Volume 232, number 2, 351–353 FEB 05883 May 1988

Evidence for the identity of anti-proteinase pulmonary protein CCSP and uteroglobin

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Received 17 March 1988

Purified Clara cell secretory protein (CCSP) from rabbit lung was analyzed by SDS gel electrophoresis, and immunoblotting with a specific anti-uteroglobin antibody as well as for its ability to bind [^{3}H]progesterone. The results obtained indicate that proteins CCSP and uteroglobin are identical.

Secretion; Uteroglobin; Proteinase; Enzyme inhibitor; (Clara cell, Lung)

1. INTRODUCTION

The small airways, connecting the alveoli and conducting airways, are lined by an epithelium mainly constituted by the so-called Clara cells. Although the exact physiological function of these cells has not yet been established, several lines of evidence suggest that Clara cells play an important role in lung functions [1]. Thus, morphological and biochemical studies [2–5] indicated that Clara cells are actively engaged in the secretion of proteins and perhaps of lipids. Therefore, knowledge of the secretory products of these cells should be of importance in gaining better understanding of their functions.

A recent study has described the purification of a low-M_r protein synthesized and secreted by Clara cells from rabbit lung [5]. This protein, termed CCSP, accounts for a considerable fraction of the total protein in the lavage of lung airways and presents proteinase-inhibitory activity. On the other hand, uteroglobin is also a small secretory protein synthesized and secreted in several organs of the rabbit under tissue-specific control of steroid hormones (reviews [6,7]). In lung, uteroglobin synthesis is under the control of glucocorticoids [8,9] and the primary structure has been established for two mammalian species [10,11].

Here, we present evidence for the identity of CCSP and uteroglobin.

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Abbreviation: CCSP, Clara cell secretory protein

2. MATERIALS AND METHODS

New Zealand White rabbits (approx. 3 kg) were used throughout. [1,2,6,7-^{3}H]Progesterone (85 Ci/mmol) was from Amersham International.

2.1. *Purification of CCSP and uteroglobin*

Rabbit CCSP was isolated as described by Gupta et al. [5]. Rabbit uteroglobin was purified as in [12].

2.2. *SDS gel electrophoresis and Western blotting*

Electrophoresis was carried out using slab gels containing 15% polyacrylamide, 0.1 M phosphate buffer, pH 7.2, 0.1% SDS and 6 M urea as recommended by the supplier (Bethesda Research Laboratories). For Western blotting, proteins separated on the gels were electrophoretically transferred onto nitrocellulose filters [13] and the blots were incubated with a specific anti-uteroglobin antibody [8,9] followed by a second, peroxidase-conjugated antibody.

2.3. *Assays on progesterone binding*

Purified CCSP or uteroglobin was reduced in the presence of 10 mM dithiothreitol [14] and then incubated with 10^{-7} M

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00145793/88/$3.50

[^{3}H]progesterone. Bound and free progesterone was separated by chromatography on small columns of Sephadex G-25 [15].

3. RESULTS

Following the protocol described by Gupta et al. [5] for the purification of pulmonary CCSP, we obtained results similar to theirs (not shown). The purified CCSP thus obtained was analyzed by SDS gel electrophoresis together with homogeneous uteroglobin for comparison (fig.1A). The electrophoretic behaviour of both proteins was identical in either the absence or presence of 2-mercaptoethanol, the latter conditions leading to dissociation of the two subunits of CCSP [5] and uteroglobin [6,12].

The immunological similarity between the two proteins was investigated by Western blotting of both whole pulmonary lavage and purified CCSP.

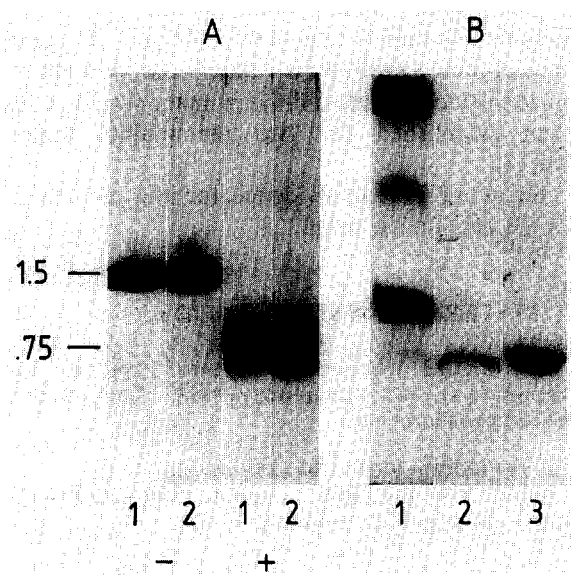

Fig.1. SDS gel electrophoresis (A) and immunoblotting (B) of CCSP pulmonary protein. (A) 10 μg of either purified CCSP (1) or uteroglobin (2) were analyzed on SDS-polyacrylamide gels in either the absence (−) or presence (+) of 2-mercaptoethanol. (B) 70 μg protein from rabbit lung lavage was analyzed by SDS gel electrophoresis and stained with Coomassie blue (1). An identical gel lane was transferred to nitrocellulose paper and probed with a specific anti-uteroglobin antibody (2). 6 μg purified CCSP run on a parallel gel lane were also transferred to nitrocellulose and immunoprobed with the anti-uteroglobin antibody (3). The M_r values for the dimer and monomer of uteroglobin are given to the left. ($\times 10^{-3}$).

