Case Report

Unusual thermal transition in the serum calorimetric profile of a patient diagnosed with multiple myeloma with secretion of monoclonal κ free light chains: a case report

Svetla Todinova¹, Sashka Krumova¹, Tonya Andreeva¹, Keranka Dimitrova², Lidia Gartcheva², Stefka Germanova Taneva¹*¹

¹Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria
²National Specialized Hospital for Active Treating of Haematological Diseases, Sofia 1756, Bulgaria

Abstract

Differential scanning calorimetry (DSC) gains speed and success in the last decade in the characterization of blood plasma and serum. Numerous publications reveal the potential of this technique to identify calorimetric markers specific for variety of diseases and their staging. In our previous works we have clearly demonstrated that DSC can serve to classify multiple myeloma cases in a number of calorimetric groups whose thermodynamic parameters are strongly affected by the level and isotype of the secreted monoclonal immunoglobulins or free light chains (FLC). In this report we present a case of multiple myeloma with secretion of monoclonal κ FLC (stage III according to ISS classification). High FLC level (about 20% from the total protein content) was found in the patient’s serum that remained persistent for the monitoring period of 1 year. The calorimetric profile of the serum revealed the occurrence of an unusual calorimetric transition at 46-47 °C, unique among nearly 500 multiple myeloma patients studied by us so far. This transition was assigned to unstable monoclonal free light chains that also led to the formation of amorphous aggregates (imaged by atomic force microscopy) in the patient’s serum. Additional studies of patients with similar calorimetric features are needed in order to relate the emergence of the 47 °C transition and protein aggregation to the disease activity status of multiple myeloma or to other pathology.

Key words: multiple myeloma, monoclonal free light chains, differential scanning calorimetry

Introduction

Polyclonal immunoglobulin free light chains (FLC) (not involved in the assembly of intact immunoglobulin molecules) are normally present in the blood stream, their concentration in the blood being determined by the balance of their production and renal clearance [1]. In certain haematological disorders (myeloma, amyloidosis, various types of lymphoma [2-5], monoclonal FLC (also denoted as Bence Jones (BJ) proteins) are secreted due to clonal plasma cell proliferation; these can readily be quantified by serum free light chains assay [1]. For the case of multiple myeloma (MM) the κ/λ FLC ratio is regarded as a reliable marker for disease progression and recurrence [3, 6, 7]; they also
proved to be reliable disease biomarkers for variety of plasma cell-proliferative disorders [8, 9]. Recently we have performed in-depth calorimetric study of patients diagnosed with multiple myeloma with secretion of monoclonal FLC (denoted BJ MM in [10]) as a part of a large-scale investigation intended to identify thermodynamic features specific for the various multiple myeloma types [10, 11] that might be used to follow the outcome of patient treatment and the progression of the disease. Differential scanning calorimetry (DSC) is a technique suitable to study the physical properties of biological molecules [12], as well as complex biological systems such as tissues [13], cells [14 - 16], cerebrospinal fluid [17]. It can also be used to precisely determine the excess heat capacity and denaturation temperature (T_m) of the major serum proteins (albumin and immunoglobulins) in their native environment - the highly complex and protein-crowded blood serum ([18, 19] and references therein). Our own investigations showed that while for about 15% of the studied BJ MM cases the calorimetric profiles did not differ dramatically from the typical healthy controls, for the rest of the BJ MM cases some common disease related characteristics can be defined. The transition assigned to albumin (T_m ~ 62 °C), splits into two transitions with higher and lower transition temperature than that in the healthy thermogram. The stabilization of the albumin transition (effect common for the various secretory MM cases as well as for non-secretory MM) is believed to be due to MM specific ligand binding. However, the nature of the transition with lower T_m is not clear yet and it is suggested to originate either from destabilized albumin or from so far unidentified protein which endothermic thermal transition emerges after the shift of the main transition to higher temperature [10]. The globulins assigned transitions (T_m above 65 °C) were found to have higher amplitude as compared to the control healthy sera and variable T_m. The BJ MM calorimetric profiles were grouped in four sets based on the transition temperature of the main (the one with the highest excess heat capacity, c_p^α, value) transition. An unique feature among all studied BJ MM cases, as well as among the other secretory and non-secretory MM cases, was observed for the calorimetric set denoted as BJ4 (11% of all BJ MM samples) consisting of the cases with the highest content of monoclonal FLC, namely the occurrence of a transition at 57 °C that was suggested to originate from ligand free destabilized FLCs [10]. In the course of time we have screened nearly 500 myeloma samples and only a single BJ MM case presented an exceptional calorimetric fingerprint that was persistent during the patient’s monitoring period. In this report we performed a retrospective study of this case and suggested a plausible explanation for the nature of this new calorimetric feature.

