Thermodynamic features of blood plasma derived from patients with neurodegenerative diseases

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Background:

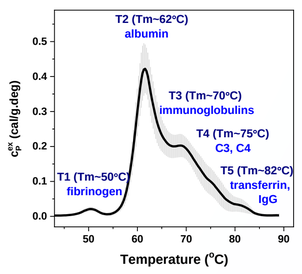
Neuro-degenerative diseases (NDDs) are an important medical and social problem as their relative share has been steadily increasing in recent years. These disorders include conditions of progressive dysfunction and/or loss of a group of neurons in the brain or peripheral nervous system. NDDs are incurable and disabling diseases, the most common being Alzheimer's disease, Parkinson's disease, Lewy body dementia and Amyotrophic lateral sclerosis. Despite advances in diagnostic and therapeutic methods, there are still no reliable biomarkers identifying the complex pathways contributing to the pathology and early diagnosis of NDDs. Therefore, the development of new approaches for early detection of these diseases is of great importance. The blood plasma proteome is extremely complex, but it is a strongly dominated by a number of proteins such as albumin, haptoglobin, immunoglobulins, fibrinogen, IgG, transferrin etc. Binding of disease-specific ligands to these plasma proteins might induce changes in their conformation that can be followed by means of differential scanning calorimetry (DSC), a technique highly sensitive to the protein concentration and conformation, as well as to the interactions of proteins with other molecules. In the recent years, this was exploited in order to identify disease specific changes in the blood plasma proteins that might serve as biomarkers.

AIM:

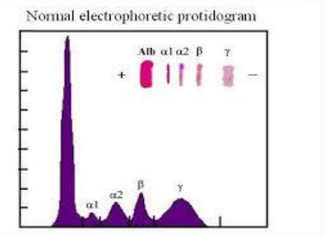
To identify specific features of the thermodynamic behavior of blood plasma derived from NDDs patients compared to healthy ones.

Methods:

The protein thermal stability was probed by means of highly sensitive differential scanning calorimeter DASM-4 (Privalov, Puschino). Blood plasma samples were linearly heated in the range 30 - 95°C with a scanning rate of 1 °C/min. The thermodynamic parameters: excess heat capacity (c_p^{ex}), transition temperature (T_m) of the successive transitions and the weighted average center of the thermogram (T_{FM}) were defined from the calorimetric profiles. Quantification of the individual plasma protein fractions (albumin, α1-, α2-, β1-, β2- and γ-globulins) was carried out by capillary electrophoresis (Capillarys2, Sebia).



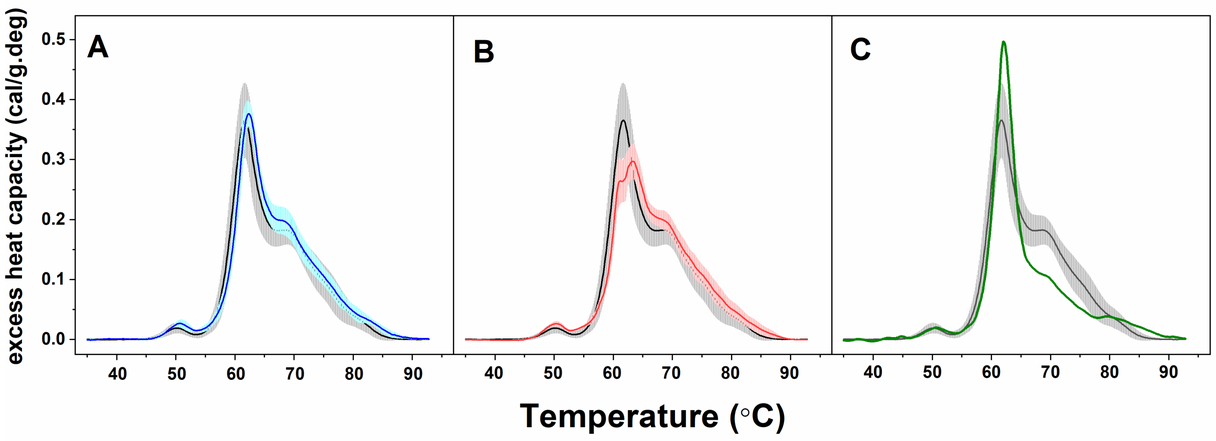
Typical DSC plasma profile obtained from healthy subjects (mean±SD). The contribution of the major plasma proteins to the calorimetric transitions is indicated



Blood plasma protein electrophoresis of healthy individual

Results

DSC profiles of blood plasma from healthy individuals and patients with neuro-degenerative diseases



