Biophysical Chemistry 29 (1988) 271–276
Elsevier

BPC 01235

Adsorption of human β_2-microglobulin at a water / mercury interface

Viktor Brabec [a] and Dušan Vučelič [b]

[a] *Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 612 65 Brno, Czechoslovakia*
and [b] *Faculty of Sciences and Mathematics, Department of Physical Chemistry, Belgrade University, Belgrade, Yugoslavia*

Received 10 July 1987
Accepted 29 October 1987

β_2-Microglobulin; Interfacial properties; Protein unfolding; Water/mercury interface; Differential capacitance

Adsorption of human β_2-microglobulin from a neutral solution of 0.15 M NaCl on a mercury surface was studied at 25 °C by measurement of the differential capacity of the electrical double layer. From the diffusion-controlled adsorption kinetics, the surface concentration and hence the area occupied by the adsorbed β_2-microglobulin molecule were determined at various potentials of the mercury surface. The results indicate unfolding or flattening of β_2-microglobulin molecules adsorbed in particular on the electrically uncharged surface. The extent of this interfacial conformational rearrangement was reduced with growing positive or negative surface charge density.

1. Introduction

Human β_2-microglobulin (β_2-m) is a low molecular mass protein (molecular mass calculated from amino acid composition: 11 815 Da) synthesized by all nucleated cells [1,2]. It occurs free as a monomer in various body fluids (urine, plasma, etc.) as a product of cellular catabolism or excess synthesis of class I HLA genes [2–4]. Elevated levels of β_2-m are found, for instance, in urine in conditions of renal tubular damage [1–5] and in serum of patients with various malignant disorders [6–9]; quite recently it has also been shown that a change in the β_2-m level in human serum is an important symptom of the acquired immune deficiency syndrome (AIDS) and AIDS-related ailments [10]. Moreover, β_2-m occurs as a constant subunit of certain classes of histocompatibility antigens [2–5] and has been found to have a primary structure that is homologous to that of the constant domains of the immunoglobulin molecule [2–5,11].

The β_2-m molecule consists of a single polypeptide chain with two half-cystinyl residues involved in an interchain disulphide bond [12]. This bond seems to be buried within the molecule and the loop formed by the disulphide bond has 55 amino acid residues [12]. The exact function of β_2-m is still unknown. Nevertheless, research so far on this protein suggests connections with immunology, nephrology and oncology. Moreover, it has been demonstrated that most β_2-m can also be non-covalently associated with cell surfaces [2–4]. With the extensive recent development of research on β_2-m there is thus increasing interest in the structure and function of this protein at the interfaces between cell surfaces or other membrane proteins on the one hand and aqueous solutions on the other, and at interfaces in general. Unfortunately, investigation of the structure and function of proteins is much more difficult at interfaces than in the bulk of the solution.

Most of the studies on the surface behaviour of

Correspondence address: V. Brabec, Institute of Biophysics, Czechoslovak Academy of Sciences, Královopolská 135, 612 65 Brno, Czechoslovakia.

proteins and polypeptides have been concerned with spread monolayers. It has been shown that there may be a significant difference between the conformation of protein molecules in the adsorption monolayers and in the bulk of the solution [13–18]. We present here a study of the interaction of human β_2-m with a charged surface. The polarized mercury dropping electrode is a suitable model for a charged surface, since the surface charge density which can be easily controlled by external polarization determines the adsorption. The behaviour of β_2-m on the mercury surface was investigated by measuring changes in the differential capacity of the electrical double layer of this surface produced by the adsorption of the protein. The entry of the surface-active compound into the mercury surface affects the capacity by changing the dielectric constant, by displacing the ions and water molecules from the surface and by introducing new charges [19,20]. From the diffusion-controlled adsorption kinetics, the overall surface concentration or the area occupied by the protein molecule can be determined [14,16]. The evaluation of these data can provide information on the conformational changes occurring on the surface at different surface charge densities.

