Volume 239, number 1, 155–158 FEB 06436 October 1988

Prosomes, small cytoplasmic RNP particles, contain glycoproteins

Wolfgang Tomek, Günter Adam* and Hans-Peter Schmid

Biologisches Institut, Universität Stuttgart, Ulmerstrasse 227, D-7000 Stuttgart 60 and **Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Arbeitsgruppe Pflanzenviren, Messeweg 11/12, D-3300 Braunschweig, FRG*

Received 6 September 1988

Prosomes, ubiquitous small ribonucleoprotein complexes, were isolated from the cytoplasm of erythropoietic mouse cells induced by Friend leucemia virus. We present evidence that some of the prosomal proteins are glycosylated. Specific reactions with the biotinylated lectins concanavalin agglutinin (Con A), *Solanum tuberosum* agglutinin (STA) and *Limulus polyphemus* agglutinin (LPA) indicate that the carbohydrate moieties contain *N*-acetylneuraminic acid, *N*-acetylglucosamine and mannosyl- or glucosyl-residues. Glycosylation of prosomal proteins could explain the resistance of prosomes to proteinase K digestion.

Prosome; Glycosylation; Lectin binding

1. INTRODUCTION

Prosomes, small ribonucleoprotein complexes (19 S scRNPs), have been isolated and described in several eucaryotic cells. Recently we discovered them in the free mRNP fractions of duck and mouse erythroblasts [1]. They have been found in the cytoplasm of mammalian cells [2], in *Drosophila* tissue cells [3], in sea urchin eggs [4], in different plant tissue cells [5,6] and even in yeast [6]. In *Xenopus* oocytes similar particles were also found in the nucleus [7]. In all cases they consist of a specific set of proteins with molecular masses ranging from 19 to 35 kDa. Some of them, e.g. the 27 kDa protein, were highly conserved during evolution, while others vary from species to species [2]. Similarly the small RNAs of prosomes are related between species but not identical [2,6]. Until now there is no evidence about the stoichiometry of the proteins in prosomes. It is possible that multimers of even one protein can form a particle. In contrast to their different RNA and protein constituents the morphology of the prosomes is universal among the various species analyzed so far [1–6]. Under the electron microscope they appear as a hollow cylinder [3] or a raspberry-like structure [1,2]. Most interestingly prosomes resist the strong detergent sarcosyl (1%), high ionic strength and 7 M urea. Even proteinase K is not able to affect prosomes under conditions where other RNPs are readily digested. This could be either due to their highly compact structure and/or to posttranslational modifications of prosomal proteins. Here we show that some of the prosomal proteins are glycosylated.

Correspondence address: H.-P. Schmid, Biologisches Institut, Universität Stuttgart, Ulmerstrasse 227, D-7000 Stuttgart 60, FRG

2. MATERIALS AND METHODS

2.1. *Cell fractionation procedure and isolation of prosomes*

The isolation of Friend virus-induced erythropoietic cells from inbred Balb/c mice and the subsequent preparation of the postmitochondrial supernatant has been described [8]. The postmitochondrial supernatant was layered over 10 ml of 30%, w/w, sucrose in TBK 100 (20 mM Tris-HCl, pH 7.4; 100 mM KCl; 3 mM $MgCl_2$; 7 mM 2-mercaptoethanol) and centrifuged to sediment ribosomes and polysomes (MSE rotor 8 × 50; 36000 rev/min; 180 min). To separate the cytoplasmic proteins (≥10 S), the resulting postpolysomal supernatant was sedimented through 5 ml of 20%, w/w, sucrose (Beckmann Ti 60 rotor, 42000 rev/min; 17 h). The pellets, postribosomal particles (PRPs), were resuspended in TBK 100 frozen at −70°C or immediately applied to a FPLC-Mono Q column, an anion

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

exchanger, equilibrated in Tris-HCl buffer, pH 7.4, then particles eluted at 380 mM KCl were concentrated and subjected to gel filtration (FPLC-Superose 6 B equilibrated in Tris-HCl buffer, pH 7.4, containing 420 mM KCl.

