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Microcalorimetry of Blood Serum Proteome: A Modified Interaction Network in the Multiple Myeloma Case

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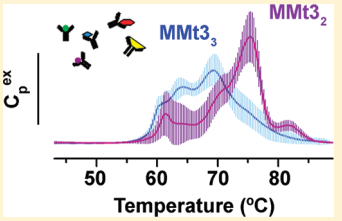
Supporting Information

ABSTRACT: Hereby we report on a novel approach in the study of multiple myeloma (MM), namely, differential scanning calorimetry (DSC) combined with serum protein electrophoresis. Distinct thermodynamic signatures describe the DSC thermograms of MM blood sera, in contrast to the unique profile found for healthy individuals. The thermal behavior of MM sera reflects a complex interplay between the serum concentration and isotype of the M protein and of albumin, and modified ligand- and/or protein−protein interactions, resulting in stabilization of globulins and at least a fraction of albumin. In all MM cases the 85 °C, transferrin-assigned transition is missing. A distinct feature of IgG isotype (κ and λ) DSC profiles only is the presence of a transition at 82 °C. A DSC-based classification of MM depicts two sets of melting patterns (MMt2 and MMt3 with two or three successive thermal transitions), and subsets within each set (MMt2$_i$ or MMt3$_i$, the subscript i = 1, 2 or 3 denotes the main transition being one of the three transitions). The results demonstrate the potential of DSC to monitor MM-related modifications of the serum proteome, even at low M protein concentrations, Bence Jones and importantly nonsecretory multiple myeloma cases, and prove DSC as a versatile tool for oncohematology.



Multiple myeloma (MM) is a monoclonal B-cell disorder associated with proliferation of malignant plasma cells in the bone marrow[1,2] and commonly with production of monoclonal (M) proteins, recognized as IgG, IgA, IgM, IgD, IgE, depending on their heavy chain class and the light chain proteins designated as kappa (κ) or lambda (λ), and Bence Jones (BJ) protein.[3] In rare cases, clones are nonsecretory and no M proteins are produced.[4]

Clinically MM is a rather heterogeneous disease, with different symptoms, like bone lesions, renal failure, immunodeficiency, etc.[5] and yet with an unknown origin. Current knowledge from MM oncogenomics points to the importance of genetic factors (multitude of chromosomal aberrations) for this clinical heterogeneity[2,6,7] and to redundancy of mechanisms involved in the disease pathogenesis.[2,8,9] On the basis of genetic and molecular characteristics, several subtypes of MM were defined and genetic testing of patients was encouraged to be incorporated in the clinical practice.[7] Heterogeneity in the gene expression and respective molecular grouping has also been recently demonstrated in human myeloma cell lines derived from newly diagnosed patients.[10] These new findings (different molecular groups of MM, related to common features in MM genetics and clinical characteristics) most probably are relevant for the specific patients′ response to a given treatment and hence to the prediction of patient survival. Besides the advances in proteomics research, the clinicians generally use the Durie/Salmon staging system[11] and International Staging System (ISS)[12] in combination with conventional cytogenetics and fluorescent in situ hybridization. Thus, different prognostic factors (serum levels of β2-microglobulin, human serum albumin (HSA), C-reactive protein, calcium, hemoglobin, the level and type of M protein, soluble syndecan-1 (CD138), and cytogenetics (with still limited application)) or combination of factors (like β2-microglobulin and albumin) are considered for the patient outcome, though there is no consensus about the most powerful prognostic factor.[7,11,13−15] Unlike other leukemias (such as chronic lymphocytic leukemia) and cancers, there are no available targeted therapies for MM yet, however, novel approaches are under investigation.[16,17]

Advanced proteomics techniques,[18,19] gene expression profiling,[6,20,21] post-translational protein modifications related to MM onset and pathogenesis,[22,23] and alteration of miRNA expression[24−27] could provide potential therapeutic strategies (see also the review of Ge et al).[28]

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Table 1. Clinical Characteristics of the MM Patients

number of patients		82
gender, M/F	M (36)	F (46)
mean age (interval)	59 (31−82)	63 (46−84)
M protein isotype	*n* (%)[a]	
IgG	47 (57.3%)	
IgA	12 (14.6%)	
IgM	6 (7.3%)	
light chain/BJ	8 (9.8%)	
nonsecretory MM	9 (11%)	
protein fraction	*n* (%)	
γ	0	
β2	3 (3.7%)	
β1	4 (4.9%)	
α2	25 (30.5%)	
α1	18 (22%)	
albumin	12 (14.6%)	

[a] *n* (%), *n* is the number of MM cases for the different M protein isotypes and for the different protein fractions in the blood serum, determined by SPE. In parentheses *n* is given in % of the total number of cases studied.