Case presentation and biophysical characterization of the protein-protein interactions in the blood serum

A 65-γ-old male was admitted at the National Specialized Hospital for Active Treating of Hematological Diseases, Sofia, Bulgaria, due to complains of pain in the back and in the ribs and he was treated conservatively with transient effect. A biopsy was made on the 10th rib and the 10th breast vertebrae; the histological study revealed brownish soft tissue material with morphology of plasmocellular myeloma. Investigation of bone marrow for suspected plasmocytosis as well as urine and blood serum for the presence of monoclonal components was recommended. The bone marrow examination showed 76% plasmocytes population and there were data for 5 % leukemization. Immunochemistry revealed presence of traces of κ free light chains in the blood serum and in the urine, the serum level of β2 microglobulin was 5.8 mg/L. Roentgenographic examination revealed multiple osteolitic lesions. The patient was diagnosed as multiple myeloma with secretion of monoclonal κ FLC (stage III according to ISS classification). As associated diseases the patient also had arterial hypertonia and polyposis established before MM diagnosis. For a year, the patient underwent a series of chemotherapy treatments starting with 6 courses of Vincristine/ Doxorubicine/Dexamethasone regimen after which the plasmocytes population was reduced to 46% indicating partial response to the treatment. The chemotherapy continued with 2 courses of Dexametazone/Cyclophosphamide/Etoposide/Cisplatine and 4 courses of bortezomid, corticosteroids and biphosphonates but unusually high, 19.2% (14.7 g/L) from the total protein content, FLC κ paraprotein was detected in the β-fraction of serum globulins indicating worsening of the condition. The patient underwent additional 4 courses of Vincristine/Doxorubicine/Dexametazone
regimen without positive effect and the FLC level remained essentially unchanged (20-22%). In the next months the patient was subjected to symptomatic therapy with bisphosphonates and erythropoietin, and transfusion therapy with erythrocyte concentrate.

At the time of our study (12 (monitoring point 1, mp1), 13 (monitoring point 2, mp2) and 25 (monitoring point 3, mp3) months after diagnosis) the patient’s clinical status did not change significantly. At the time of mp3 the patient was hospitalized in worsened overall condition and the laboratory data showed thrombocytopenia. After the measurement at mp3 the patient was subjected to symptomatic therapy with diuretic, ulcoplor, corticosteroids, antiendemic therapy with mannitol, antibiotic therapy, transfusion therapy with erythrocyte concentrate and a hemodialysis session was performed.

The data from the paraclinical investigations for the three monitoring points are presented in Table 1. During the monitoring period the monoclonal κ FLC content varied in the range 23 - 24% from the total protein content (16.6-20.31 g/L, Table 1), albumin was below the reference limits (<36 g/L, Table 1), hemoglobin concentration also progressively decreased (from 76 to 42 g/L), while creatinine (382 - 973 µmol/L), total calcium (2.33 - 3.01 mmol/L) and

Table 1. Serum protein levels, monoclonal (κ) FLC content, paraclinical data and chemotherapy regimens