2.2. *One- and two-dimensional gel electrophoresis*

Electrophoresis of proteins in one-dimensional SDS-polyacrylamide gels was performed according to [9]; two-dimensional gel electrophoresis as described by [10]. Molecular mass markers were: phosphorylase *b* (94 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa), carbonic anhydrase (29 kDa) and soybean trypsin inhibitor (20 kDa).

2.3. *Probing of protein blots with lectins*

The following set of biotinylated lectins was used: concanavalin A (ConA), *Dolichus biflorus* agglutinin (DBA), *Lens culinaris* agglutinin (LCA), *Limulus polyphemus* agglutinin (LPA), *Pisum sativum* agglutinin (PSA), *Phaseolus vulgaris* agglutinin (PHA), peanut agglutinin (PNA), soybean agglutinin (SBA), *Solanum tuberosum* agglutinin (STA), *Ulex europaeus* agglutinin (UEA_I) and wheatgerm agglutinin (WGA). The LPA was obtained from Sigma, all other lectins were obtained from Kem-En-Tec. As probe for the biotinylated lectins streptavidin-horseradish peroxidase complexes from Amersham were used.

After separation on gels, prosomal proteins were transferred electrophoretically from the gel onto nitrocellulose (Schleicher and Schüll BA 85) according to [11]. After the transfer the remaining binding sites on the nitrocellulose were blocked by incubation in 20 mM Tris-HCl, pH 7.4; 0.5 M NaCl, 2% Tween 20 for 2 min [12]. The nitrocellulose was then washed twice in TBS (20 mM Tris-HCl, pH 7.4; 0.5 M NaCl) for 5 min. After a short wash in lectin buffer (10 mM Tris-HCl, pH 7.4; 1 M NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM NaN_3), the blots were incubated overnight with biotinylated lectins (2 μg/ml) in the same buffer. The nitrocellulose sheets were then washed twice with TTBS (TBS with 0.05% Tween 20) for 5 min and thereafter incubated with horseradish peroxidase-streptavidin complexes (Amersham) for 1 h, using TTBS. After 3 times washing in TBS the blots were assayed for peroxidase activity by incubating them with peroxidase substrate buffer (0.05 M citrate, pH 5.0; 0.25 mg/ml carbazol; 0.5% H_2O_2). The reaction was stopped by rinsing with tap water and the nitrocellulose was dried between Whatman filter paper.

3. RESULTS

To examine if prosomal proteins become post-translationally modified by glycosylation, we have separated them by one-dimensional gel electrophoresis, transferred them to nitrocellulose by electroblotting and probed the filters with 11 different biotinylated lectins of different carbohydrate specificity (see section 2). Among the 11 lectins only four reacted with prosomal proteins (fig.1a,c,e). The positively reacting lectins were PSA (not shown) and ConA, specific for mannosyl- and glucosyl-containing residues; LPA, specific for neuraminic acid residues and