The goal of the present work is to validate the use of a novel approach, differential scanning calorimetry (DSC), for study of MM combining it with the traditionally applied serum protein electrophoresis (SPE). DSC has been classically used in studies of protein thermal stability/unfolding, lipid phase transitions, and more complex biological systems as well as in drug design.[29,30] Garbett et al.[31] first put forward the possibility of DSC application in early diagnostics and monitoring of diseases. The authors investigated the thermal behavior of blood plasma from healthy and diseased individuals and showed a typical DSC thermogram of healthy individuals that is indeed drastically modified for plasma of patients with rheumatoid arthritis, Lyme disease, cervical cancer, etc.[31−33] Michnik et al.[34] furthermore claimed that there were pronounced changes in thermograms of blood serum from patients with chronic obstructive pulmonary disease compared to healthy subjects.

Here, we explore this approach for the case of MM. The thermal denaturation profiles of blood sera from patients with MM, cases with different M isotypes (IgG, IgA, and IgM, λ and/or κ), BJ protein (monoclonal immunoglobulin light chain), and nonsecretory MM, are investigated by DSC for the first time. Our findings clearly demonstrate that DSC of the serum proteome from MM patients possesses specific thermodynamic features associated with the presence of monoclonal globulins and altered albumin content as well as the modified macromolecular interaction network. The data provide a novel estimate of MM and reveal so far unsuspected signatures for the MM subtypes.

EXPERIMENTAL SECTION

Blood Serum Samples. Study Population. Blood serum samples were prepared by a 15 min centrifugation of blood at 300 RCF in BD Vacutainer SST/5 mL tubes. The samples were stored no longer than 48 h at 4 °C prior the measurements.

Blood was derived from 82 patients, diagnosed with multiple myeloma and from 8 healthy individuals. Approval for the investigation and informed consent from the patients was obtained by the ethics committee of the National Specialized Hospital for Active Treating of Haematological Diseases, Sofia.

The diagnosis of MM is based on clinical criteria and information for each individual (age, gender, and concentrations of serum proteins for each sample) was collected (Table 1).

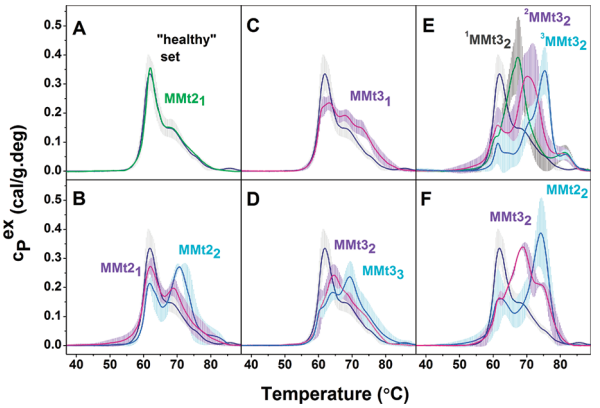

Figure 1. Characteristic DSC profiles of blood serum from patients with multiple myeloma. Average subsets (solid lines) of thermograms and standard deviations (shadows) for blood sera from healthy individuals (dark blue solid line and light gray shadow) and MM patients (green, blue, purple, and dark gray) (A−F). The range and the average M protein concentration for the MM subsets are given in Table 2. The designations of the subsets are shown in the corresponding color in each panel (A−F). For clarity, the three subsets in panel E, varying in the T_m of their main transition, are denoted with superscripts. For comparison, the control set thermogram is presented in each panel. The thermograms are recorded by scanning from 30 to 95 °C with a scan rate of 0.8 °C/min.

