The effect of a ribonuclease inhibitor from human placenta on the in vitro synthesis of human placental proteins

W. Hiranyavasit and T. Kusamran*

Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

Received 29 September 1982; revision received 13 December 1982

Addition of a ribonuclease inhibitor (10 μg/ml) from human placenta caused 2–3-fold increase of [^{3}H]leucine incorporation in the wheat germ extract as directed by human placental poly (A)-mRNA. Analysis of the translated products by sodiumdodecylsulfate/polyacrylamide gel electrophoresis/fluorography revealed that the inhibitor preferentially increased the yields of the larger proteins, particularly those of larger than M_r 40 000. In the presence of the inhibitor, yields of two placental proteins (human placental lactogen and human chorionic gonadotropin) were increased about 70–80% as detected by immunoprecipitation with specific homologous antisera. The method provided an improvement of translation system for studying biosynthesis of other human placental proteins.

Human placental RNA *Placental ribonuclease inhibitor* *hPL* *hCG* *In vitro translation* *Wheat germ*

1. INTRODUCTION

The in vitro translation of mRNA from human placenta has been investigated in many laboratories especially in relation to the biosynthesis of two major peptide hormones secreted by the placenta, i.e., human placental lactogen (hPL) and human chorionic gonadotropin (hCG) [1–4]. The synthesis of the hormones in the wheat germ cell-free system coded by their corresponding mRNAs has frequently been taken as a measure of the biosynthetic capacity of the placental tissue. However, the presence of ribonuclease both in the wheat germ system itself [5] and more especially in the human placental extract [6] might well jeopardise the interpretation of the results.

Recently, a ribonuclease inhibitor isolated from human placenta has been shown to increase the translational activity of dog pancreas mRNA in the wheat germ extract [5]. Here, the effect of the inhibitor on the translation of mRNA from early and full term human placentas was investigated. The in vitro translational efficiency of the placental mRNA was markedly enhanced by the presence of the inhibitor.

* To whom correspondence should be addressed

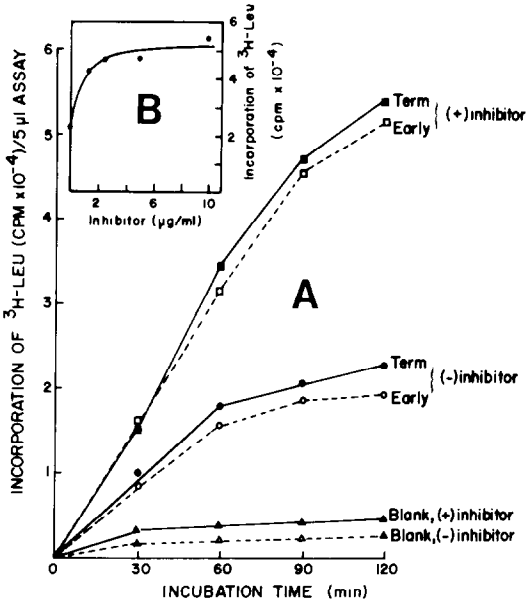
2. MATERIALS AND METHODS

Total RNA from human early (10–12 weeks) and full term placentas was prepared essentially as in [4]. The tissue (15 g) was homogenized in the buffer containing 5 M guanidine hydrochloride to minimize the ribonuclease activity. The poly(A)-mRNA was separated from the phenol-extracted total RNA by oligo(dT)-cellulose chromatography [7] and concentrated by ethanol precipitation. The mRNA was pelleted by ultracentrifugation, dried and kept at −70°C.

The mRNA was translated in the S_{30} wheat germ extract prepared as in [8]. The reaction mixture (100 μl) including 20 μCi of [^{3}H]leucine (110 Ci/mmol, New England Nuclear), 1.5 mM magnesium acetate, 25 mM potassium acetate and 800 μM spermine was incubated at 22°C. Placental RNase inhibitor was added to a final concentration of 10 μg/ml, unless otherwise indicated. Incorporation of radioactivity into total protein was measured in 5-μl aliquots as in [9].

Published by Elsevier Biomedical Press
00145793/83/0000—0000/$3.00

The total synthesized products were analyzed by SDS–polyacrylamide gel electrophoresis on 10–15% slab gels [10]. The radioactivity following the electrophoretic separation was visualized by the fluorographic method on Kodak RP-X-Omat film [11].



The synthesized hPL and hCG were sequentially immunoprecipitated from the incubation mixture with specific homologous rabbit antisera (Dako, Denmark) and formaldehyde-fixed *Staphylococcus aureus* [12]. To minimize non-specific immunoprecipitation, the incubation mixture was first treated with non-immune serum and the *S.*

Fig.1. (A) Effect of placental ribonuclease inhibitor on [^{3}H]leucine incorporation in a wheat germ extract coded by human placental mRNA. Poly(A)-containing RNA isolated from early (○,□) or term (●,■) placentas was translated in the cell-free system in the presence (10 µg/ml: □,■), or absence (○,●) of the inhibitor. About equal OD_{260} of early and term placental mRNA was incubated at 22°C. Duplicate aliquots (5 µl) were removed at the time intervals and the radioactivity determined on a Whatman filter paper. The blanks (△,▲) were similarly incubated, but without placental mRNA added; (B) Optimum stimulatory effect of the ribonuclease inhibitor on [^{3}H]leucine incorporation in wheat germ system programmed by term placental mRNA. 15 µl of reaction mixture contained equal amount of mRNA as in (A) and various concentrations of the inhibitor. After 120 min of incubation at 22°C, 5-µl duplicate aliquots were removed and processed as in (A).