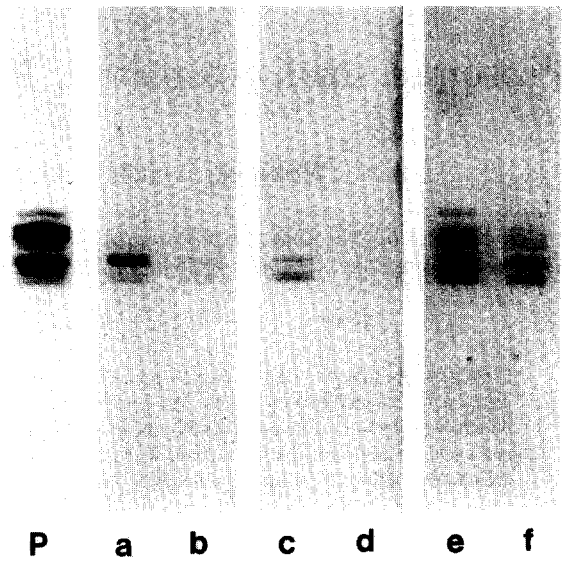

Fig.1. Competition of lectin binding by corresponding carbohydrates. Prosomal proteins were separated by SDS-gel electrophoresis and transferred to nitrocellulose. Strips were cut and probed with biotinylated STA, LPA or ConA in the presence or absence of *N*-acetylglucosamine, *N*-acetylneuraminic acid or methyl-α-D-glucose. Thereafter the filters were washed, incubated with horseradish peroxidase-conjugated streptavidin and assayed for remaining peroxidase activity. (a,c,e) Protein blots probed by: (a) STA; (c) LPA; (e) ConA. (b,d,f) Protein blots incubated with lectins and 0.5 M of the corresponding carbohydrates: (b) STA + *N*-acetylglucosamine; (d) LPA + *N*-acetylneuraminic acid; (f) ConA + methyl-α-D-glucose. (P) Prosomal proteins stained with Coomassie blue.

STA, which reacts specifically with *N*-acetylglucosamine. Since the lectin reactions were carried out after blocking the nitrocellulose with Tween 20, unspecific binding due to hydrophobic interaction between the lectins and the prosomal proteins could be excluded [12].

In order to ascertain the specificity of the lectin reactions, competition tests with the corresponding specific monosaccharides for the positive lectins were conducted. For this approach the lectins were incubated with and without 0.5 M of the specific monosaccharides. As shown in fig.1b,d,f, the specific monosaccharides reduced the amount of lectin bound to the proteins, however, to different extents. Whereas *N*-acetylneuraminic acid and *N*-acetylglucosamine strongly inhibited the lectin binding to prosomal glycoproteins (fig.1b,d), methyl-α-D-glucopyranosid had only a partial

competitive effect. This is due to the fact that monosaccharides are not the optimal competitors for the interaction between lectins and oligocarbohydrate moieties [12].

In order to determine which of the different prosomal proteins is glycosylated, two-dimensional gel electrophoresis was employed, and after blotting of the proteins to nitrocellulose the lectin reactions were carried out with the 3 positively reacting lectins. The results are summarized in fig.2. One prosomal protein (fig.2b; closed triangles) reacted very strongly with STA. The same protein was detected by LPA and ConA (fig.2c,d). A second, more basic protein with a lower molecular mass reacted only with LPA and ConA (fig.2c,d; open triangles). These were the most prominent reacting proteins, however, as could already be deduced from the one-dimensional tests, several other prosomal proteins showed slight reactions with ConA and LPA (fig.2c,d).