Serum Protein Electrophoresis. All MM patients were screened for M proteins and the serum levels of HSA, α1-, α2-, β1-, β2-, and γ-globulins were determined by serum protein electrophoresis (MINICAP, Sebia). The technique (Figure S-1 in the Supporting Information) allows the separation of serum proteins by their size to charge ratio in a capillary filled with an electrolyte and the precise determination of the concentrations of the above-mentioned serum proteins and of the type of M protein (immunotyping).[35]

Differential Scanning Calorimetry. DSC experiments, to examine the thermal unfolding of blood serum proteins, were performed on a DASM1 (Biopribor, Pushchino)-build in highly sensitive microcalorimetric system. The serum samples were diluted 2−3 times in PBS buffer before each measurement and were scanned from 30 to 95 °C with a scan rate of 0.8 °C/min. The excess heat capacity curves were corrected for the instrumental (buffer/buffer) baseline, routinely recorded before each experiment, smoothed and normalized to the total protein concentration. The thermograms were analyzed using Origin software package. All DSC thermograms, presented in Figure 1A−F, are the average of several thermograms (the number of averaged cases is given in Table 2) included in the respective subsets (Figure 1 and Table 2). For each mean value, the corresponding standard deviation is also plotted.

RESULTS

Thermal Denaturation of Blood Serum from Healthy Individuals. DSC experiments show typical endothermic unfolding transitions of serum proteins for healthy individuals, with

Table 2. Thermodynamic Parameters of the Depicted MM Subsets and the Control Set R of Thermograms[a]

subsets Figure 1	no. of cases	M protein (%)	M protein ⟨%⟩	$\langle T_{m1}\rangle$ (°C)	$\langle c_P^{ex}{}_1\rangle$	$\langle T_{m2}\rangle$ (°C)	$\langle c_P^{ex}{}_2\rangle$	$\langle c_P^{ex}{}_1/c_P^{ex}{}_2\rangle$
A R (control)	8			61.9 ± 0.7	0.37 ± 0.05	68.4 ± 0.3	0.15 ± 0.03	2.56 ± 0.72
A $MMt2_1$	8	2−18	3.9 ± 1.2	62.0 ± 0.3	0.37 ± 0.05	68.4 ± 0.6	0.15 ± 0.03	2.46 ± 0.22
B $MMt2_1$	9	1−25	11.7 ± 7.2	61.8 ± 0.7	0.28 ± 0.03	67.9 ± 2.5	0.21 ± 0.04	1.34 ± 0.24
B $MMt2_2$	3	0−19	13.0 ± 7.4	61.8 ± 1.1	0.21 ± 0.04	71.0 ± 1.0	0.28 ± 0.01	0.74 ± 0.14
C $MMt3_1$	11	0−32	4.8 ± 5.0	61.6 ± 1.0	0.23 ± 0.03	63.9 ± 1.1	0.20 ± 0.02	1.17 ± 0.08
D $MMt3_2$	7	0−32	6.2 ± 6.7	61.3 ± 0.9	0.16 ± 0.05	64.6 ± 0.8	0.25 ± 0.04	0.66 ± 0.20
D $MMt3_3$	13	1−22	12.3 ± 6.9	60.6 ± 0.5	0.14 ± 0.04	64.2 ± 0.8	0.19 ± 0.03	0.78 ± 0.27
E $^1MMt3_2$	4	33−41	36.8 ± 3.8	62.1 ± 2.4	0.19 ± 0.14	66.9 ± 0.9	0.40 ± 0.11	0.48 ± 0.29
E $^2MMt3_2$	9	33−43	35.7 ± 3.9	61.5 ± 1.3	0.19 ± 0.03	70.8 ± 1.7	0.39 ± 0.10	0.53 ± 0.20
E $^3MMt3_2$	3	33−36	35.1 ± 1.8	61.1 ± 0.5	0.12 ± 0.08	75.6 ± 0.6	0.31 ± 0.10	0.60 ± 0.35
F $MMt3_2$	2	33−43	38.1 ± 6.4	61.8 ± 0.6	0.16 ± 0.02	68.8 ± 0.3	0.34 ± 0.01	0.49 ± 0.08
F $MMt2_2$	5	33−54	40.8 ± 11.2	62.7 ± 1.1	0.18 ± 0.08	74.4 ± 0.9	0.40 ± 0.12	0.70 ± 0.72

[a] The averaged values and their standard deviations are given for the transition temperatures (T_{m1} and T_{m2}), heat capacity changes ($c_P^{ex}{}_1$ and $c_P^{ex}{}_2$) and their ratio ($c_P^{ex}{}_1/c_P^{ex}{}_2$) at the first and second thermal transitions. The designations of the subsets correspond to the ones presented in Figure 1A−F. The interval of the respective M protein concentration is given for each subset as well as its mean value and standard deviation.