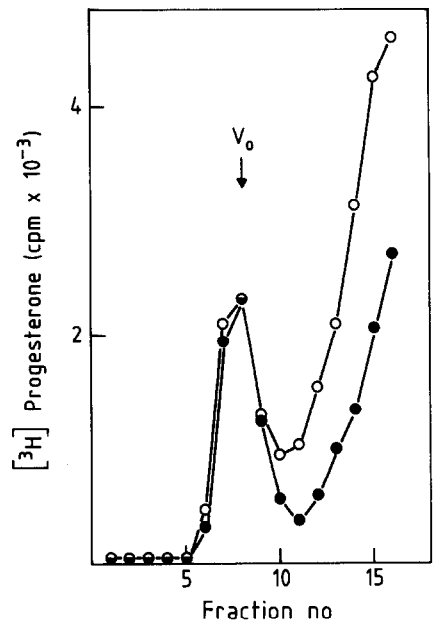

Fig.2. Comparison of the progesterone-binding abilities of CCSP and uteroglobin. Either protein (10 μg/ml) was reduced with 10 mM dithiothreitol and then incubated with [^{3}H]progesterone. Protein-bound steroid was separated by rapid chromatography on Sephadex G-25 mini-columns. (○) Uteroglobin, (●) CCSP. V_0, void volume.

When the blot was probed with a specific antibody against uteroglobin, CCSP was shown to react specifically with the antibody (fig.1B).

A striking property exhibited by reduced uteroglobin is the ability to bind progesterone with relatively high affinity [7,14]. Therefore, we determined whether CCSP also displayed this property. The results in fig.2 demonstrate that CCSP bound progesterone to the same extent as that of uteroglobin.

4. DISCUSSION

Our results demonstrate that the protein CCSP secreted by pulmonary Clara cells is uteroglobin. This identification appears of importance, since the characterization of a specific protein from Clara cells can help in gaining an understanding of their hitherto unknown physiological role. Besides the present data other properties described for CCSP are shared by uteroglobin. Thus, the amino acid sequence of the N-terminus of CCSP indicated Gly-Ile-X-Pro-Arg-Phe-Ala- [5], the same

as that for uteroglobin (where X is Cys) [7,10]. Also, the amino acid composition of CCSP was very similar to that of uteroglobin [12], including the lack of tryptophan. CCSP was found to have an M_r value of 15000 in gel filtration experiments and of 12400 from SDS-gel electrophoresis determinations. These values are exactly those determined for uteroglobin by use of the same techniques [12]. Upon gel filtration chromatography under reducing conditions, CCSP still behaved as a dimer [5], a property similar to that described for reduced and carboxymethylated uteroglobin, whose subunits are still bound by non-covalent forces [12].

Moreover, CCSP has been reported to be a proteinase inhibitor [5] in the same way that some studies have assigned this property to uteroglobin [16].

Despite characterization of the uteroglobin gene and its hormonal regulation, the physiological role of this protein remains obscure. The identification of anti-protease CCSP as uteroglobin should stimulate more efforts aimed at the study of this interesting protein.

Proteinase inhibitor polypeptides of low M_r have been described in secretions of the genital tract and bronchial tree of several species [17]. Since uteroglobin is secreted in the same organs of the rabbit, it is possible that this protein could be involved in similar functions.

Acknowledgements: This work was supported by grants from the CAICYT and FIS.

REFERENCES

[1] Van Scott, M.R., Hester, S. and Boucher, R.C. (1987) Proc. Natl. Acad. Sci. USA 84, 5496–5500.
[2] Devereux, T.R. and Fortis, J.R. (1980) In Vitro 16, 958–968.
[3] Massaro, G.D., Fischman, C.M., Chiang, M.J., Amado, C. and Massaro, D. (1981) J. Clin. Invest. 67, 345–351.
[4] Singh, G. and Katyal, S.L. (1984) J. Histochem. Cytochem. 32, 49–54.
[5] Gupta, R.P., Patton, S.E., Jetten, A.M. and Hook, G.E.R. (1987) Biochem. J. 248, 337–344.
[6] Beato, M., Arnemann, J., Menne, C., Müller, H., Suske, G. and Wenz, M. (1983) in: Regulation of Gene Expression by Hormones (McKerns, K.W. ed.) pp.151–175, Plenum, New York.
[7] Westphal, U. (1986) in: Steroid-Protein Interactions, II (Gross, F. et al. eds) pp.357–386, Springer, Berlin.
[8] Fernández-Renau, D., Lombardero, M. and Nieto, A. (1984) Eur. J. Biochem. 144, 523–527.
[9] López de Haro, M.S. and Nieto, A. (1985) Biochem. J. 225, 255–258.
[10] López de Haro, M.S. and Nieto, A. (1985) FEBS Lett. 193, 247–249.
[11] López de Haro, M.S. and Nieto, A. (1986) Biochem. J. 235, 895–898.
[12] Nieto, A., Ponstingl, H. and Beato, M. (1977) Arch. Biochem. Biophys. 180, 82–92.
[13] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[14] Beato, M. (1976) J. Steroid Biochem. 7, 327–334.
[15] López de Haro, M.S. and Nieto, A. (1983) Arch. Biochem. Biophys. 226, 539–547.
[16] Beier, H.M., Mootz, U. and Fischer, B. (1980) in: Steroid Induced Uterine Proteins (Beato, M. ed.) pp.47–67, Elsevier/North-Holland, Amsterdam, New York.
[17] Grütter, M.G., Fendrich, G., Huber, R. and Bode, W. (1988) EMBO J. 7, 345–351.