Human Pregnancy Zone Protein and \(\alpha_2\)-Macroglobulin

HIGH-AFFINITY BINDING OF COMPLEXES TO THE SAME RECEPTOR ON FIBROBLASTS AND CHARACTERIZATION BY MONOCLONAL ANTIBODIES*

(Fred Van Leuven, Jean-Jacques Cassiman, and Herman Van den Berghe)

From the Center for Human Genetics, University of Leuven, Campus Gasthuisberg, O&N6, Herestraat, B-3000 Leuven, Belgium

Pregnancy zone protein (PZP) was isolated from late pregnancy serum and examined for binding to normal skin fibroblasts in culture. A high-affinity binding site on these cells is demonstrated for PZP reacted with methylamine. Experiments with \(\alpha_2\)-macroglobulin (\(\alpha_2\)M) and PZP, both modified by methylamine, showed this receptor to be identical to the previously characterized receptor for \(\alpha_2\)M-proteinase complexes (Van Leuven, F., Cassiman, J. J., and Van den Berghe, H. (1979) J. Biol. Chem. 254, 5155-5160). With available monoclonal antibodies directed toward \(\alpha_2\)M and prepared toward PZP, only a limited cross-reaction was observed. We obtained a monoclonal antibody which defines a neo-antigenic site on PZP-methylamine, completely analogous to the monoclonal antibody PZ2B2, which was previously shown to define a neo-antigenic site on \(\alpha_2\)M complexes (Marynen, P., Van Leuven, F., Cassiman, J. J., and Van den Berghe, H. (1981) J. Immunol. 127, 1782-1786).

These results provide evidence for the homologous function of \(\alpha_2\)M and PZP as proteinase scavengers. The need for an extra proteinase inhibitor of the \(\alpha_2\)M-type in pregnancy is discussed. The monoclonal antibodies now available will prove helpful in quantitation and eventually isolation of proteinase complexes of \(\alpha_2\)M and PZP.

Pregnancy zone protein (PZP), first described by Smithies (1969), is quantitatively one of the most important pregnancy-associated plasma proteins in humans; at term, plasma levels of 1-2 mg/ml are not uncommon. The characterization of this protein has been hampered by the difficult separation from body F2B2, which was previously shown to define a neo-antigenic site on PZP-methylamine. Experiments with \(\alpha_2\)-macroglobulin (\(\alpha_2\)M) and PZP, both modified by methylamine, showed this receptor to be identical to the previously characterized receptor for \(\alpha_2\)M-proteinase complexes (Van Leuven, F., Cassiman, J. J., and Van den Berghe, H. (1979) J. Biol. Chem. 254, 5155-5160). With available monoclonal antibodies directed toward \(\alpha_2\)M and prepared toward PZP, only a limited cross-reaction was observed. We obtained a monoclonal antibody which defines a neo-antigenic site on PZP-methylamine, completely analogous to the monoclonal antibody PZ2B2, which was previously shown to define a neo-antigenic site on \(\alpha_2\)M complexes (Marynen, P., Van Leuven, F., Cassiman, J. J., and Van den Berghe, H. (1981) J. Immunol. 127, 1782-1786).

These results provide evidence for the homologous function of \(\alpha_2\)M and PZP as proteinase scavengers. The need for an extra proteinase inhibitor of the \(\alpha_2\)M-type in pregnancy is discussed. The monoclonal antibodies now available will prove helpful in quantitation and eventually isolation of proteinase complexes of \(\alpha_2\)M and PZP.

The abbreviations used are: PZP, pregnancy zone protein; \(\alpha_2\)M, \(\alpha_2\)-macroglobulin; \(\alpha_2\)M-MA, \(\alpha_2\)M reacted with methylamine; PZP-MA, pregnancy zone protein reacted with methylamine; mAb, monoclonal antibodies; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; HPLC, high pressure liquid chromatography; NHF, normal human fibroblasts.

*This work was supported by Grant 3.0055.83 from the National Fund for Scientific Research, Belgium, by Grant "Geconcerteerde Acties" from the Belgian Government, and by a research grant from the American Cystic Fibrosis Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed.