a sharp major transition with midpoint transition temperature, T_m, at around 62 °C (Figure 1A) that can be attributed to the most abundant protein carrier HSA (∼60% of the total plasma protein). Other proteins, like haptoglobin or α1-antitrypsin, could also contribute to this transition, though at a much lower extent.[33] This main transition is followed by two transitions/shoulders at ∼68 and ∼75 °C that can be assigned to globulins (∼40% of the total protein content) and a low-enthalpy high-temperature transition, with midpoint T_m at ∼85 °C, attributed to serum transferrin (with a serum concentration of 3−4% of the total protein), respectively. The average DSC profile derived for healthy individuals forms the reference set (R or "healthy" thermogram), also referred to as the control set (shown in all panels A−F of Figure 1 for comparison).

Sets of DSC Thermograms of Serum from Patients with Multiple Myeloma. The DSC thermograms of serum from patients with MM differ from the "healthy" one and can be categorized based on different parameters: the number of unfolding transitions (assigned to albumin, immunoglobulins, transferrin etc.), their corresponding T_m, excess heat capacity c_P^{ex}, and/or the ratios of the excess heat capacities of the transitions, corresponding to the denaturation of albumin and immunoglobulins, etc.

The clinical characteristics of the MM patients are given in Table 1. The concentrations of albumin and α1-, α2-, β1-, and β2-globulins were determined by serum protein electrophoresis (SPE).[35]

Typically two or three well-resolved transitions can be distinguished in MM DSC thermograms (Figure 1A−F) with T_m and corresponding c_P^{ex} that differ depending on the serum level of both the M protein and HSA. Rarely an additional fourth transition, or commonly a shoulder, is also observed (5% of the studied cases, data not shown).

According to the number of the resolved transitions, two sets of thermograms are defined: MMt2 and MMt3 with two and three transitions, respectively. The ratio between the heat capacity changes at the low (below 65 °C)- and high (above 65 °C)-temperature regions depicts subsets ($MMt2_1$, $MMt2_2$, $MMt3_1$, $MMt3_2$, and $MMt3_3$, the subscripts, 1−3, denote the transition at which the c_P^{ex} has a maximal value) within each set, illustrated in Figure 1 (average DSC profiles, and standard deviation, for each subset are presented).

The excess heat capacity is higher at the first low- compared to the high-temperature transition(s) ($c_P^{ex}{}_1/c_P^{ex}{}_2 > 1$), as in the control thermogram, for 37% of the cases studied. The opposite trend ($c_P^{ex}{}_1/c_P^{ex}{}_{2,3} < 1$, i.e., the heat capacity value is substantially lower for the first transition than for the successive second and/or third high-temperature transitions) is characteristic for the remaining 63% of the MM cases. The first transition, detected at ∼61−62 °C, has a c_P^{ex} value comparable (Figure 1A, $MMt2_1$), slightly (Figure 1B, $MMt2_1$) or substantially lower (Figure 1B $MMt2_2$, Figure 1C−F) than that of the control, depending on the M protein concentration (Table 2).

The successive transitions and/or shoulders, after the first one, in the MM thermograms are observed at 63.5−64.6 °C, 68−70 °C, or 73−75 °C, and only for MM cases with the IgG isotype, a low-enthalpy transition appears at 81−82 °C (Figure 1E). It is worth noting that the last transition in the "healthy" thermogram, at ∼85 °C, is missing in all MM cases studied, which might be due either to strong reduction in the serum level of transferrin, that is expected to unfold at this temperature,[33] or to a ligand binding-induced shift in its thermal unfolding. It is of high interest, but beyond the scope of this work, to investigate whether this feature common for all studied MM cases can serve as a malignancy indicator.

The subsets of thermograms, shown in Figure 1, cover two ranges of M protein serum level from 1.3 to 32%, panels A−D, and above 32%, panels E and F. The thermograms for M protein concentrations below 32% include different M protein isotypes, BJ, and nonsecretory MM cases (Figure 1A−D), while those for concentrations higher than 32% (Figure 1E,F) correspond to the IgG, panel F, and IgA, panel E, cases.

All MM DSC thermograms strongly differ from the control set with the exception of eight cases (only 9% of the cohort, one nonsecretory MM, three IgG κ/λ, and three BJ cases, with relatively low M protein level, in the range of 2.4−4.7%, and one case of 18% IgG isotype (Figure 1A)). Despite the strong resemblance to the healthy DSC profile, these thermograms do not display the last 85 °C transition.