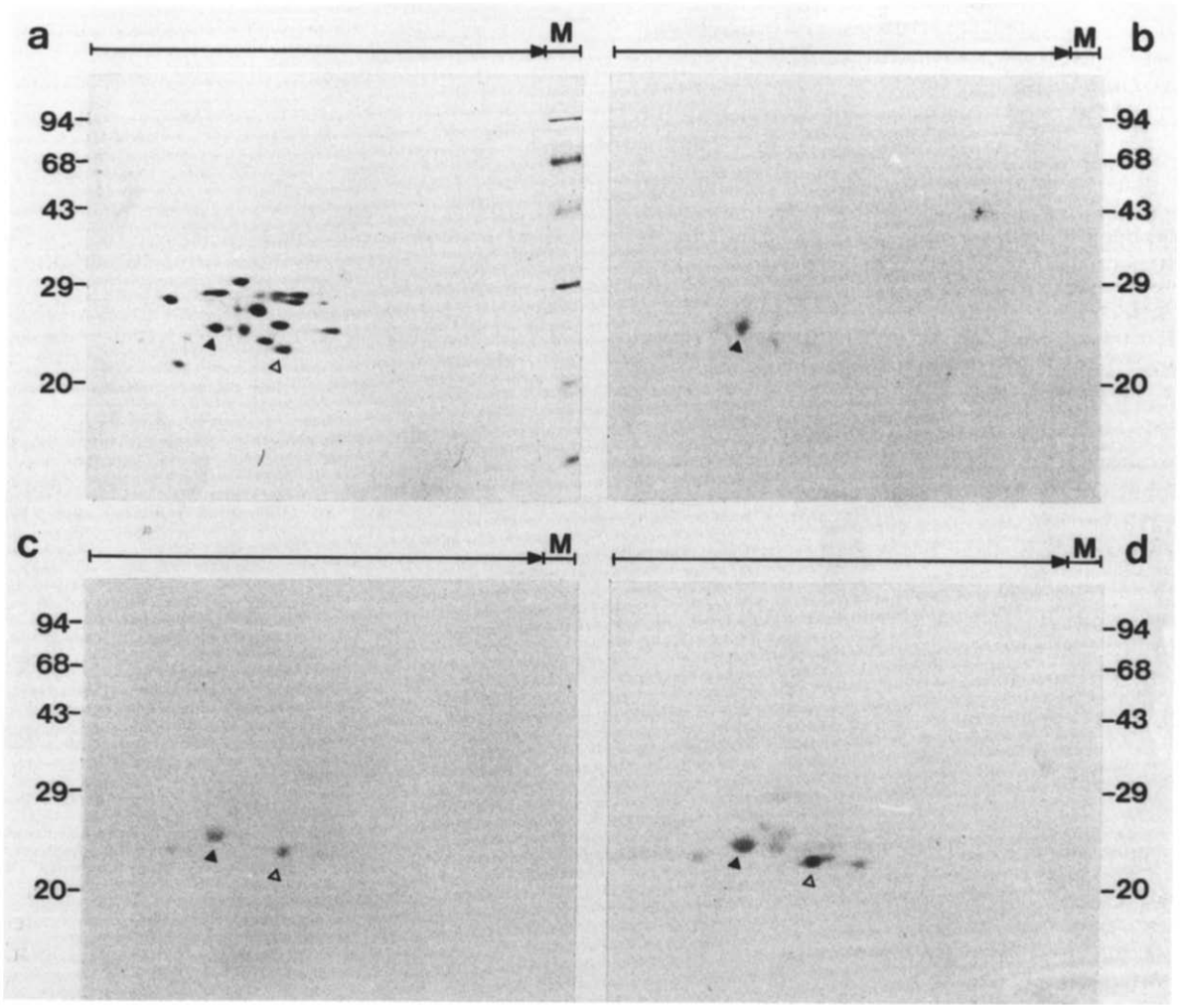

Fig.2. 2D protein blots of prosomes probed with lectins. Prosomes purified by gel filtration were separated by 2D gel electrophoresis and transferred to nitrocellulose by electroblotting. After blocking with Tween 20, the blots were incubated overnight with biotinylated lectins (2 μg/ml lectin buffer). Thereafter the filters were washed and incubated for 1 h with horseradish peroxidase-conjugated streptavidin. Finally all papers were washed and assayed for peroxidase activity. (a) Prosomal proteins stained with Coomassie blue. (b–d) 2D protein blots probed by: (b) STA, (c) LPA, (d) ConA. (▶) Prosomal protein reacting with all three lectins; (▷) prosomal protein reacting with STA and ConA.

4. DISCUSSION

Our results demonstrate that prosomes, which belong to the group of small cytoplasmic ribonucleoprotein particles, contain glycoproteins. This is in contrast with the prevailing evidence that the bulk of carbohydrate moieties are either localized on proteins exposed to the extracellular face of cellular membranes or within the lumen of intracellular organelles (e.g. Golgi apparatus or endoplasmic reticulum). However, recently published studies of Davis and Blobel [13] and of Holt and Hart [14], as well as investigations of other groups [15–17], also indicated the existence of glycoconjugates in cytoplasmic and nuclear compartments. How glycosylation of prosomal proteins is implicated in the function of prosomes remains speculative, since we know only little about their role in cellular events, except that prosomes are involved in translational control of messenger RNA. Recently we reported that prosomes have an unusually high affinity for viral messengers and can inhibit protein synthesis of viral messengers in vitro, under conditions where translation of cellular mRNA (e.g. HeLa mRNA or globin mRNA) is not affected [18,19]. On the other hand, prosomes of *Drosophila* and HeLa cells were reported to have specific proteinase activity [20,21]. In the latter case glycosylation could protect prosomes against self-digestion. Indeed prosomes are highly resistant to proteinase K digestion (unpublished). Taking all data together we postulate that prosomes are particles with multiple enzymatic functions. The complexity of protein and RNA components of prosomes reflects a situation which is known for spliceosomes or ribosomes.

