Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1753 (2005) 131 – 140
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
http://www.elsevier.com/locate/bba

Review

Interactions of charged ligands with β_2-microglobulin conformers in affinity capillary electrophoresis

Niels H.H. Heegaard[a,*], Ersilia De Lorenzi[b]

[a]*Department of Autoimmunology, Statens Serum Institute, Bldg. 81, Rm. 536, Artillerivej 5, DK-2300 Copenhagen S, Denmark*
[b]*Department of Pharmaceutical Chemistry, University of Pavia, Pavia, Italy*

Received 15 June 2005; received in revised form 8 July 2005; accepted 12 July 2005
Available online 22 July 2005

Abstract

Alternative conformations of β_2-microglobulin (β_2m) are involved in its transformation from soluble monomeric precursor molecules to the insoluble polymeric material that constitutes β_2m amyloid. Accordingly, non-native conditions such as low pH or high ionic strength promote β_2m amyloid formation in vitro. The early events in these processes are not well known, partly because of the paucity of techniques available for the characterization of transient folding intermediates in proteins. We have used high-resolution separations in capillaries (capillary electrophoresis, CE) to resolve putative conformer fractions in native and structurally modified β_2m and to show the induction of alternatively folded β_2m under different experimental conditions. The conformer fractions are observed as distinct peaks in the separation profiles and thus it is possible to probe for the reactivity of these individual β_2m species with specific ligands that, upon binding, alter analyte mobility in affinity capillary electrophoresis experiments. Interactions were shown in this way for the negatively charged substances heparin, Congo red, and suramin, as well as for Cu^{2+} ions. Marked differences in the binding behavior of the β_2m conformational variants compared with native β_2m could be demonstrated. This approach for conformer separation and binding characterization is a valuable starting point for the assessment of various ligand molecules, or analogues thereof, as agents capable of perturbing the mechanisms of fibril formation.

Keywords: β_2-microglobulin; Amyloid; Protein conformation; Capillary electrophoresis; Affinity

1. Introduction

It is well established that semidenatured or structurally modified β_2m – in contrast to intact β_2m under physiological conditions – is capable of amyloid formation [2,3,27–29,34], but despite the fact that it has been known since 1985 that intact β_2m is involved in amyloid formation in dialysis-related amyloidosis [10,12], the conformational changes leading to amyloidogenicity in vivo are still largely unknown. Recently, however, it was found that capillary electrophoresis (CE), a high-resolution, yet native, free-solution separation method [24], was capable of separating populations of alternatively folded species existing in intact preparations of β_2m at neutral pH, at low ionic strength, or in the presence of various organic solvents as well as in different structural variants of β_2m. The β_2m conformers were observed as additional peaks in the CE analyses that were not due to dimerization or impurities or degradation artifacts [1,6,18,20,21]. Free-solution CE is useful for the study of protein folding by virtue of its highly efficient separations under highly controllable conditions, where separation does not depend on interactions with any kind of solid phases or other types of secondary equilibria [22]. Furthermore, CE in the affinity mode [15,16] could be utilized to qualitatively and quantitatively examine interactions of the separated fractions with various charged ligands such as glycosaminoglycans, Congo red, suramin, and Cu^{2+}-ions [6,18,20,21]. The outcome of these experiments further supported that the origin of the detected analyte heterogeneity in CE is differently folded β_2m species because the binding characteristics

* Corresponding author. Tel.: +45 32683378; fax: +45 32683876.
E-mail address: nhe@ssi.dk (N.H.H. Heegaard).

1570-9639/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbapap.2005.07.005

(i.e., binding constants) of the fractions were found to be different. These studies have benefited greatly from the quantitative aspects, the minute sample consumption, and the highly reproducible separation conditions of CE. There presently is no other simple approach for simultaneous assessment of the presence of conformers and their interaction profiles in a given protein sample, and – as reviewed here – these types of analyses have provided a means of characterizing conformations that probably represent very early stages on the amyloid misfolding pathway.