More drastic differences in the DSC profiles are found at higher M protein levels (33−54.7%) for either M protein isotype (IgA, M and G, κ and λ). Three transitions are typically observed, the midpoint transition temperatures of the second and/or third one being shifted to higher temperatures compared to those

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observed for lower M protein levels (Figure 1E,F and Table 2). The HSA concentration for these MM cases varies in the range 30.2−45.9%, i.e., it is lower than the cutoff level (55.8−66.1%).

The three subsets of IgG (κ and λ) and one subset of IgA (κ and λ) MM thermograms (all $MMt3_2$ subsets) show three well-resolved transitions with the highest c_P^{ex} at the second one (Figure 1E,F). The three IgG $MMt3_2$ subsets differ in the T_m of the main transition. Taking into account the HSA concentrations and the $c_P^{ex}{}_1$ values of those three subsets and that of the control set, it can be estimated that ∼20% of albumin for $^1MMt3_2$ and $^2MMt3_2$, while ∼52% for $^3MMt3_2$ subsets does not unfold at 62 °C but at higher temperature. Thus, the stronger shift in the main T_m of $^3MMt3_2$ compared to $^1MMt3_2$ and $^2MMt3_2$ correlates with a higher percentage of thermally stabilized albumin which strongly suggests a change in the albumin-globulin binding state. The same holds for the IgA $MMt3_2$ subset (Figure 1F) where the estimate shows that about 30% of albumin should unfold at higher T_m.

In contrast, biphasic thermograms with highly cooperative high-temperature transition, $MMt2_2$ subtype, are only observed for several MM cases with high IgA κ levels (Figure 1F, $MMt2_2$). The c_P^{ex} at the second transition is almost 3-fold higher than at the first one (Table 2), and its difference from the control c_P^{ex} value indicates that ∼25% of the albumin has shifted T_m. In general, the thermograms of IgA κ present a smaller number of transitions and/or shoulders, i.e., they are featureless compared to the IgG thermograms, particularly the second, most strongly shifted average subset (Figure 1F, $MMt2_2$ subset).

A distinct feature of the IgG myeloma cases is the presence of a transition at 82 °C that is missing in the IgA and IgM cases. Therefore, it seems that not only the concentration but also the M protein isotype is reflected in the DSC profiles, at least at high concentrations.

The c_P^{ex} values for the transitions below 68 °C, as can be expected from the T_m of unfolding of individual isolated immunoglobulins,[33] did not depend on the concentrations of M proteins, whereas those at 68, 75, and 82 °C generally increased with the increase of M protein concentrations (compare Figure 1D with Figure 1E,F where the M protein concentration is up to 32% and above 32%, respectively).

Importantly, the higher c_P^{ex} values at the second transition are accompanied by a substantial shift of the corresponding transition temperature toward higher values (from 67 to 75 °C for IgG and from 69 to 74 °C for IgA cases). Most probably these result from protein− and/or ligand−globulin interactions modifying the melting pattern by stabilization of the globulins unfolding in this temperature range.

Furthermore, a relationship is found between c_P^{ex} of the main thermal transition, being one of the three successive calorimetric transitions, and the M protein concentration (Figure 2). The c_P^{ex} increases with the increase in the M protein concentration while the M protein isotype is randomly divided and does not seem to have a specific relation with the value of c_P^{ex}. The same trend is also observed for free monoclonal light chains (BJ protein, Figure 2), and the effect is pronounced even though BJ appears in rather low concentrations (1.3−8% in this study) compared to the cases of different M protein isotypes, which might be related to the fact that light-chain myeloma is more aggressive than the typical MM form.[36,37]

On the other hand, a 3-fold increase in the M protein concentration was estimated to correspond to a ∼3-fold increase in the c_P^{ex} value for the transition at 68 °C, for those MM cases for

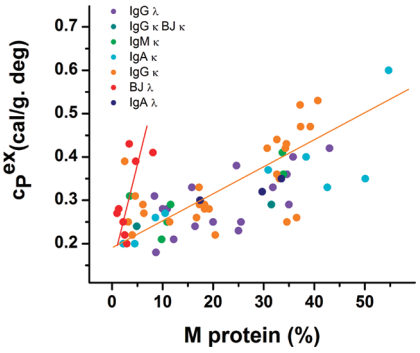

Figure 2. Dependence of the excess heat capacity of the main thermal transition, c_P^{ex}, in the DSC profiles on the serum level of the M protein. The different M isotypes are represented by different colors (see inset), and the M protein concentration is expressed in % of the total protein content. Straight lines are used to guide the eye for the two types of dependences observed (one characteristic for BJ and another one for all other isotypes of M proteins).

which it was observed (Figure S-2 in the Supporting Information). This strongly suggests a major contribution of the M protein to the 68 °C thermal transition.

