Supplementary materials

Structural Features of Amyloid Fibrils Formed From the Full-length and Truncated Forms of Beta-2microglobulin Probed by Fluorescent Dye Thioflavin T

Anna I. Sulatskaya ¹, Natalia P. Rodina ¹, Dmitry S. Polyakov ^{2,3}, Maksim I. Sulatsky ¹, Tatyana O. Artamonova ⁴, Mikhail A. Khodorkovskii ⁴, Mikhail M. Shavlovsky ^{2,3}, Irina M. Kuznetsova ¹ and Konstantin K. Turoverov ^{1,5,*}

- ¹ Laboratory of Structural Dynamics, Stability and Folding of Proteins, Institute of Cytology of the Russian Academy of Science, Tikhoretsky ave. 4, St. Petersburg 194064, Russia; ansul@mail.ru (A.I.S.); natalia240994@gmail.com (N.P.R.); m_sulatsky@mail.ru (M.I.S.); imk@incras.ru (I.M.K.)
- ² Department of Molecular Genetics, Institute of Experimental Medicine, Pavlov str. 12, St. Petersburg 197376, Russia; ravendoctor@mail.ru (D.S.P.); mmsch@rambler.ru (M.M.S.)
- ³ Chair of Medical Genetics, North-Western State Medical University named after I.I. Mechnikov, Piskarevskij prospect 47, St. Petersburg 195067, Russia
- ⁴ Research and Innovation Complex "Nanobiotechnologies", Peter the Great St.-Petersburg Polytechnic University, Polytechnicheskaya 29, St. Petersburg 195251, Russia; artamonova@nanobio.spbstu.ru (T.O.A.); khodorkovskii@mail.ru (M.A.K.)
- ⁵ Institute of Physics, Nanotechnology and Telecommunications, Peter the Great St. Petersburg Polytechnic University, Polytechnicheskaya 29, St. Petersburg 195251, Russia
- * Correspondence: kkt@incras.ru; Tel.: +7-812-297-19-57

1. Full-length and Truncated Forms of β2M Expression and Purification

To obtain soluble recombinant beta-2-microglobulin (β 2M), the vector pETb2m6.8 was created. The vector was designed in such way that β 2M is synthesized with the bacterial PelB "leader peptide" transporting the protein to the periplasmic space of the bacteria and subsequently cleaved by the action of bacterial proteases. This method provided β 2m without an additional methionine at the N-terminus; instead, it began with isoleucine (first amino acid of human β 2m) (Table S 1).

Native Human β2m	10	20	30	40	50
	IQRTPKIQVY SRHPAENGKS NFLNCYVSGF HPSDIEVDLL KNGERIEKVE				
	60	70	80	90	99
	HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM				
Recombinant β2m	10	20	30	40	50
	IQRTPKIQVY SRHPAENGKS NFLNCYVSGF HPSDIEVDLL KNGERIEKVE				
	60	70	80	90	100
	HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDMH				
	104				
	НННН				
Recombinant ∆N6β2m	10	20	30	40	50
	MIQVY SRHPAENGKS NFLNCYVSGF HPSDIEVDLL KNGERIEKVE				
	60	70	80	90	100
	HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDMH				
	104				
	НННН				
Recombinant ΔN10β2m	10	20	30	40	50
	M SRHPAENGKS NFLNCYVSGF HPSDIEVDLL KNGERIEKVE				
	60	70	80	90	100
	HSDLSFSKDW SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDMH				
	104				
	НННН				

Table S1. The amino acid sequence of the full-length and truncated forms of β 2M.

Recombinant full-length β 2M (β 2m) was prepared from *E*.coli cells strain BL21 (DE3) transformed with the pETb2m6.8 vector. *E*.coli cells were grown overnight in 10 ml of Luria-Bertani (LB) medium, containing 1 mg of ampicillin, and after were introduced into 500 ml of fresh medium. The cultivation was continued at 37 °C under aeration conditions until the optical density of A (600) = 0.6–1.0. β 2m synthesis was induced by adding IPTG to a final concentration of 0.5 mM, and subsequent cultivation overnight at 26 °C under aeration conditions. After deposition of the bacterial cells by centrifugation for 25 min at 10,000 g, the periplasmic fraction was obtained by the "osmotic shock" method [1].

As expected, soluble β 2m was found in the periplasm. The protein contained a polyhistidine sequence at the C-terminus, which made it possible to purify the protein very quickly and efficiently using affinity chromatography on a nickel metal chelate-agarose sorbent (the volume of column was 1.5 mL). For this aim, phenylmethylsulfonyl fluoride and imidazole (final concentration 1 mM and 10 mM, respectively) were added to the resulting contents of the bacterial periplasm. The solution was filtered through a metal chelate sorbent. The ballast proteins were successively washed with 0.5 m NaCl solution in 0.1 M K-phosphate buffer, pH = 8.0, containing 20–30 to 40 mM imidazole. Further, the protein was eluted with 0.15 M NaCl, pH = 7.4, containing 200 mM imidazole. The extraction of the periplasmic fractions and the subsequent affinity chromatography yielded β 2m with a purity greater than 98%.