2. Materials and methods

β_2-m was purified from the urine of a patient with Balkan nephropathy disease by gel and ion-exchange chromatography [1,2]. The concentration of the protein was determined spectrophotometrically with a Beckman DU-8 spectrophotometer using the value for the extinction coefficient previously published [2]. The apparent diffusion coefficient, $D_{20,w}$, of human β_2-m is 13.3×10^{-7} cm^2 s^{-1} [2].

Differential capacitance measurements were made by the phase-selective alternating current (a.c.) polarographic method [21,22]. The working electrode was a PARC model 303A static mercury drop electrode with an area of 0.01455 cm^2. A saturated calomel electrode (SCE) at 25 °C was used as reference electrode and a platinum wire as counter electrode.

The appropriate test or background solution (0.15 M NaCl plus 20 mM sodium phosphate, pH 7.0) at 25 °C was deaerated with water-saturated argon for approx. 15 min. After this time a phase-selective a.c. polarographic curve or a.c./time curve at a constant potential was recorded with the aid of Endim model 620.02 recorder.

Calculation of the surface concentration of β_2-m is based on the assumption that its adsorption is diffusion-controlled [23,24]. Thus, if insufficient time is allowed for this adsorption process to run to completion the surface will be only partly covered and the surface excess of the solute Γ is given, in the case of diffusion-controlled adsorption, by the following expression [16,25]:

$$\Gamma = 1.13 D^{1/2} t^{1/2} c, \quad (1)$$

where D is the diffusion coefficient, t the time allowed for the adsorption and c the bulk concentration of solute. Any measured surface property (for instance, differential capacity) is proportional to Γ and thus to $t^{1/2}$ if measurements are made at constant c, and to c if measurements are made at constant t. With sufficiently long times t or at high solute bulk concentrations c, when the surface becomes fully covered, there is negligible variation in Γ with further increase in t or c.

An extrapolation of the differential capacity at short times t, where it is proportional to surface coverage, to a time at which the surface just becomes fully covered is thus possible. The intercept of the linear plot of differential capacity vs. $t^{1/2}$ (obtained for short times) with almost constant capacity values at long times gives the limiting time t_{sat} at which the surface becomes fully covered at the solute bulk concentration in question. From this intercept and with the aid of eq. 1 the surface concentration Γ_{sat} of the fully covered surface can be calculated.

3. Results and discussion

The plots of differential capacity, C vs. potential (capacitance curves) in the presence of protein molecules can provide substantial information about the configuration of these biomacromolecules on the mercury surface. The capacitance curve in the presence of 93.1 μM β_2-m using a

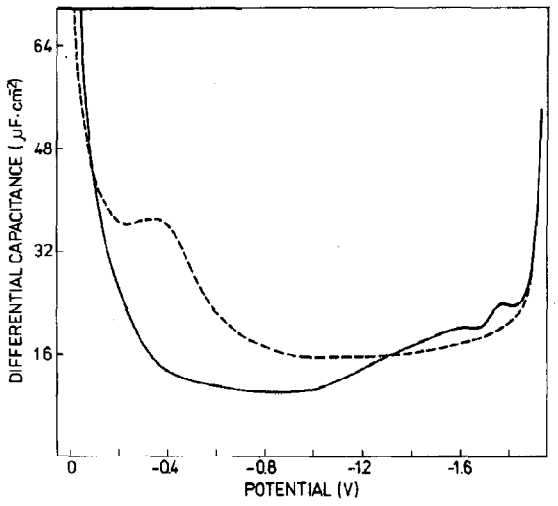

Fig. 1. Differential capacity vs. potential in the presence of 0.15 M NaCl plus 20 mM sodium phosphate, pH 7.0 and of 93.1 μM β_2-microglobulin. Drop time was 5.0 s. (- - - - -) Background electrolyte curve.