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/L)</td>
<td>58.0-85.0</td>
<td>83.6</td>
<td>76.7</td>
<td>71.0</td>
<td>68.7-78.3</td>
</tr>
<tr>
<td>monoclonal FLC (g/L)</td>
<td>-</td>
<td>20.31</td>
<td>17.9</td>
<td>16.6</td>
<td>1.8-8</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>40.2-47.6</td>
<td>35.8</td>
<td>33.0</td>
<td>30.57</td>
<td>40-50.4</td>
</tr>
<tr>
<td>α1-globulins (g/L)</td>
<td>2.1-3.5</td>
<td>4.51</td>
<td>4.7</td>
<td>4.33</td>
<td>3.6-5.0</td>
</tr>
<tr>
<td>α2-globulins (g/L)</td>
<td>7.1-11.8</td>
<td>8.95</td>
<td>8.0</td>
<td>7.39</td>
<td>6.7-10.7</td>
</tr>
<tr>
<td>β-globulins (g/L)</td>
<td>5.7-9.9</td>
<td>11.2</td>
<td>10.1</td>
<td>9.38</td>
<td>8.2-14.8</td>
</tr>
<tr>
<td>γ-globulins (g/L)</td>
<td>8.13-15.5</td>
<td>2.8</td>
<td>3.0</td>
<td>2.77</td>
<td>2.5-5</td>
</tr>
<tr>
<td>β2-microglobulin (µg/ml)</td>
<td>1.2-4</td>
<td>30</td>
<td>n/a</td>
<td>14.9</td>
<td>10.2-25.8&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>34-134</td>
<td>381.8</td>
<td>973</td>
<td>767.2</td>
<td>58-319</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.1-2.62</td>
<td>3.01</td>
<td>n/a</td>
<td>2.33</td>
<td>1.3-2.63</td>
</tr>
<tr>
<td>Lactatdehydrogenase (U/L)</td>
<td>125-220</td>
<td>266</td>
<td>326</td>
<td>410</td>
<td>460-849</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>140-180</td>
<td>76.1</td>
<td>59.9</td>
<td>41.6</td>
<td>n/a</td>
</tr>
<tr>
<td>FLC kappa (mg/L)</td>
<td>3.3-19.4</td>
<td>very high</td>
<td>very high</td>
<td>n/a</td>
<td>4.2-6.7</td>
</tr>
<tr>
<td>FLC lambda (mg/L)</td>
<td>5.71-26.3</td>
<td>0.61</td>
<td>0.95</td>
<td>n/a</td>
<td>5.5-very high</td>
</tr>
<tr>
<td>FLC κ/λ</td>
<td>0.26-1.65</td>
<td>very low</td>
<td>very low</td>
<td>n/a</td>
<td>very low-1.2</td>
</tr>
<tr>
<td>IgD (mg/L)</td>
<td>77-132</td>
<td>&lt;0.66</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>IgE (mg/L)</td>
<td>0-100</td>
<td>&lt;0.30</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Reference intervals for the serum protein levels are determined by means of capillary electrophoresis. Monoclonal (κ) FLC content is measured by means of SPAPLUS analyzer. Paraclinical data and chemotherapy regimens are given for the presented BJ MM case at three monitoring points (mp, dates of sampling are presented in brackets), for the BJ4 cohort (n=10) and where applicable for healthy individuals (n=18), n/a means not applicable.

<sup>1</sup>applicable only for 2 patients in this group, n/a for the rest of the patients

<sup>2</sup>patients in this group underwent different chemotherapy regimens that included: Bortezomid/Dexamethazone; Cyclophosphamide/Dexamethasone/bisphosphonate; Endoxan/corticosteroids/bisphosphonate, erythropoietin; Vincristine/Doxorubicine/Dexamethasone

\[ \beta_2 \text{-microglobulin (14.9 - 30 µg/ml)} \] were well above the norm indicating progression of the disease. Capillary electrophoresis revealed that at the three monitoring points the level of \( \alpha_1 \)-globulins was above the reference values, while that of \( \gamma \)-globulins was about 4 times below the norm (Table 1).

Turbidimetry analysis, performed on SPAPLUS analyzer, showed very high FLC \( \kappa \) level. Serum thermograms for the studied case (henceforth denoted as BJ MM case), as well as for the patients included in the BJ4 calorimetric set (n=10) and for healthy controls (n=18) were recorded by means of DASM-4 microcalorimeter with 1 °C/min scanning rate. The thermograms of the BJ MM case at the three mps were characterized with five well resolved endothermic transitions as opposed to three well resolved transitions in the healthy sera (Fig. 1). The transitions occur at temperatures 46-47 °C \( (T1) \), 61 °C \( (T2) \), 69-70 °C \( (T3) \), 74-79 °C \( (T4) \) and 85-90 °C \( (T5) \). The \( T2-T5 \) transitions appeared at positions close to those found for the BJ4 set (Fig. 1, Table 2). However, the \( T1 \) transition was observed at 46-47 °C and not at 57 °C as for the BJ4 calorimetric set. This is a unique feature among the 88 BJ MM patients (reported in [10]) and a total of 409 cases of secretory (IgG, IgM, IgA isotype) and non-secretory myeloma [10, 11, 20] characterized by us so far, revealing a pathology that has not been characterized up to this moment.

