Primary structure of rabbit lung uteroglobin as deduced from the nucleotide sequence of a cDNA

Maria S. López de Haro and Antonio Nieto*

Centro de Biología Molecular (CSIC-UAM) Universidad Autónoma, Canto Blanco, 28049 Madrid, Spain

Received 8 October 1985

Double-stranded cDNA was synthesized from partially purified uteroglobin mRNA from rabbit lung. A cDNA coding for lung uteroglobin was then cloned in the plasmid pUC18 and both the nucleotide sequence and the derived amino acid sequence were determined. This allowed us to demonstrate unequivocally that uteroglobins from lung and uterus are identical proteins.

Uteroglobin *Steroid-induced protein* *(Lung)* *Amino acid sequence* *cDNA*

1. INTRODUCTION

The hormonal regulation of the synthesis of the protein uteroglobin in the rabbit endometrium is being currently studied as a suitable model for the mechanism of progesterone action [1]. Besides the endometrium, uteroglobin is also synthesized in important amounts in the lung, although in this organ the synthesis of the protein is under the control of glucocorticoids [2,3].

The function of uteroglobin remains to be established, although in the uterus it has been related, in some way, to the facilitation of blastocyst implantation [4,5]. Nevertheless, the presence of uteroglobin in lung suggests that this protein may have other functions. Although several types of indirect evidence suggest that uteroglobins from lung and uterus are identical [6,7], a demonstration is so far lacking. Therefore, it appears important to establish whether both uteroglobins are the same protein. Here, we report the amino acid sequence of rabbit lung uteroglobin, as deduced from the nucleotide sequence of a cloned cDNA, unequivocally demonstrating that it is identical to the uterine protein.

* To whom correspondence should be addressed

2. MATERIALS AND METHODS

Enzymes were obtained either from Bio-Labs, USA or from Boehringer Mannheim. Nitrocellulose filters were from Schleicher and Schuell. Low-gelling-temperature agarose (type VII) was from Sigma, USA. [α-^{32}P]- and [γ-^{32}P]dATP were obtained from the Radiochemical Centre, Amersham.

2.1. *Isolation of RNA*

Poly(A)-containing RNA was obtained from rabbit lung as described [8]. The RNA was centrifuged through 5–20% sucrose gradients [9] and the fractions assayed for uteroglobin mRNA content by dot-blot hybridization to labelled pUG10 DNA, a uterine cDNA probe [2]. The fractions containing uteroglobin mRNA were pooled and precipitated with ethanol.

2.2. *Synthesis and cloning of cDNA*

Double-stranded cDNA was synthesized as described [10] and cloned at the *Pst*I site of pBR322 by dG/dC tailing. Recombinants were screened with the labelled pUG10 probe by colony hybridization [11]. The inserts containing uteroglobin sequences were obtained after *Pst*I digestion of plasmid DNA and electrophoresis on

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

low-melting agarose [12]. The recovered uteroglobin cDNA was then subcloned at the *Pst*I site of pUC18.

2.3. *DNA sequencing*

Plasmid DNA was digested with the appropriate restriction enzyme (see section 3) and labelled at the 5′-ends with polynucleotide kinase [13]. After secondary digestion, the labelled fragments were isolated by agarose gel electrophoresis as described above and sequenced according to Maxam and Gilbert [14]. Both the sense and antisense strands were sequenced and each strand was read at least twice.

3. RESULTS AND DISCUSSION

Double-stranded cDNA synthesized from lung poly(A)$^+$ RNA enriched in uteroglobin sequences was inserted into the *Pst*I site of pBR322 and bacterial transformants were screened by hybridization with the pUG10 probe. Five, out of 1300 transformants, gave positive signals. Taking into account that uteroglobin mRNA accounts for about 0.1% of the lung mRNA population [15], this number of transformants would be expected from our enriched fraction of mRNA. After analysis of the size of the inserted cDNAs, a clone, designated pUG119 and which contained an insert of about 300 bp, was selected for further studies. To facilitate sequencing, this insert was subcloned into the *Pst*I site of the pUC18. The sequence of the clone was then obtained after labelling at the 5′-ends of either the *Bam*HI or *Hin*dIII sites of the pUC18, secondary cleavage with *Hin*dIII or *Bam*HI, respectively (fig.1), and isolation of the labelled fragments.

