Forensic determination of blood sample age using a bioaffinity-based assay

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Electronic Supplementary Information (ESI)

EXPERIMENTAL SECTION

Chemicals and reagents used

All enzymes and substrates were purchased from Sigma-Aldrich and used with no further treatment: Creatine Phosphokinase from rabbit muscle, Type I (CK; E.C. 2.7.3.2), Glutamic-Pyruvic Transaminase from porcine heart (ALT; E. C. 2.6.1.2), Pyruvate Kinase from rabbit muscle, Type II (PK; E.C. 2.7.1.40), L-Lactic Dehydrogenase from rabbit muscle, Type II (LDH, E.C. 1.1.1.27), serum from a human male (Type AB), creatine anhydrous (Crt), adenosine 5'-triphosphate disodium salt hydrate (ATP), phospho(enol)pyruvic acid monopotasium salt (PEP), L-alanine (Ala), α-Ketoglutaric acid disodium salt dihydrate (KTG), and β-Nicotinamide adenine dinucleotide reduced dipotassium salt (NADH). The water used for all experimental procedures was ultrapure water (18.2 MΩ•cm), obtained from PURELAB flex, ELGA water purification system.

Instrumentation and measurements

The spectrophotometer UV/Vis temperature-controlled plate reader (SpectraMax Plus 384, Molecular Devices, CA), was used to take optical measurements of the samples at $\lambda = 340$ nm. A microtiter polystyrene (PS, Thermo Scientific) plate was utilized. The samples were incubated in MyTemp™ Mini Digital Incubator (VWR).

Composition and operation of the model systems:

Scheme 1, creatine kinase (CK) and alanine transaminase (ALT) were used in a concerted manner to determine the age of bloodstain samples.[1-4] The mean concentrations of the markers CK (100 mU/mL and ALT (20 mU/mL) were based on their physiological levels present in a healthy adult person. Scheme 1A/B (in the manuscript) represents the CK/ALT biocatalytic cascade, which follows the enzymatic activities of both biomarkers within the aging process. The biocatalytic cascade was performed in 50 mM glycine-glycine buffer solution at pH 7.95. CK/ALT biocatalytic reaction was combined with PK to yield pyruvate followed with the oxidation of NADH by LDH. The optical signal in the cascade is generated by the production of pyruvate and the consumption of NADH. Pyruvate is the product of CK and ALT. The concentrations of the substrates were as follows: Crt 15 mM,

ATP 10 mM, PEP 6 mM, KTG 1 mM and NADH 0.25 mM. The concentration of PK and LDH were 2 U/mL and 150 mU/mL, respectively. The measurements were taken from samples incubated at following temperatures 18 °C, 25 °C and 40 °C. The time intervals were: 0, 6, 24, 48, 96 and 120 hours. After incubation (aging phase) the samples were immediately subject to a continuous optical measuring at λ = 340 nm, to monitor the consumption of NADH. The reaction and optical measurements were performed at 37°± 0.1 °C.

Serum stains analysis:

To mimic blood samples, serum from human male (type AB; Sigma-Aldrich) was spiked with levels of biomarkers relevant for healthy adults. The bioanalytic cascade had two different biomarkers dissolved in the serum, CK (100 mU/mL), and ALT (20 mU/mL). There were three sets of parallel samples incubated at 18 °C, 25 °C and at 40 °C for the following times: 0, 6, 24, 48, 96 and 120 hours. The mimicked samples, naturally dried by the environment on a glass surface, were then re-suspended with water. The biocatalytic cascade was activated by mixing the incubated sample with the substrates that were previously dissolved in 50 mM glycine-glycine buffer at pH 7.95. The concentrations of the substrates were as follows: Crt 15 mM, ATP 10 mM, PEP 6.0 mM, KTG 1mM and NADH 0.25 mM. CK was coupled with, PK (2 U/mL). The samples were immediately subjected to a continuous optical measuring at λ = 340 nm, to monitor the consumption of NADH. The measurement was taken at 37 °C for both sets of samples to optimize the activity of the enzymes. They were also pre-incubated at 37 °C before the measurement.

References

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