To obtain $\beta 2M$ without the 6 ($\Delta N6\beta 2m$) and 10 ($\Delta N10\beta 2m$) N-terminal amino acids of the polypeptide chain (Table S1), gene expression constructs were created. Instead of the PelB "leader peptide", the start codon ATG was inserted before the nucleotide sequence encoding $\Delta N6\beta 2m$ and $\Delta N10\beta 2m$ in a bacterial plasmid. As a result, the protein synthesized from the resulting gene expression construct accumulated in inclusion bodies and had an additional methionine (due to the start codon) at the N-terminus.

The vector for obtaining recombinant human β 2m without the 6 N-terminal amino acids (Δ N6 β 2m) was constructed using forward primer 5'catatgattcaggtttactcagt3' [2] and backward primer 5'gtcaagcttatcagtgatggtgatggtgatggtcagtcccactt3'. As a template during the PCR a plasmid comprising full-length wild type β 2m was used [3]. The vector for the production of recombinant human β 2m without the 10 N-terminal amino acids (Δ N10 β 2m) was constructed similarly, but the forward primer had the sequence: 5'ctgcatatgtcagtcagtcaga3'. The PCR products were treated with restriction enzymes NdeI and HindIII (Fermentas) and inserted into the plasmid pET22b (+) (Novagen), treated with appropriate restriction enzymes.

Recombinant $\Delta N6\beta 2m$ and $\Delta N10\beta 2m$ were prepared from E.coli cells strain BL21 (DE3), transformed with the appropriate vector. *E*.coli cells were grown overnight in 10 mL of Luria-Bertani (LB) medium, containing 1 mg of ampicillin, and after were introduced into 500 mL of fresh medium. The cultivation was continued at 37° C under aeration conditions until the optical density of A (600) = 0.6-1.0. Protein synthesis was induced by adding IPTG to a final concentration of 0.5 mM, and subsequent cultivation overnight at 37° C under aeration conditions. After deposition of the bacterial cells by centrifugation for 25 minutes at 10 000 g, the cells were once washed with saline (PBS) followed by precipitation at 10 000 g. To the laundered cell pellet PBS was added and cells were disrupted by sonication (3 kHz frequency range 3 to 30 s.) in cooling conditions adding water with ice. For a more complete destruction 1/5 volume of glass beads was added to a suspension (Glass Beads, 500 microns, Sigma).

For obtaining initial soluble proteins, β 2M truncated forms, renaturation directly on the affinity column was carried out. For this purpose, the inclusion bodies, after washing up from bacterial lysate, were dissolved in 8 M urea in the presence of mercaptoethanol. After separation of insoluble aggregates by centrifugation, the supernatant was supplied to a nickel agarose column. The urea was washed with buffer with decreasing concentration of urea. Complete removal of urea was achieved by washing the column with 0.15 M sodium chloride with 30 mM imidazole. Proteins were eluted by 200 mM imidazole solution. The elution of proteins was tested by absorbance of the eluate at 280 nm. Imidazole was removed from preparations of soluble protein (monomer) by ultrafiltration through membranes retaining a material with a molecular weight more than 5 kD. The yield of the fusion proteins was 30 mg of 1 liter of bacterial culture.

Obtained the full-length and truncated forms of β 2M were analyzed by electrophoretic separation in polyacrylamide gel electrophoresis under denaturing conditions and by the mass-spectral analysis.

2. Principle of Equilibrium Microdialysis Experiment



Figure S1. Principle of equilibrium microdialysis experiment [4,5]. Equilibrium microdialysis was performed using a Harvard Apparatus/Amika (USA) device that consists of two chambers (500 μ L each) that are separated by a membrane that is impermeable to particles larger than 10,000 Da. Equilibrium microdialysis implies the allocation of two interacting agents, a ligand and receptor, in two chambers (#2 and #1, respectively) that are divided by a membrane permeable to the ligand and impermeable to the receptor. The essence of the method is as follows: amyloid fibrils are placed in the buffer solution at concentration *C*_P (concentration of the protein that is used to prepare the amyloid fibrils) in chamber #1, and the dye is placed in the same buffer and at an initial concentration *C*₀ in chamber #2. After equilibration, the free ThT concentrations in chambers #1 and #2 become equal (*C*_t), whereas the total ThT concentration in chamber #1 exceeds that in chamber #2 by the concentration of bound dye (*C*_b). Thus, using the method of equilibrium microdialysis, sample and reference solutions can be prepared; these solutions can be used to determine the absorption spectrum of the ThT bound to amyloid fibrils and the concentrations *C*_f and *C*_b.

References

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