At mp1 - mp3 the total enthalpy of the BJ case thermogram was significantly higher than that of the control and the BJ4 set (Table 2). At mp1 the transition temperatures of the successive thermal transitions did not shift significantly as compared to the BJ4 calorimetric set, however at mp2 and mp3 the transitions \( T3 \), \( T4 \) and \( T5 \) are shifted to higher temperatures and there was a notable decrease in the amplitude of the \( T2-T5 \) transitions, while \( T1 \) remained unchanged (Table 2). Due to the occurrence of a transition at 46-47 °C the shape of the thermograms deviated strongly from that of the BJ4 calorimetric set as well as from the healthy ones.

Next we probed the reversibility of the calorimetric transitions, having in mind that the denaturation of isolated BJ protein is reversible [21 - 23]. The samples were subjected to sequential heating (up to temperature slightly above the \( Tm \) of the respective transition) and cooling cycles (the annealing procedure was performed according to [24]) which allowed for estimation of the enthalpy of the individual transitions. All transitions in the thermograms of healthy individuals were irreversible (data not shown), while for the samples from the BJ4 set only the transition centered at 56 °C was reversible (72% reversibility, Fig. 2A), thus confirming that it indeed originates from FLC \( \kappa \) denaturation as already suggested in [10]. However, after heating to 56 °C the reversibility of the 61 °C transition was reduced to ca. 63% of the original one.

### Table 2. Thermodynamic parameters derived from the calorimetric profiles

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy control</th>
<th>BJ case mp1 (03.02.2012)</th>
<th>BJ case mp2 (21.02.2012)</th>
<th>BJ case mp3 (22.02.2013)</th>
<th>BJ4 set*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T1m ) (°C)</td>
<td>-</td>
<td>47.0</td>
<td>46.0</td>
<td>46.0</td>
<td>56.4±0.85</td>
</tr>
<tr>
<td>( T2m ) (°C)</td>
<td>61.9±0.7</td>
<td>60.8</td>
<td>61.3</td>
<td>61.3</td>
<td>68.2±0.9</td>
</tr>
<tr>
<td>( T3m ) (°C)</td>
<td>68.4±0.3</td>
<td>69.0</td>
<td>70.3</td>
<td>70.7</td>
<td>77.1±1.3</td>
</tr>
<tr>
<td>( T4m ) (°C)</td>
<td>75.4±0.91</td>
<td>74.8</td>
<td>78.8</td>
<td>78.8</td>
<td>76.2±0.95</td>
</tr>
<tr>
<td>( T5m ) (°C)</td>
<td>85.6±1.77</td>
<td>85.1</td>
<td>89.9</td>
<td>89.1</td>
<td>85.9±0.91</td>
</tr>
<tr>
<td>( \Delta H_{cal} ) (cal/g)</td>
<td>3.10±0.30</td>
<td>4.3</td>
<td>5.5</td>
<td>4.9</td>
<td>3.60±0.60</td>
</tr>
</tbody>
</table>

\( Tm \) – transition temperatures of the successive thermal transitions, \( \Delta H_{cal} \) – total calorimetric enthalpy, mp - monitoring points (dates of sampling are presented in brackets). n=18 (mean values ± SD) for healthy controls, n = 10 (mean values ± SD) for the calorimetric BJ4 set (Todinova et al., 2014).
suggesting an interaction between the monoclonal FLC proteins and the molecules that melted at 61 °C.