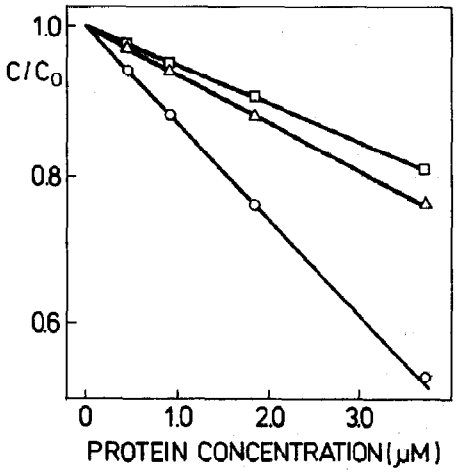

Fig. 2. Reduced differential capacity, C/C_0 (C_0, differential capacity in the presence of the background electrolyte only) plotted vs. bulk concentration of β_2-microglobulin at the drop time $t = 2.0$ s: (△); −0.25 V; (○): −0.45 V; (□) −0.8 V. Other conditions were the same as in fig. 1.

drop time of 5 s is shown in fig. 1. The addition of β_2-m to the background electrolyte caused a marked decrease in differential capacity, in particular around the potential of zero charge (E_{ECM}) (E_{ECM} of background electrolyte, −0.47 V as determined by electrocapillary measurements [22]). This capacitance fall indicates adsorption of β_2-m molecules on the mercury surface. At much more positive and negative potentials β_2-m molecules do not interact with the surface or affect the capacity of the double-layer. The nature of the peaks appearing on capacitance curves of β_2-m at −1.5 to −1.8 V has not been examined yet but it appears likely that their origin could be connected with an adsorption/desorption process or with faradayic currents due to catalytic hydrogen evolution.

The linear dependences of capacity on the bulk concentration of β_2-m at constant time allowed for adsorption (t) and at various constant potentials are shown in fig. 2. Similarly, the linear dependences of the capacity on $t^{1/2}$ at constant bulk concentration of β_2-m at various constant potentials are shown in fig. 3. It is evident, therefore, that differential capacity in the potential range examined (−0.2 to −1.0 V) is proportional to surface concentration. The latter linear dependences give an indication that at bulk concentrations and the times t where the surface is not yet

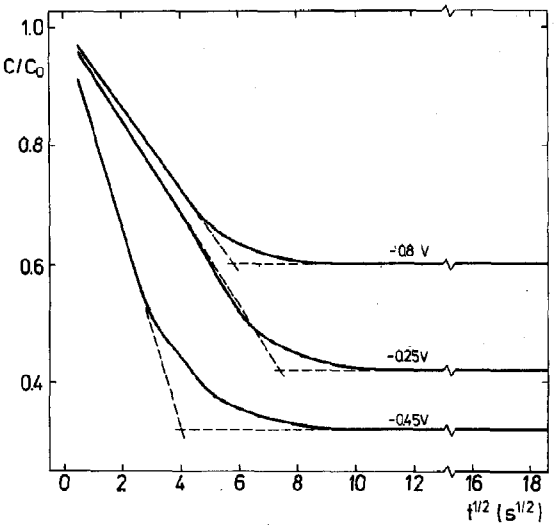

Fig. 3. Reduced differential capacity, C/C_0 plotted vs. the square root of the time allowed for adsorption at different potentials (indicated in the graph). The bulk concentration of β_2-microglobulin was 18.6 μM. Other conditions were the same as in fig. 1.

fully covered, the capacitance curves of β_2-m are a function of the diffusion coefficient of the adsorbing β_2-m molecules. This means that the adherence to the surface, including necessary structural rearrangement of the β_2-m molecules in the adsorbed state, is very fast and that diffusion to the surface is the rate-controlling step.