2. The conformational heterogeneity of normal, truncated, mutated, and cleaved β_2m revealed by capillary electrophoresis

Analysis of recombinant, full-length β_2m by CE at neutral pH consistently reveals sample heterogeneity in that two electrophoretic peaks are observed: the first one, which is most abundantly populated, corresponds to the native protein and the second, minor one, shows lower mobility [1] (Fig. 1). This was also observed, but to a lesser degree, in native wild-type (wt) β_2m purified from urine [21]. The electrophoretic mobility of the slower peak indicates that this species either has an overall more negative charge than the species represented by the main peak and/or a different size and/or shape. This observation and the ratio of folding and unfolding rate constants [2] support the assignment of the second peak to a partially structured species, a slow converting intermediate I_2, which is in equilibrium with the native form N [1,6]. While charge and mass of protein conformers are the same, the changes in shape, charge distribution including local ionization equilibria, and also the hydrophobicity characteristics of differently folded species often is enough to enable separation by CE. The capability of CE to separate these conformers as distinct peaks on a time scale of minutes indicates that the interconversion rate is slow compared with the peak appearance time [22], but still too fast to allow other separation techniques to visualize the phenomenon. Importantly, this less populated species has a higher propensity to aggregate than the native form, as demonstrated by the thioflavin T test and light scattering measurements in the presence of fibrils extracted from patients [1]. This latter result led us to use CE for the analysis of mutated, truncated, or cleaved β_2m that have amyloidogenic potentials that are different from wt β_2m [6], or known to be generated in vivo by an activated complement system [18]. In Fig. 1 shows the CE-analyses of some of these variants including two truncated species (ΔN3 and ΔN6) lacking the first 3 and 6 N-terminal amino acids, respectively. In these variants, the conformational stability has been reduced by the absence of the first and the second basic amino acid lodged in the first strand of β_2m [6,9]. Also shown are the analyses of three mutants, created by replacing histidine in position 31 with either tyrosine (H31Y) or serine (H31S) or by replacing arginine in position 3 with alanine (R3A).

For all protein species, folding stability experiments were carried out by monitoring the secondary structure by circular dichroism upon denaturation with increasing concentrations of guanidinium chloride. The ΔN3-variant was found to be the least stable, whereas H31Y was more stable than wt β_2m. As H31S β_2m was less stable than H31Y β_2m, the presence of tyrosine in position 31 rather than the absence of histidine in that position is important for stabilizing the molecule. The values of free energy of unfolding in the absence of denaturant, obtained by analysis of these experiments, were found to be inversely correlated to the percent peak area of the partially unfolded conformer, as measured by capillary electrophoresis [6].

The desLys^{58} β_2m variant and its precursor Lys^{58} β_2m (β_2m cleaved after lysine-58) can readily be generated by an activated complement system [31] and also display the peak heterogeneity behavior described above (cf. Fig. 1). The cleaved variants show distinct changes in their circular dichroism spectra when compared to wt β_2m and this is compatible with the peaks being two conformations in equilibrium with each other under physiological conditions exactly as observed for the other β_2m species described above [18].

Conclusively, the conformational instability inducible in wt β_2m has been found by CE to be more or less spontaneously existing in significant fractions of β_2m destabilized by structural alterations such as mutations, truncations, and cleavages [6,17–19].

3. Augmentation of variant species by non-physiological conditions

The possibility that the second peak of β_2m represents partially unfolded molecules was supported by the chance observation that treatment of wt β_2m with denaturing compounds (e.g., acetonitrile, trifluoroethanol, and ethanol) considerably enlarged the amount of the second species [1,20,21] (Fig. 2). Indeed, the presence of the above-mentioned β_2m conformer was first evidenced when natural, wt-β_2m was treated with 50% acetonitrile [21]. The two peaks observed were called f (fast) and s (slow) and their dependency on the acetonitrile concentration was studied by CE in samples treated with varying organic solvent concentrations [1]. As seen in Figs. 2A and B, there is a clear shift of the equilibrium of the two peaks toward the less structured species upon addition of increasing concentrations of acetonitrile: within the range of 30–40% acetonitrile, a remarkable decrease in the area ratios between the first and the second peak occurs, which also corresponds to a conformational transition from a structured state to a globally more unfolded one, as