For the presented BJ MM case the 46 °C transition was fully reversible, while all other sequential transitions, including the one at 61 °C, were irreversible (Fig. 2 B, C). The 46 °C transition is characterized with a very high calorimetric enthalpy (\( \Delta H_{\text{cal}} = 1.08 \text{ cal/g} \)) comparable to that of the T3 transition (\( \Delta H_{\text{cal}} = 1.18 \text{ cal/g} \)) that is ca. 25% from the total enthalpy of the thermogram, well corresponding to the monoclonal FLC concentration in the serum (22-24%). This strongly suggests that the 46-47 °C transition originates from destabilized FLC molecules and furthermore demonstrates that the FLC denaturation does not affect the thermodynamic properties of the major serum proteins.

To this point our results indicate that the transition at ca. 46-47 °C in the presented BJ MM case originates from thermodynamically unstable form of FLCs. This might be due to protein misfolding that was reported to lead to formation of aggregates with complex morphology. In order to probe this possibility we imaged the blood serum derived from the studied BJ MM case, samples from the BJ4 set and from healthy controls by atomic force microscopy (AFM). Sera were spread on freshly cleaved mica, incubated for 20 min, washed with PBS buffer and gently dried under nitrogen stream. The AFM-imaging was performed in tapping mode with

Fig. 1. (A) Thermograms derived for the presented BJ MM case at monitoring point 1 (red line), the cases in the BJ4 calorimetric set (blue line, cyan shadow, mean±SD) and the typical healthy thermogram (black line, gray shadow, mean±SD). (B) Comparison of the thermograms recorded for the BJ MM case at different monitoring points (mp1, red line; mp2, purple line and mp3, green line). The sequential transitions of the BJ MM case thermograms are denoted as T1-T5.
standard silicon nitride (Si3N4) probe tips with tip radius <10 nm (Budget Sensors, Innovative solutions Ltd., Bulgaria). Each sample was examined at 5 different locations all over the surface exploring the areas of 5×5 µm. The images (512×512 pixels) were captured in height and deflection modes and analyzed by NanoScope 6.13R1 software. As can be seen in Fig. 3 the control sample was characterized with homogenous proteins distribution, while that of the BJ MM case had large amorphous aggregates with a height of 8.36 ± 1.6 nm SD. AFM imaging of sera from the BJ4 set revealed formation of much

Fig. 2. Successive annealing of thermograms recorded for BJ MM case sera. (A) DSC profile of blood serum from a patient included in the BJ4 calorimetric set (a) and profiles after successive annealing (b, c). For the annealing procedure the first scan was stopped at 58 °C (b), cooled to 30 °C and sequentially heated up to 96 °C (c). (B) Thermogram of blood serum from the BJ MM case (a) and calorimetric profiles successively registered after heating (to different temperatures) and cooling cycles (b – f). For clarity the curves are displaced on the vertical axis. (C) Deconvolution of the BJ MM case thermogram according to the annealing procedure [15]. The individual transitions are presented with dash dot lines, while the original scan is shown with a solid line.
lower (4.79 ± 0.5 nm SD height) and fewer aggregates. Comparison of the roughness (indication of the averaged height distribution of the imaged objects) of the images revealed that the areas surrounding the large aggregates in the BJ MM case have a roughness value (0.62 ± 0.11 nm SD) that is very similar to the control one (0.68 ± 0.10 nm SD). On the contrary the roughness of the area surrounding the small aggregates in the BJ4 set samples was higher (0.85 ± 0.11 SD, p = 0.04) than the control.

**Discussion**

The most significant finding uncovered in this study is the unusual thermal transition centered at 46-47 ºC in the calorimetric profile of multiple myeloma patient characterized with high (above 20% of the total protein content) level of monoclonal κ FLCs and β2-microglobulin concentration 10 times above the reference limit. This was accompanied by formation of large protein aggregates imaged by AFM in the blood serum.