Information on the conformation and configuration in the mercury surface of β_2-m molecules can be obtained from the limiting surface concentration and the calculated area occupied by the protein molecule on the mercury surface. The limiting surface concentrations Γ_{sat} have been derived from the adsorption kinetics by the method described in section 2. Plots of differential capacity vs. time t at constant β_2-m bulk concentrations and at different constant potentials are given in fig. 3. The intercepts give the critical times (t_{sat}) at which the mercury drop at constant β_2-m bulk concentration is just fully covered. These critical adsorption times are dependent on the electrode potential with a minimum around the potential of zero charge. The surface concentrations of the fully covered surface are now calculated from these critical adsorption times with the aid of eq. 1. The surface concentration and the areas per β_2-m molecule calculated in this way are summarized in table 1 and fig. 4. As seen from table 1 and fig. 4, the areas occupied by one β_2-m molecule vary from 8.2 to 16.7 nm^2, with a maximum at potentials around -0.5 V, i.e., at a potential

Table 1

Parameters of diffusion-controlled adsorption on a mercury electrode of 18.6 μM β_2-microglobulin in 0.15 M NaCl with 20 mM sodium phosphate, pH 7.0

Potential (V)	$t_{sat}^{1/2}$ ($s^{1/2}$) [a]	Γ_{sat} (M m^{-2}) [b]	S (nm^2) [c]	$\bar{S}$ (nm^2) [d]
−0.25	7.45	1.81×10^{-15}	9.1	9.0±0.3 [e]
−0.45	4.10	9.94×10^{-16}	16.7	16.7±0.2 [e]
−0.80	5.80	1.41×10^{-15}	11.8	11.7±0.4 [e]

[a] Time at which saturation surface coverage occurred.
[b] Surface concentration of the fully covered surface.
[c] Area per molecule.
[d] Mean values of the area per molecule estimated on the basis of three independent determinations for bulk concentrations of the protein of 4.7, 9.3, 18.6, and 37.2 μM.
[e] Standard deviation.

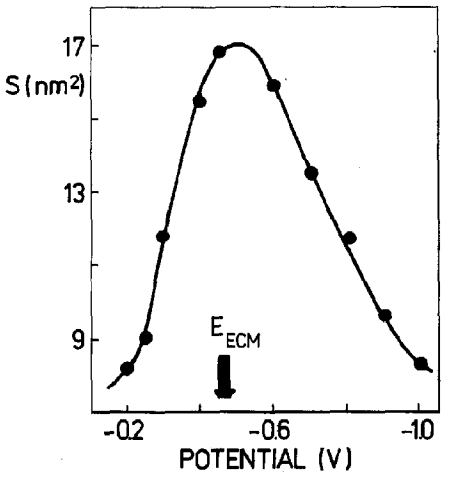

Fig. 4. Values of the area per β_2-microglobulin molecule adsorbed on the mercury drop, $\bar{S}$ plotted vs. potential. The $\bar{S}$ values represent the mean values estimated on the basis of three independent determinations for bulk concentrations of the protein of 4.7, 9.3, 18.6, and 37.2 μM.

close to that of zero charge of the mercury surface. If the surface charge became more positive or more negative the area per β_2-m molecule decreased and at -0.2 and -1.0 V was close to that corresponding to the cross-section of the β_2-m molecule in the bulk of solution (the low frictional ratio, $f/f_0 = 1.16$ [2], indicates that the β_2-m molecule in the bulk of solution has an almost spherical shape; considering the Stoke's radius of the β_2-m molecule as 1.6 nm [2], the cross-section of this biomacromolecule in the native state is around 8.04 nm^2). The most likely explanation for the latter observation is that β_2-m molecules adsorbed at the mercury surface polarized to more extreme values of potentials relative to E_{ECM} (-0.2 and -1.0 V) preserve to a significant extent their native conformation.

