Characterization of α_1-microglobulin in human colostrum and milk

I. Bernier, A. Dautigny and P. Jollès[1,2]

Laboratoire des Protéines, Université de Paris V, 45, rue des Saints-Pères, F-75270 Paris Cedex 06 (France), 27 May 1980

Summary. The mean concentration of α_1-microglobulin in human colostrum and milk, estimated by electroimmunoassay, was found to be about 0.4–0.6 mg/l and 0.1–0.2 mg/l, respectively. Both liquids contained α_1-microglobulin in mono- and dimeric forms, while the presence of higher polymeric forms, as characterized in plasma, could not be demonstrated.

α_1-Microglobulin, a low molecular weight glycoprotein, was first isolated from the urine of patients with tubular proteinuria by Ekström et al.[3]. Its presence was further described in plasma and cerebrospinal fluid[3-5]. The present note deals with a first series of data concerning the presence of α_1-microglobulin in human colostrum and milk.

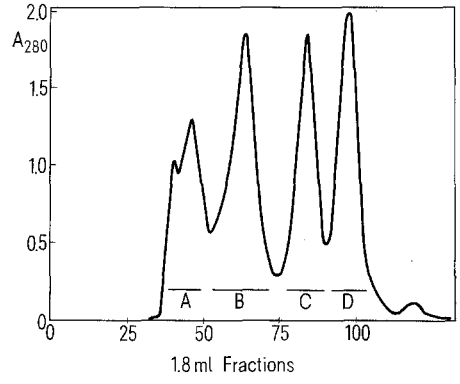
Material and methods. Human colostrum was collected from healthy women just after parturition. Colostrum and milk were immediately frozen until required. After thawing, and stirring for 30 min, the preparations were centrifuged for 30 min at 5000 × g in order to remove fat and a small precipitate. The caseins were precipitated from skimmed milk at 4 °C and pH 4.6; after centrifugation for 30 min at 16,000 × g, Hyflo Super Cel (1% w/v) was added to the clear supernatant. The suspension was stirred at room temperature for 30 min and centrifuged. The supernatant was adjusted to pH 8.0 with NH_4OH and concentrated by ultrafiltration (Amicon, PM-10). Gel filtration was performed on Sepharose CL-6B columns using a 0.05 M Tris-HCl buffer, pH 8.0, containing 0.5 M NaCl. Electrofocusing was carried out with ampholines (pH 3.5–10), using a LKB column of 110 ml. An anti-α_1-microglobulin antiserum was prepared in rabbits with α_1-microglobulin purified from urine as described previously[6]. The quantitative determination of α_1-microglobulin was performed by electroimmunoassay[7]. Albumin and IgA were characterized by immunodiffusion in gel according to Ouchterlony[8].

Results and discussion. The presence of α_1-microglobulin in human colostrum and milk was established and quantified by electroimmunoassay on concentrated samples. The mean concentration of α_1-microglobulin was found to be 0.4–0.6 mg/l in colostrum and 0.1–0.2 mg/l in milk.

Concentrated colostrum and milk samples, corresponding to about 10 ml of the untreated material, were submitted to gel filtration on Sepharose CL-6B (figure). The elution profiles were similar for colostrum and milk. In both cases, the major part of the α_1-microglobulin (about 2 µg for colostrum and 0.7 µg for milk) was detected in peak C, corresponding to the elution volume of albumin. In peak D, α_1-microglobulin was present in a lower concentration (about 0.9 µg and 0.3 µg for colostrum and milk, respectively): its elution volume was the same as that of monomeric urinary α_1-microglobulin. In the more rapidly eluted peaks, no α_1-microglobulin could be detected, neither in peak A, nor in peak B, which contained IgA. This observation is noteworthy, because the presence of α_1-microglobulin in forms with high mol.wt (together with IgA) had been demonstrated in plasma[3,4]. Our results suggest that α_1-microglobulin is only present in mono- and dimeric forms in human colostrum and milk.

The charge heterogeneity of α_1-microglobulin from human colostrum and milk has been demonstrated by electrofocusing. Its presence was detected in a pH range of 3.6–4.7, corresponding to isoelectric points described for urinary α_1-microglobulin[4].

Information about the origin and the biological role of α_1-microglobulin is still very limited. Its previously described presence on the surfaces of lymphocytes[9] could not be corroborated and the reactivity of anti-α_1-microglobulin antisera with cell surface components was explained by the presence of minor impurities in the purified α_1-microglobulin preparations used as antigen and corresponding either to α_1-acid glycoprotein[10] or to human histocompatibility antigens (HLA)[6], co-purified with α_1-microglobulin from urine[11]. Our observation on the approximately threefold decrease of α_1-microglobulin in human milk compared with colostrum suggests a possible biological function in newborns, especially during the first few hours.



Filtration on Sepharose CL-6B of human colostrum (10 ml). The column (60 cm × 2.2 cm) was equilibrated with a 0.05 M Tris-HCl buffer, pH 8.0, containing 0.5 M NaCl. The flow rate was 7.6 ml/h. Peaks A–D were concentrated on PM-10 membranes (AMICON), before immunochemical analysis, in order to determine the presence of α_1-microglobulin, albumin and IgA.

1 To whom correspondence should be addressed.

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