‡Portions of this paper (including "Experimental Procedures," "Results," Figs. 1-8, and Table 1) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 86M-1834, cite the authors, and include a check or money order for $4.80 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.
extended: aminolysis of the internal thiol esters in PZP also brings about the expression of a receptor recognition site. Although not explicitly demonstrated here, it is obvious that complexes of proteinases with PZP will be bound to this receptor and cleared by receptor-mediated endocytosis. Preliminary experiments with PZP-trypsin and PZP-chymotrypsin complexes showed little difference from PZP-MA in binding to skin fibroblasts. This would mean that PZP is a proteinase scavenger like α2M and uses the same receptor-mediated pathway for clearance.

The "raison d'être" of PZP must then be sought in those characteristics which are different from α2M. The major difference is its temporary appearance in the circulation: in normal conditions, only pregnancy is characterized by highly increased levels of PZP (for review, see Von Schoultz and Stigbrand, 1982). This "acute phase"-like increase in concentration has been interpreted (Sand et al., 1986) to be analogous to acute phase α2M homologues in species other than man, e.g. α2M in the rat (Gauthier and Mouray, 1976). The similarity is only apparent in terms of "induced synthesis" but not where actual triggers are concerned: in rat, the synthesis of acute phase α2M is induced by interleukin 1 and macrophage-derived factors (Bauer et al., 1984, 1985), whereas in humans, the appearance of PZP is triggered by estrogens (Von Schoultz and Stigbrand, 1982, and references therein). A role for PZP in pregnancy would only be meaningful if PZP and α2M are complementary in their ability (in kinetic terms) to inhibit proteinases and mediate their clearance.

Another major difference between α2M and PZP is evident from rate electrophoresis: whereas α2M is well-known to be tetrameric (in fact dimeric), PZP behaves like a dimer in the native state. Reaction with methylamine but not with trypsin induces further aggregation to species similar in mobility to α2M. It is hard to imagine any physiological consequence, unless major differences in interaction with the receptor between dimeric and tetrameric PZP complexes exist. No evidence for this was obtained, although this ambiguity in PZP structure poses a problem. Indeed, since the preparations of PZP-MA used in the experiments described contained both dimeric and tetrameric forms, it was impossible to calculate exactly the binding affinity and receptor number. By gel filtration, no separation of the two forms was possible (Sand et al., 1985), which indicates either a highly asymmetric structure for PZP or an association to tetramers in solution. The observation of dimers in rate electrophoresis would then constitute an aberrant behavior of PZP in this system. None of these explanations are satisfactory, however, to account for all observations (Sand et al., 1985). Unless the problem is solved, we cannot decide whether dimer-tetramer formation has any physiological importance.

The cross-reaction with PZP of monoclonal antibodies prepared against α2M was less than expected from the extreme sequence homology. mAb F2B2 and F12A3, which were shown to define epitopes in the receptor recognition site at the carboxyl terminus of α2M complexes (Marynen et al., 1981; Van Leuven et al., 1983, 1986b), did not react with PZP or PZP-MA in rate electrophoresis (Fig. 8), radioimmunoassay, or reversed dot blotting (Van Leuven et al., 1986a). Hence, the similar high-affinity receptor binding obviously still allows for highly different immunochennical characteristics of the receptor recognition site in these two homologous proteins.

From a panel of 59 mAb directed toward α2M which we have thus far characterized, only four reacted with PZP with comparable affinity, whereas four other mAb cross-reacted with decreased affinity for PZP.3 Preparation of mAb toward PZP revealed a similar pattern: of 61 mAb reacting with PZP and/or PZP-MA, only five reacted also with α2M and/or α2M-MA. Two of these were further characterized together with the PZP-MA-specific mAb F57.12F5. The latter is in fact the equivalent for PZP of mAb F2B2 and F12A3 for α2M: they define neo-antigenic sites not present on the native proteins. mAb F57.9D11 and F57.13G5 also define neo-antigenic sites on PZP-MA, but they also react with α2M-MA.