Adsorption of the β_2-m molecules at the mercury surface polarized to potentials corresponding to zero or very small surface charge density (around E_{ECM}), leads to larger areas per adsorbed β_2-m molecule extending well beyond the cross-section calculated from the physicochemical properties of native β_2-m [2]. This observation could indicate unfolding or flattening of β_2-m molecules under the influence of interface

energy. In the latter case the degree of this interfacial conformational rearrangement appears to be consistent with a lowering of the standard free energy of adsorption ΔG^0 [26]; ΔG^0 is expected, under the experimental conditions used throughout our work, to have minimum value around the potential of zero charge. The latter conclusion is justified, since the average net charge per β_2-m molecule at neutral pH is negative but in the medium of 0.15 M NaCl used as the background electrolyte most of the charged amino acid residues at the aqueous periphery of β_2-m molecules were screened and thus neutralized by the counterions of the background electrolyte. It is thus likely that β_2-m is adsorbed on the mercury surface from the medium used throughout our work as an almost electroneutral surface-active compound. The electroneutral surface-active compounds are adsorbed at the mercury surface most strongly if this surface is polarized to potentials around E_{ECM} [27]. The conclusion that β_2-m was adsorbed as an almost electroneutral compound is also supported by our adsorption studies performed in a medium of 0.15 M NaCl with 20 mM sodium acetate, pH 5.8 and 4.5. The results obtained at pH values when the average net charge of the β_2-m molecule was considerably reduced (isoelectric point of β_2-m: around 5.8 [2]) or even had the opposite sign were practically identical to those obtained in neutral medium.

Another explanation for our observation that around the potential of zero charge the area per adsorbed molecule of β_2-m was larger than the cross-section of this native biomacromolecule in the bulk of solution could be based on the existence of possible lateral repulsive forces between the adsorbed molecules. These repulsive forces could cause the proteins to form only a loose layer on the electrode. However, this explanation does not appear to be justified because there is no reason for stronger lateral repulsion between the protein molecules adsorbed on a surface polarized specifically only to potentials around E_{ECM}. Also, it has been shown on other solid surfaces that lateral repulsions between adsorbed protein molecules are negligible at ionic strengths as high as that used in our work [28]. Moreover, as already mentioned above, we also performed the experiments at pH 5.8 when average net charge per β_2-m molecule was reduced so that any lateral repulsion between adsorbed molecules was necessarily much weaker than at pH 7.0. However, even in this case, a marked increase in area occupied by one β_2-m molecule on the mercury surface polarized to potentials around E_{ECM} was observed. This increase was approximately the same as that observed for adsorption from neutral solutions.

The results described in this work thus appear to be mostly consistent with the conclusion that β_2-m molecules adsorbed on a mercury surface polarized to potentials corresponding to an uncharged surface or a surface having only a small charge density (around the E_{ECM}) from dilute neutral solutions of the protein are significantly unfolded or flattened. If the adsorption of β_2-m is observed on a surface charged more positively or negatively the extent of this interfacial conformational rearrangement decreases and at fairly extreme surface charge densities of the adsorbent most if not all adsorbed molecules of β_2-m maintain their native conformation. The reason for extensive unfolding or flattening of the β_2-m molecules on a surface with only a low electric charge density may be rather strong adsorption of the proteins on such a surface. Thus, more hydrophobic amino acid residues buried inside the native protein molecule could be forced to adhere to the mercury surface. The conformational rearrangement of many globular proteins has already been described at various interfaces, including that of mercury/water [13–18].

4. Conclusions

The results of this work support the view that conformational perturbations may occur in β_2-m molecules as a result of their interaction with a surface. In addition, it has been shown that increasing surface charge density lowers the extent of this interfacial conformational rearrangement. A considerable body of evidence indicates that β_2-m molecules function in association with cell surfaces or their components. It is therefore apparent that the results reported in this paper could be useful in attempts to clarify the mechanism of

functioning of β_2-m on the cell surface or in a membrane situation. The question of which domains of the β_2-m molecule can undergo interfacial conformational rearrangement (e.g., whether only the loop of β_2-m occurring in this protein as a consequence of the disulphide bond becomes more accessible with or without an alteration in the loop domain conformation or whether this interfacial unfolding takes place only in the remainder of the biomacromolecule forming two tails) remains, however, to be resolved.