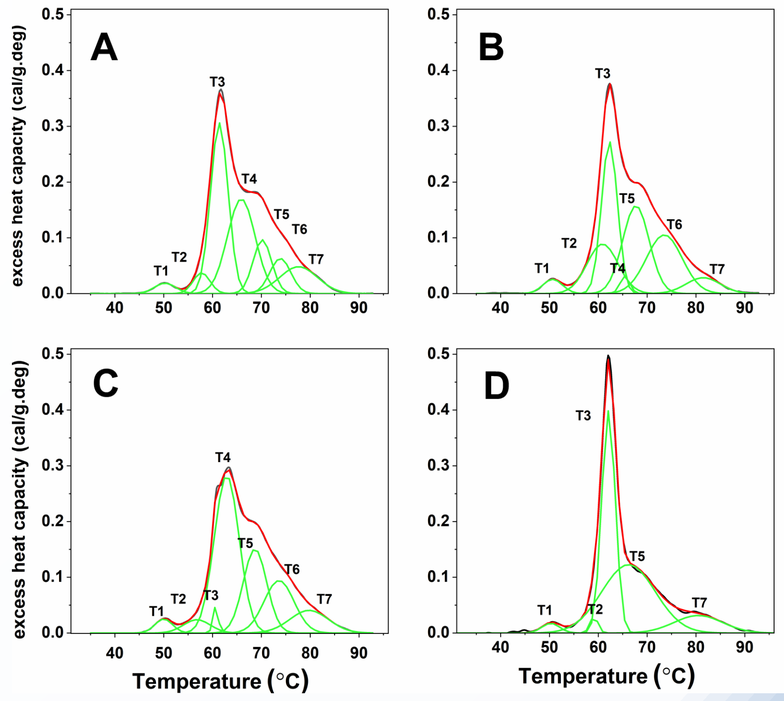
Averaged DSC profiles (solid lines) and standard deviations (shading) are shown for the healthy individuals (black line/light gray shading, all panels) and for Group1 (blue line/cyan shading) - panel A, Group2 (red line/pink shading) – panel B and Case 1 (green line) – panel C of NDD patients.

Thermodynamic parameters (mean value ± SD): excess heat capacity (c_p^{ex}), transition temperature (T_m) of the successive transitions, estimated from the calorimetric profiles of blood plasma from healthy controls and patients with neuro-degenerative disease (Group 1, Group 2 and case 1).

Groups	Tm^{Fib} (°C)	C_p^{Fg} (cal/g.K)	Tm^{Alb} (°C)	C_p^{Alb} (cal/g.K)	Tm^{Igs} (°C)	C_p^{Igs} (cal/g.K)	$Tm^{IgG/Tf}$ (°C)	$C_p^{Ig/Tf}$ (cal/g.K)	Cp^{Alb} / Cp^{Igs}
Controls	50.2±0.01	0.02±0.02	61.3±0.06	0.36±0.04	69.1±0.02	0.18±0.02	81.7±0.01	0.03±0.001	2.0±0.1
Group 1	51.0±0.1*	0.026±0.006	62.5±0.04*	0.38±0.01	68.7±0.4	0.18±0.01	83.2±0.4	0.04±0.001	2.1±0.1
Group 2	50.4±0.1	0.02±0.005	63.6 ±0.06*	0.30±0.02*	68.9±0.4	0.20±0.01	80.9±0.5	0.05±0.001	1.5± 0.1*
Case 1	50.7	0.02	62.05	0.49	69.56	0.10	80.08	0.03	4.9*

*indicates statistically significant difference from the Controls, p < 0.05

Examples of deconvolution analysis of DSC profiles of blood plasma from healthy control and NDD patients



Gaussian fits of the scans of healthy individual (A), patient from Group1 (B), patient from Group2 (C) and ungrouped case (D). The original DSC scan is presented in black, the individual Gauss components (T1-T7) are plotted in green and the product of their sum is shown in red.

Thermodynamic parameters determined by Gaussian fitting of the calorimetric blood plasma scans recorded for the different groups of individuals: healthy controls and NDD patients (Group1; Group2 and Case1). The calorimetric enthalpy (ΔH) and the denaturation temperature (Tm) of the individual components (denoted as 1 - 7 subscript) are shown as mean value ± SD.