The transition at 46-47 ºC was associated with a dramatic stabilization of the T5 transition at mp2 and mp3; these effects were not found in a large-scale screening of BJ MM patients performed by us and reported in [10, 11]. Furthermore, such low temperature transition in the thermograms of blood sera has not been reported so far for any other disease or disorder [10, 11, 20, 25-40]. In our calorimetric classification of BJ MM cases [10], those with exceptionally high monoclonal FLC content were clustered in one group – BJ4 that differed most from the typical healthy profile as well as from the profiles of all other secretory and non-secretory MM cases. The BJ4 group was unique among the BJ MM calorimetric groups due to the presence of a
transition at 57 °C that was hypothesized to originate from the denaturation of free BJ proteins, while a portion of the BJ proteins was suggested to form complexes with other molecules and to melt at higher temperatures overlapping with the globulins transitions [10]. This suggestion is in line with the reported denaturation temperature at about 57 °C for isolated FLCs recorded by circular dichroism [21]. Indeed, the annealing procedure performed in our study for sera from the BJ4 calorimetric set as well as the AFM imaging of blood sera does demonstrate that for this set of sera the FLCs (or at least a major portion of these proteins) interact with other molecules, most probably those melting at 61 °C; in depth characterization of these interactions will be a subject of our future work.

In the BJ MM case that we are presenting here, the transition at 46-47 °C must originate from a protein that is highly abundant in the serum since its enthalpy is about 25% of the total thermogram enthalpy, thus the possible candidates are albumin and FLCs. Contribution of the major serum protein, albumin, is highly unlikely since for all other cases with secretion of monoclonal FLCs studied by us [10] we have observed albumin stabilization and corresponding shift of its denaturation temperature towards higher values, and no destabilized forms were detected. On the contrary, good correspondence between the enthalpy (ca. 25% from the total thermograms area) of the 46-47 °C transition and the level of FLCs is observed since their concentration is about 20% from the total protein content (Table 1). Those forms of FLCs are destabilized (by 10 °C) in comparison to the cases in the BJ4 calorimetric group where the free FLCs denature at 57 °C [10]. Several reasons for this destabilization can be pointed out. For example numerous reports show that light chain destabilization is correlated with increased amyloidogenicity [41 - 46]. Khurana et al. [47] have reported that different conformational states of partially unfolded light chain intermediates can result in the formation of aggregates with different structure (amyloid fibrils/amorphous aggregates). Light chains instability can also be induced by somatic mutations [22, 48]. However, it should be noted that recent findings contradict this hypothesis and demonstrate that for the case of AL-2 protein mutants the amyloidogenic forms are thermodynamically stabilized [48]. FLC glycosylation can also be excluded since it is shown to increase the thermal stability of a number of proteins (reviewed in [49]).

We probed the plausible presence of amyloid fibrils of FLCs or other types of aggregates in the serum of the BJ MM case by means of AFM and provided compelling evidence that the strongly destabilized FLCs are forming amorphous aggregates (similar to those imaged by Khurana et al. [47]) that are not found in healthy individuals and the rest of the studied MM cases with secretion of monoclonal FLCs and hence are specific for the presented BJ MM case. Furthermore, it appears that the FLCs aggregates are segregated from the rest of the serum proteins since no difference in the roughness of the adhered serum proteins was established between the BJ MM case and the control samples that is in line with our DSC observations.

To this point we can speculate that the appearance of the 46 – 47 °C calorimetric transition and the formation of amorphous aggregates of FLCs is associated with severe progression of multiple myeloma since at the three monitoring points where these features are present the paraclinical parameters indicate fast progression of the disease and poor prognosis (characteristic for FLC type MM). However, more patients with similar presentations have to be studied in order to confirm this hypothesis. Nevertheless, the presented study helps us complement our knowledge on the specific calorimetric features characteristic for multiple myeloma and demonstrates the potential of DSC to detect the appearance of unstable forms of FLCs in multiple myeloma and possibly also in other pathologies.

**Conclusions**

In this work we present a case report of BJ MM patient that exhibited unusual calorimetric transition assigned to destabilized forms of FLCs that denature at 46 – 47 °C and most probably are responsible for the observed protein aggregates in the patient’s blood serum. Further studies are needed to reveal the relation of the observed calorimetric features and protein aggregation to the disease status of multiple myeloma or the plausible presence of other pathologies.

**Abbreviations list:**
AFM, Atomic force microscopy
BJ proteins, Bence Jones proteins
$\Delta H_{\text{cal}}$, Calorimetric enthalpy
Tm, Denaturation temperature
DSC, Differential scanning calorimetry

References