The transition temperatures and excess heat capacities for the MM and control (R) sets are summarized in Table 2. Comparison of the excess heat capacities at the first transition (T_m ∼ 62 °C) of the MM thermograms with the HSA concentrations determined by SPE (both normalized to the corresponding values of the control, i.e., $c_P^{ex}{}_{1MM}/\ c_P^{ex}{}_{1control}$ and $[HSA]_{MM}/[HSA]_{control}$) shows that the normalized c_P^{ex} is more strongly reduced than the normalized serum level of HSA in almost all MM cases (Figure S-3 in the Supporting Information). This further supports the notion that the first transition, assigned to HSA, should correspond to unfolding of only a fraction of HSA and not the whole HSA amount. The rest of HSA appears to be stabilized by the serum interactions network and thus should melt at higher temperatures probably overlapping with the other high-temperature transitions, including those of globulin denaturation. Nevertheless, the possibility of a shift of the melting transition of the entire amount of albumin cannot be ruled out, meaning that the low-temperature transition might correspond to another serum protein. A shift in the albumin unfolding transition is in line with the findings that it can bind a multitude of serum proteins and/or cofactors.[38−41] For example binding of the ligand bromocresol green to blood plasma has been found to induce a strong, ligand-concentration dependent shift in the main thermal transition, similar to that observed upon its binding to isolated albumin.[33] Stabilization of albumin was also reported for octanoate, caprylate, *N*-Ac-L-tryptophanate and more hydrophobic fatty acids.[42,43] Furthermore, biphasic unfolding of HSA was demonstrated for palmitate/HSA binding, and the authors stated that the greater the affinity of HSA for a ligand, the greater is the tendency for biphasic denaturation.[43] However, to our knowledge MM-related ligand binding to HSA was not reported so far and thus presents a new direction for further investigations.

Importantly, even when only traces of M protein were resolved by SPE (for example, IgA κ ∼1−2%) the DSC profile is already modified. It should also be pointed out that the nonsecretory MM cases (9.5% from the total MM thermograms, Table 1) do exhibit modified DSC profiles compared to the "healthy" thermogram (those are included in the average subsets in Figure 1C,D).

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In only one such case the thermogram is almost identical to the control one (the sole difference between them being the absence of the 85 °C transition in the MM case). In the rest of the cases, the c_P^{ex} is reduced for the major transition, $T_m \sim 62$ °C, while it is significantly enhanced for the high-temperature transitions, as observed for the majority of MM cases with M proteins. It should also be noted that the MM cases of BJ protein secretion (BJ concentration in the range 1.3−8% of the total protein) possess strongly modified but not a common atypical, BJ-related profile and thus are included in the averaged sets in Figure 1B−D. One of the BJ cases (BJ protein concentration ∼3%) (Figure S-4 in the Supporting Information) strongly resembles the $^3MMt3_2$ average profile of IgG cases (Figure 1E), with the highest T_m of the main transition at ∼75 °C. It is the sole BJ case in this study with such a strong shift in the T_m of the main transition, the latter remains at $T_m \sim 62$ °C for all other cases. Taking into account the fact that the BJ concentration, relative to the total protein, is rather low, whereas the HSA concentration is similar to the control one, it is plausible that the reduction of $c_P^{ex}{}_1$ and the shift of T_m are related to alteration of albumin binding state and hence to its unfolding at higher temperature.

Deconvolution of the control and MM thermograms revealed that most, but not all, of the sets yield five peaks with T_m that differ significantly between MM cases and control as well as within each set (data not shown), providing an additional evidence for the strongly altered interaction network. However, direct assignment of the deconvolved transitions to the serum proteins would be speculative given the high complexity of serum composition and requires additional information and in-depth investigations.