Acknowledgements: We thank Dr Bistra Dineva for the analysis of prosomal RNA, Professor K. Köhler and Dr M. Schönfelder for helpful discussions. This work has been supported by grants from the Deutsche Forschungsgesellschaft (Schm 620/1-2; Ad 51/3-2) and by the Hasselblad Foundation.

REFERENCES

[1] Schmid, H.-P., Akhayat, O., Martins de Sa, C., Puvion, F., Köhler, K. and Scherrer, K. (1984) EMBO J. 3, 29–34.
[2] Martins de Sa, C., Grossi de Sa, M.-F., Akhayat, O., Broders, F., Scherrer, K., Horsch, A. and Schmid, H.-P. (1986) J. Mol. Biol. 187, 479–493.
[3] Arrigo, A.-P., Darlix, J.-L., Khandjian, E.W., Simon, M. and Spahr, P.-F. (1985) EMBO J. 4, 399–406.
[4] Akhayat, O., Grossi de Sa, M.-F. and Infante, A.A. (1987) Proc. Natl. Acad. Sci. USA 84, 1595–1599.
[5] Kremp, A., Schliephacke, M., Kull, U. and Schmid, H.-P. (1986) Exp. Cell. Res. 166, 553–557.
[6] Arrigo, A.-P., Simon, M., Darlix, J.-L. and Spahr, P.-F. (1987) J. Mol. Evol. 25, 141–150.
[7] Kleinschmidt, J.A., Hügle, B., Grund, C. and Franke, W.W. (1983) Eur. J. Cell. Biol. 32, 143–156.
[8] Schmid, H.-P., Köhler, K. and Setyono, B. (1982) J. Cell. Biol. 93, 893–898.
[9] Laemmli, U.K. (1970) Nature 227, 680–685.
[10] O'Farrell, P.Z., Goodman, H. and O'Farrell, P.H. (1977) Cell 12, 1133–1142.
[11] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[12] Adam, G., Heegard, P., Bog-Hansen, T.C. and Mundry, K.-W. (1987) J. Virol. Methods 17, 263–275.
[13] Davis, L.I. and Blobel, G. (1986) Cell 45, 699–709.
[14] Holt, G.D. and Hart, G.W. (1986) J. Biol. Chem. 261, 8049–8057.
[15] Kan, F.W.K. and Pinto da Siva, P. (1986) J. Cell. Biol. 102, 576–586.
[16] Snow, C.M., Senior, A. and Gerace, L. (1987) J. Cell. Biol. 104, 1143–1156.
[17] Holt, G.D., Snow, C.M., Senior, A., Haltiwanger, R.S., Gerace, L. and Hart, G.W. (1987) J. Cell. Biol. 104, 1157–1164.
[18] Horsch, A., Köhler, K. and Schmid, H.-P. (1985) Z. Naturforsch. 40c, 449–450.
[19] Horsch, A., Spindler, E., Martins de Sa, C., Köhler, K. and Schmid, H.-P. (1987) Eur. J. Cell. Biol. 43, 27.
[20] Falkenburg, P.-E., Haass, C., Kloetzel, P.-M., Niedel, B., Kopp, F., Kühn, L. and Dahlmann, B. (1988) Nature 331, 190–192.
[21] Arrigo, A.-P., Tanaka, K., Goldberg, A.L. and Welch, W.J. (1988) Nature 331, 192–194.