many important metabolites, suggesting species-specific adaptation pathways.

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