Groups	ΔH_1 (cal/g)	Tm_1 (°C)	ΔH_2 (cal/g)	Tm_2 (°C)	ΔH_3 (cal/g)	Tm_3 (°C)	ΔH_4 (cal/g)	Tm_4 (°C)	ΔH_5 (cal/g)	Tm_5 (°C)	ΔH_6 (cal/g)	Tm_6 (°C)	ΔH_7 (cal/g)	Tm_7 (°C)
Controls	0.1±0.01	50.26±0.15	0.16±0.50	57.75±0.10	1.37±0.15	61.4±0.3	1.20±0.05	65.88±0.60	0.46±0.15	70.2±1.05	0.34±0.10	73.88±2.02	0.47±0.02	77.62±0.31
Group1	0.12±0.05	51.37±0.29*	0.55±0.54*	59.41±0.61*	1.14±0.10*	62.1±0.8*	0.13±0.01*	67.44±0.93*	1.08±0.07*	76.5±2.11	0.97±0.06*	78.16±1.23*	0.24±0.06*	85.32±1.00*
Group2	0.11±0.01	50.00±0.23	0.16±0.65	56.77±0.77*	0.07±0.01	60.0±0.1	1.79±0.60	63.00±1.10*	0.91± 0.34	68.69±1.12	0.72±0.10	73.61±1.91	0.40±0.07	79.77±1.35*
Case 1	0.08	50.25	0.06	60.00*	1.38	62	-	-	1.68*	66.1	-	-	0.40	80.4

*indicates statistically significant difference from the Controls, p < 0.05

Thermodynamic parameters (mean ± SD): change of the total calorimetric enthaply and the weighted average center of the thermogram (T_{FM}), similarity parameters (r, P, ρ) and results from Wilcoxon test, estimated from the DSC profiles of blood plasma derived from healthy controls and NDD patients

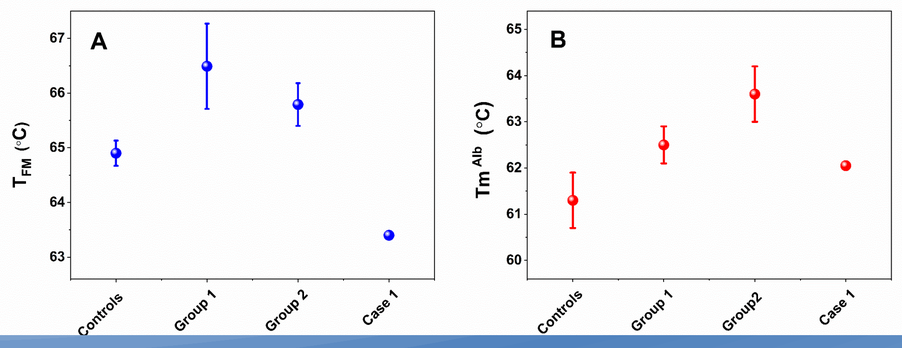
Groups	Δ H (cal/g)	T_{FM} (°C)	r	P	ρ	Wilcoxon test (55-67°C)	Wilcoxon test (68-87°C)
Controls	4.10±0.21	64.9±0.29	-	-	-		
Group 1	4.31±0.35	66.49±0.78*	0.88±0.05	0.83±0.04	0.85±0.03	P>0.05	P<0.05
Group 2	4.17±0.45	65.79±0.39*	0.88±0.05	0.82±0.04	0.83±0.04	P<0.05	P<0.05
Case 1	3.60*	63.4*	0.88	0.78	0.80	P>0.05	P<0.05

*indicates statistically significant difference from the Controls, p < 0.05

Concentration of the main plasma proteins fractions (mean ± SD) determined by capillary electrophoresis and presented as percentage of the total protein content for the groups under study

Group 1	Albumin (%)	Alpha-1 (%)	Alpha-2 (%)	Beta-1 (%)	Beta-2 (%)	Gamma (%)
Reference	55.8-66.1	2.9-4.9	7.1-11.8	4.7-7.2	3.2-6.5	11.1-18.8
Group 1	58.41±2.54	4.73±1.10	9.45±1.01	7.15±1.37	7.63±2.08	12.62±2.43
Group 2	58.69±1.04	4.90±0.45	10.28±1.24	6.96±0.74	6.82±0.77	12.33±1.00
Case 1	56.80	4.00	10.00	6.50	11.40*	11.30

Thermodynamic parameters for the different calorimetric groups



Selected thermodynamic parameters (T_{FM} – weighted average center of the thermograms and Tm - temperature of the main transition) determined from the DSC profiles of blood plasma derived from healthy controls and NDD patients.

CONCLUSIONS

- The obtained results reveal that for most of the studied NDD cases the plasma thermograms exhibit shift of fibrinogen and albumin assigned transitions to higher temperatures (by 1 – 2 °C) as compared to those of healthy individuals.
- Two groups of NDD plasma thermograms are defined, based on the thermodynamic parameters. For the first group the application of Wilcoxon non-parametric test shows statistically significant difference in globulin assigned region (68 – 87 °C) compared to the healthy controls, whereas for the second group difference is also detected for the albumin assigned transition (55 – 67 °C) and in addition, a new peak at ca. 60 °C is detected.
- Statistically significant shift of the weighted average center (T_{FM}) of the thermograms for the NDD cases of Group 1 and Group 2 reveals stabilization of the plasma proteome as compared to healthy controls.

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