The nucleotide sequence of clone pUG119 and the derived amino acid sequence are shown in fig.1. The 5′-end begins just at the codon corresponding to glycine, the N-terminal amino acid of lung uteroglobin [6]. This should be expected as the uteroglobin gene contains an internal *Pst*I recognition sequence at this site [16] and we subcloned the cDNA after digestion with this enzyme. The clone includes both the sequence coding for the mature protein and part of the 3′-untranslated tract of the uteroglobin mRNA. A comparison of this sequence with those previously reported for uterine uteroglobin cDNA and the

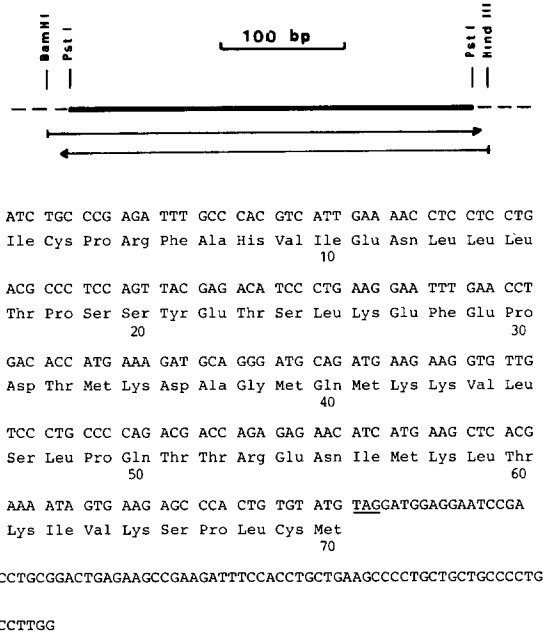

Fig.1. Schematic structure, nucleotide sequence and derived amino acid sequence of clone pUG119. Dashed lines indicate pUC18 DNA. Only the restriction sites used in subcloning or end-labelling are shown. Arrows indicate the sequence strategy. The codons and their corresponding amino acids are numbered starting with the N-terminal glycine. The termination codon is underlined.

chromosomal gene [16–18] indicates complete homology both in the coding and the untranslated regions. Previous studies have reported evidence that uteroglobins from lung and uterus might be identical. Such evidence, coming from immunological studies [7], amino acid analysis [6] and so on, is indirect and not conclusive. On the other hand, the fact that the uteroglobin gene appears to be present as a single copy does not exclude the possibility of differential expression in diverse tissues. Thus, different fibronectins with slightly different properties are expressed in liver and fibroblasts by alternative splicing of the primary transcript of the unique fibronectin gene [19]. It is conceivable that uteroglobin might have different functions in lung and uterus taking into account, among other considerations, its different hormonal regulation and different temporal expression. Then, it could be possible that slight differences exist between both uteroglobins. The

results reported here unequivocally demonstrate that lung and uterine uteroglobins are identical proteins encoded by the same gene.

ACKNOWLEDGEMENTS

This work was supported by grants from Comisión Asesora para el Desarrollo de la Investigación Científica y Técnica and Fondo de Investigaciones Sanitarias (FIS). M.S.L. de H. is a fellow of FIS.

REFERENCES

[1] 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.
[2] Fernandez Renau, D., Lombardero, M. and Nieto, A. (1984) Eur. J. Biochem. 144, 523–527.
[3] Lopez de Haro, M.S. and Nieto, A. (1985) Biochem. J. 225, 255–258.
[4] Krishnan, R.S. and Daniel, J.C. jr (1967) Science 158, 490–492.
[5] Mukherjee, D.C., Agrawal, A.K., Manjunath, R. and Mukherjee, A.B. (1983) Science 219, 989–991.
[6] Torkkeli, T., Krusius, T. and Jänne, O. (1978) Biochim. Biophys. Acta 544, 578–592.
[7] Beato, M. and Beier, H.M. (1978) J. Reprod. Fertil. 53, 305–314.
[8] Lombardero, M. and Nieto, A. (1981) Biochem. J. 200, 487–494.
[9] Arnemann, J., Heins, B. and Beato, M. (1977) Nucleic Acids Res. 4, 4023–4036.
[10] Wickens, M.P., Buell, G.N. and Schimke, R.T. (1978) J. Biol. Chem. 253, 2483–2495.
[11] Grunstein, M. and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. USA 72, 3961–3965.
[12] Langridge, J., Langridge, P. and Berquist, P.L. (1980) Anal. Biochem. 103, 264–271.
[13] Maniatis, T. (1982) Molecular Cloning, pp.122–123, Cold Spring Harbor, NY.
[14] Maxam, A. and Gilbert, W. (1980) Methods Enzymol. 65, 499–560.
[15] Savouret, J.F., Loosfelt, H., Atger, M. and Milgrom, E. (1980) J. Biol. Chem. 255, 4131–4136.
[16] Suske, G., Wenz, M., Cato, A.C.B. and Beato, M. (1983) Nucleic Acids Res. 11, 2257–2271.
[17] Chandra, T., Bullock, D.W. and Woo, S.L.C. (1981) DNA 1, 19–26.
[18] Bailly, A., Atger, M., Atger, P., Cerbon, M., Alizon, M., Vu Hai, M.T., Logeat, F. and Milgrom, E. (1983) J. Biol. Chem. 258, 10384–10389.
[19] Kornblihtt, A.R., Umezawa, K., Vibe-Pedersen, K. and Baralle, F.E. (1985) EMBO J. 4, 1755–1759.