In conclusion, the observations reported here prove that PZP, like α2M, can be modified at the internal thiol esters by methanamine to express a receptor recognition site. The modified PZP shares with α2M-MA the high-affinity receptor on human skin fibroblasts. The homology is also apparent from the reactivity with monoclonal antibodies. Moreover, as with α2M, a mAb defining a neo-antigenic site related to the receptor recognition site on PZP-MA was obtained. These mAbs are likely to become useful tools in the quantitative measurement and the isolation of α2M- and PZP-proteinase complexes. The demonstration of specific proteinases bound to PZP and to α2M should eventually clarify their physiological role.

Acknowledgments—We thank L. Stas, K. Merckx, L. Mekers, and M. Willems for expert technical assistance and K. Rondou for the photography. We thank Prof. Dr. A. Van Asche, Head of the Department of Gynecology and Obstetrics, University of Leuven, and the nurses for their interest and their cooperation.

REFERENCES

Bauer, J., Birsnelin, M., Northoff, G.-H., Northemann, W., Trän-Thi, T., Ueberberg, H., Decker, K., and Heinrich, P. C. (1984) FEBS Lett. 177, 89-94.
Bauer, J., Welser, W., Trän-Thi, T., Northoff, G.-H., Decker, K., Gerok, W., and Heinrich, P. C. (1985) FEBS Lett. 190, 271-274.
Bohn, H., and Winscleger, W. (1976) Biol. 23, 377-388.
Bolton, A. E., and Hunter, W. M. (1973) Biochem. J. 133, 529-539.
Folkerse, J., Teisner, B., Ahrons, S., and Svemag, S.-E. (1978) J. Immunol. Methods 20, 117-125.
Gauthier, F., and Mouray, F. (1979) Biochem. J. 159, 661-665.
Lasmulli, U. K. (1979) Nature 227, 680-685.
Marynen, P., Van Leuven, F., Cassiman, J. J., and Van den Bergh, H. (1981) J. Biol. Chem. 256, 1792-1796.
Sand, O., Folkerse, J., Westergaard, J. G., and Sottrup-Jensen, L. (1985) J. Biol. Chem. 260, 15727-15737.
Siniosch, M. J., Davey, M. W., Teisner, B., and Grudzinskas, J. G. (1983) Biochem. Int. 7, 33-42.
Smithies, O. (1986) Adv. Protein Chem. 41, 65-113.
Sottrup-Jensen, L., Ste anik, T. M., Kristensen, T. E., Wierzbicki, D. P., and Eiberg, H. (1984a) Proc. Natl. Acad. Sci. U. S. A. 81, 7333-7337.
Stigbrand, T., Damber, M.-G., and Von Schoultz, B. (1978) Acta Chem. Scand. Ser. B Org. Chem. Biochem. 32, 717-719.
Stimson, W. H., and Farquharson, D. M. (1978) Int. J. Biochem. 9, 839-843.
Van Leuven, F. (1982) Trends Biochem. Sci. 7, 175-177.
Van Leuven, F., Cassiman, J.-J., and Van den Bergh, H. (1978) Exp. Cell Res. 117, 275-280.
Van Leuven, F., Cassiman, J.-J., and Van den Bergh, H. (1979) J. Biol. Chem. 254, 5101-5106.
Van Leuven, F., Cassiman, J.-J., and Van den Bergh, H. (1981) J. Biol. Chem. 256, 9016-9022.
Van Leuven, F., Cassiman, J.-J., and Van den Bergh, H. (1982a) Biochem. J. 201, 119-129.
Van Leuven, F., Marynen, P., Cassiman, J.-J., and Van den Bergh, H. (1982b) Biochem. J. 203, 405-416.
Van Leuven, F., Marynen, P., Cassiman, J.-J., and Van den Bergh, H. (1983) Ann. N. Y. Acad. Sci. 421, 434-441.
Van Leuven, F., Cassiman, J.-J., and Van den Bergh, H. (1985) Sci. Tools 32, 41-43.
Van Leuven, F., Marynen, P., Cassiman, J.-J., and Van den Bergh, H. (1986a) J. Immunol. Methods 90, 125-130.
Van Leuven, F., Marynen, P., Cassiman, J.-J., and Van den Bergh, H. (1986b) J. Biol. Chem. 261, 13169-13173.
Von Schoultz, B., and Stigbrand, T. (1974) Biochem. Biophys. Acta 359, 303-310.
Von Schoultz, B., and Stigbrand, T. (1982) in Pregnancy Proteins (Grudzinskas, J. G., Teisner, B., and Seppälä, M., eds) Academic Press, Sydney, Australia.