DISCUSSION

Calorimetric studies of blood plasma have recently revealed a typical thermogram for healthy individuals,[31,33,44] and it was established that its shape can be reconstructed by adding up the thermograms of the major blood plasma protein fractions weighted by their characteristic amounts in plasma. We also obtained a "typical" DSC profile from blood sera of healthy subjects that much resembles that of plasma (as stated by Garbett et al.)[31] and in which the first low-temperature transition in the plasma profile, corresponding to fibrinogen,[33] is missing. Our "healthy" serum DSC thermogram is slightly different from the one reported by Michnik et al.,[34] nevertheless, the main features are very similar.

The calorimetric data of blood serum from patients with MM show specific changes in the thermograms, in the number of transitions and their midpoint transition temperatures and respective excess heat capacities compared to the control. Typically a reduced c_P^{ex} at the first transition and an appreciable increase in c_P^{ex} at the second and/or third transitions are observed at high levels of M proteins.

We did not find a unique DSC profile associated with MM but found distinct sets of profiles reflecting disease-induced changes of serum protein content and proteome composition. The MM-associated DSC profiles are found to be M protein concentration-dependent and M protein isotype-dependent, at least for high M protein concentrations. It is clear that the increase in M protein concentration causes a rise of c_P^{ex} at the high-temperature (globulin-related) transition(s) and more importantly is associated with a shift in the transition temperature, which is generally related to protein stabilization that might be caused by ligand binding,[29,45] indicating alteration of the binding state of the globulins. Our data furthermore reveal that at least a fraction of HSA should have T_m shifted toward higher temperatures. This might also be due to specific ligand/protein(s) binding to albumin. It is worth noting that the MM case (IgG) with the most severe reduction in the 63 °C transition (included in the $^3MMt3_2$ subset in Figure 1E) is also characterized by 71% atypical plasmacytic population (clinical data) and fast progression of the disease. Thus, the diversity of MM DSC thermograms observed is obviously related to the variety of modifications in the serum proteome that constitutes an interaction network involving protein−protein and protein−ligand interactions ("interactomics").[38,46] This MM thermogram heterogeneity is not surprising and quite agrees with the description of multiple myeloma "as not one disease but rather many", with each one of the subtypes largely defined by the specific genetic and cytogenetic aberrations.[7,10]

It should be noted that the concentration of β2-microglobulin is higher than the cutoff value in ∼50% of the MM cases (clinical data); however, these cases are distributed in all sets/subsets of thermograms. Therefore, we did not find a correlation between the MM grouping on calorimetry bases and the prognostic factor β2-microglobulin. This can be expected since the thermograms are dominated by the highly abundant serum proteins, and the thermal transitions of low abundant proteins, such as β2-microglobulin (concentration ∼2 mg/L), could not be resolved. Apparently DSC reveals distinct features of the MM serum proteome, as compared to the ones followed by the ISS and Durie/Salmon systems, and offers a novel DSC based classification.

To our knowledge this is the first calorimetric study on multiple myeloma sera and of hematological diseases in general. However, DSC has been recently applied[47] to follow the drug response in nuclear fractions isolated from chronic lymphocytic leukemia B cells. The present study strengthens our understanding of MM and complements the serum protein electrophoresis analysis. It also provides new insights into the calorimetric approach in disease investigation. The particular thermodynamic features of MM serum can also be helpful as a tool for diagnostic and follow-up of MM patients, in particular when new therapies (such as monoclonal antibodies) and/or autologous stem cell transplantation are being applied.[48,49]

CONCLUSIONS

In conclusion, this biophysical study reveals a strong MM-induced modification of blood serum thermodynamics. The MM serum DSC profiles reflect a complex interplay between the serum concentration and isotype of M protein and albumin and interprotein or ligand−protein interactions, resulting in the following events: (i) stabilization of at least a fraction of HSA; (ii) stabilization of globulins; and (iii) disappearance of the 85 °C, transferrin-assigned transition. The presence of a transition at 82 °C appears to be a distinct feature for the IgG isotype (κ and λ) proteins only.

Because of the design and relatively small scale of this study, we would not be able to confirm the diagnostic capability of this method, but our data provide a proof of principle and a thermodynamic portrait of the heterogeneous multiple myeloma. We expect that DSC would be validated as an efficient tool for diagnostics and monitoring of MM and other malignancies. This can be achieved by thorough investigations of a large

number of diseases and of a reasonably large number of cases for each disease.

■ ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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