References

1 I. Berggard and A.G. Bearn, J. Biol. Chem. 243 (1968) 4095.
2 B. Berggard, L. Björck, R. Cigén and L. Lögdberg, Scand. J. Clin. Lab. Invest. 40, Suppl. 154 (1980) 13.
3 M.D. Poulik, in: The plasma proteins, vol. I, ed. F.M. Putnam (Academic Press, New York, 1975) p. 433.
4 F.A. Karlson, L. Wibell and P.E. Evrin, Scand. J. Clin. Lab. Invest. 40, Suppl. 154 (1980) 27.
5 L. Wibell, P.E. Evrin and I. Berggard, Nephron 10 (1973) 320.
6 K. Kithier, J. Cejka, J. Belamaric, M. Al-Sarraf, W.D. Peterson, Jr, V.K. Vaitkevicius and M.D. Poulik, Clin. Chim. Acta 52 (1974) 293.
7 J. Shuster, P. Gold and M.D. Poulik, Clin. Chim. Acta 67 (1976) 307.
8 C. Teasdale, A.M. Mander, R. Fifield, J.W. Keyser, R.G. Newcombe and L.E. Hughes, Clin. Chim. Acta 78 (1977) 135.
9 R. Bataille and J. Grenier, Presse Méd. 15 (1986) 1723.
10 G.R.F. Krueger, H.F. Ostmeier, A. Ramon, B. Koch and B. Raftery, in: AIDS Bericht, vol. 2, ed. G.K. Steigleder (Grosse Verlag, Berlin, 1986) p. 38.
11 B.A. Cunningham and I. Berggard, Transplant. Rev. 21 (1974) 21.
12 B.A. Cunningham, J.L. Wang, I. Berggard and P.A. Peterson, Biochemistry 12 (1973) 4811.
13 P.R. Musselwhite and J. Palmer, J. Colloid Interface Sci. 28 (1968) 168.
14 I.R. Miller, in: Progress in surface and membrane sciences, vol. 4, eds. J.F. Danielli, M.D. Rosenberg and D.A. Cadenhead (Academic Press, New York, 1971) p. 299.
15 F. Scheller, M. Jänchen and H.J. Prümke, Biopolymers 14 (1975) 1553.
16 B.A. Kuznetsov and G.P. Shumakovitch, Elektrokhimiya 20 (1984) 147 (in Russian).
17 W. Norde, in: Adhesion and adsorption of polymers, part B, ed. L.-H. Lee (Plenum Press, New York, 1980) p. 801.
18 J.D. Andrade, in: Surface and interfacial aspects of biomedical polymers, vol. 2, ed. J.D. Andrade (Plenum Press, New York, 1985) p. 1.
19 I.R. Miller and D.C. Grahame, J. Am. Chem. Soc. 78 (1956) 3577.
20 I.R. Miller and D.C. Grahame, J. Am. Chem. Soc. 79 (1957) 3006.
21 H. Kinoshita, S.D. Christian and G. Dryhurst, J. Electroanal. Chem. 83 (1977) 151.
22 V. Brabec, S.D. Christian and G. Dryhurst, J. Electroanal. Chem. 85 (1977) 389.
23 F. MacRitchie, Adv. Prot. Chem. 32 (1978) 283.
24 B. Aizenbud, V. Volterra and Z. Priel, J. Colloid Interface Sci. 103 (1985) 133.
25 J. Koryta, Collect. Czech. Chem. Commun. 18 (1953) 206.
26 V. Brabec, S.D. Christian and G. Dryhurst, J. Electrochem. Soc. 125 (1978) 1236.
27 D.M. Mohilner, in: Electroanalytical chemistry, vol. 1, ed. A.J. Bard (M. Dekker, New York, 1966) p. 241.
28 T. Suzawa and T. Murakami, J. Colloid Interface Sci. 78 (1980) 266.