Continued on next page.
Pregnancy Zone Protein and ααM

Table 1: Displacement of monoclonal antibodies by ααM-MA and ααM-MA from isolated receptors

| MAb | ααM-MA (μg/ml) | ααM-MA (μg/ml) |
|-----|----------------|----------------|
| F926 | 10              | 1000           |
| FST 1375 | 40          | 10             |
| FST 1375 | 10          | 10             |
| FST 1375 | 100          | 5             |
| FST 1375 | 500          | 2             |

All cells were preincubated with 125I-labeled ααM-MA or 125I-labeled ααM-MA in 0.4 μg/ml for 6 hours in PBS at 4°C. The medium was removed and cell layer washed four times with cold medium (PBS). Cells were further incubated with the ααM-MA in the indicated final concentration. After 45 minutes cell layers were washed three times and cell-bound radioactivity determined.

Effect of concentration of ααM-MA on binding to NIP. Separate cell layers were incubated for 3 hours with various concentrations of 125I-labeled ααM-MA. Bound radioactivity was determined in duplicate as described under experimental procedures. Results presented in Fig. 4 were obtained at 0.4 μg/ml of ααM-MA. The specificity of the ααM-MA was assessed by using isolated ααM-MA.

Fig. 4: Effect of concentration of ααM-MA on binding to NIP. Separate cell layers were incubated for 3 hours with various concentrations of 125I-labeled ααM-MA. Bound radioactivity was determined in duplicate as described under experimental procedures. Results presented in Fig. 4 were obtained at 0.4 μg/ml of ααM-MA. The specificity of the ααM-MA was assessed by using isolated ααM-MA.
Pregnancy Zone Protein and α₂M

Fig. 5

Inhibition by α₂M MA and FPI-MA of binding of 125I-labeled PPI-MA to NFS. Binding of 125I-labeled PPI-MA at 0.3 μg/ml to NFS cell layer at 0°C was measured as described under experimental procedures, in the presence of α₂M MA or FPI-MA in the concentration indicated. Specific binding is shown relative to specific binding of 125I-labeled PPI-MA at 0.3 μg/ml without further additions.

Fig. 6

Inhibition by PPI and PPI-MA of binding of 125I-labeled α₂M MA to NFS. Binding of 125I-labeled α₂M MA (0.3 μg/ml) to NFS cell layer was determined at 0°C as described under experimental procedures, in the presence of PPI or PPI-MA in the concentrations indicated. Specific binding is shown relative to specific binding of 125I-labeled α₂M MA at 0.3 μg/ml without further additions.

Fig. 7

Screening for reversed dot blotting of antibodies with PPI and PPI-MA. Reversed dot blotting was done with rabbit anti-human anti-bodies immobilized on nitrocellulose paper. Anti-relevant with nitrocellulose paper, each well of the microtiter plates were incubated by incubation with rabbit 125I-labeled PPI (panel A) or 125I-labeled PPI-MA (panel B). Autoradiographs, taken overnight on Kodak XAR film are shown. Antibody producing monoclonal antibodies which reacted immunologically with PPI-MA are indicated by arrows. Hybridization with 125I by using the same technique of reversed dot blotting using a mixture of 125I-labeled α₂M and α₂M MA (panel C).

Fig. 8

Characterization of monoclonal antibodies for zona intercalating (unlabeled α₂M MA (panel A) or α₂M MA (panel B) or α₂M MA (panel C)) were reacted with 125I-labeled PPI (panel A), or α₂M MA (panel B) or α₂M MA (panel C). Hybridization with nitrocellulose paper, each well of the microtiter plates were incubated by incubation with rabbit 125I-labeled PPI-MA (panel A), α₂M MA (panel B), or α₂M MA (panel C). Autoradiographs, taken overnight on Kodak XAR film are shown. Hybridization with 125I-labeled PPI and PPI-MA (corresponding to about 2.0 μg/ml) are reacted with 5 μg of the Mat (0.5 μg for FPI). After reverse dot blots were made with the same technique of reversed dot blotting using a mixture of 125I-labeled α₂M and α₂M MA (panel C).