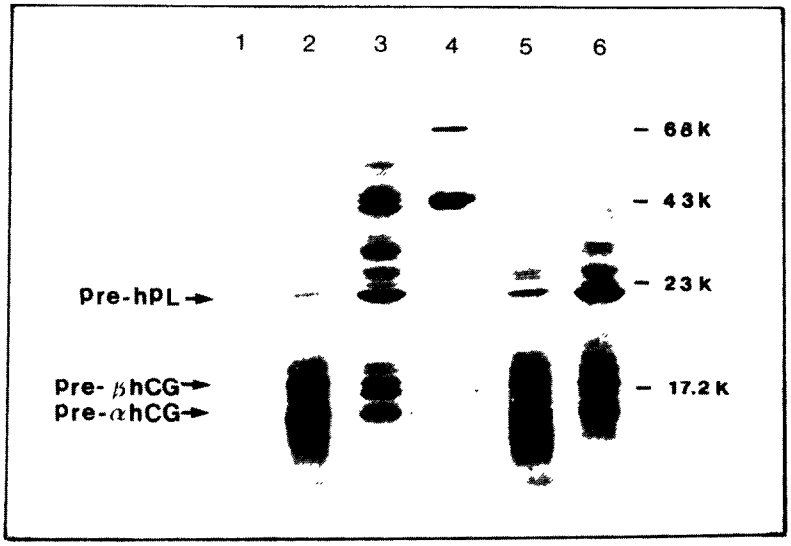


Fig.2. Fluorograph of the in vitro proteins synthesized in wheat germ programmed by early and term placental mRNA. Following the incubation, the reaction mixtures in fig.1 which contained: (1) no placental mRNA, no inhibitor; (2) early mRNA, no inhibitor; (3) early mRNA and inhibitor; (5) term mRNA, no inhibitor; (6) term mRNA and inhibitor; were analyzed on SDS–polyacrylamide slab gel. (4) is ^{14}C-labelled M_r markers, bovine serum albumin (68000) ovalbumin (43000), L-chain γ-globulin (23000) and myoglobin (17200). Arrows indicate the positions of pre-hPL (M_r ~22000), pre-βhCG (M_r ~18000) and pre-αhCG (M_r ~14000).

aureus. Each of the protein hormones was immunoprecipitated twice, dissolved in buffer containing sodium dodecylsulfate and the radioactivity measured on Whatman filter paper in trichloroacetic acid [9].

3. RESULTS

The incorporation of [^{3}H]leucine into protein synthesized in vitro by the wheat germ extract as directed by human placental poly(A)-mRNA from early and full term placentas was similar and almost linear throughout the first 60 min of incubation after which it approached a plateau (fig.1A). Addition of placental ribonuclease inhibitor (10 μg/ml) both prolonged the rate of the incorporation and increased the extent of protein synthesis (fig.1A). In general, a 2–3-fold increase was observed at 120 min and the stimulation was even more at longer periods of incubation. Maximum stimulation was obtained at 10 μg/ml of the inhibitor in the incubation mixture (fig.1B).

Analysis of the total synthesized products using sodium dodecylsulfate polyacrylamide gel electrophoresis followed by fluorography revealed the increased intensity of the protein bands in the presence of the ribonuclease inhibitor, particularly those of larger M_r (fig.2 compare channels 2 and 3, 5 and 6). Neither of the placental mRNA preparations synthesized protein larger than 40000 M_r unless the inhibitor was added. The relative amount of pre-hPL synthesized ($M_r \simeq 22000$) was higher in term placental mRNA than in the early one both in the absence (channels 2 and 5) and the presence of inhibitor (channels 3 and 6). The pre-hCG was normally synthesized as two separate non-identical subunits, pre-βhCG ($M_r \simeq 18000$) and pre-αhCG ($M_r \simeq 14000$), which appeared to be present in all gels but their proportions were not clearly identified.

In order to quantitate the amount of synthesized pre-hPL and pre-hCG, the proteins from the same reaction mixture used for fig.1A and 2 were immunoprecipitated by homologous antisera. Radioactive incorporation into pre-hPL from both early and term placental mRNA was increased by the inhibitor 78% and 85%, respectively (table 1). A similar stimulatory effect of 73% and 81% by the inhibitor was observed for the pre-hCG immunoprecipitated with rabbit anti-intact hCG (table 1). Since the synthesis of total protein was also increased by the same magnitude, the relative proportions of the pre-hPL and pre-hCG in total synthesized products were similar in the presence and absence of the nuclease inhibitor (table 1).

4. DISCUSSION

The presence of the placental ribonuclease inhibitor led to an almost 3-fold increase of total

Table 1

Effect of placental ribonuclease inhibitor on the in vitro synthesis of total proteins, pre-hPL and pre-hCG, by mRNA from early and full term placentas

	[^{3}H]Leu incorporation (cpm)		% Increase
	(−)inhibitor	(+)inhibitor	
Early placental mRNA:			
Total protein	113960	192670	69
Pre-hPL	15630	27850	78
Pre-hCG	2140	3719	73
Term placental mRNA:			
Total protein	11660	209850	81
Pre-hPL	20400	35900	75
Pre-hCG	915	1660	81

Aliquots containing about the same amount of the radioactivity were removed from the incubation mixture used in fig.1A. Pre-hPL and pre-hCG were sequentially immunoprecipitated from the same aliquot. The values of the radioactive incorporation represented the average of duplicates. Increase synthesis by the inhibitor was calculated as % increase

protein synthesized by placental mRNA after 120 min of incubation (fig.1A). Scheele reported only a 40% stimulation by the inhibitor of total protein synthesized in vitro by dog pancreas mRNA [5]. Greater stimulation by the inhibitor here may be due to a relatively higher contamination of ribonuclease activity in the placental mRNA preparations. This is supported by the absence of high M_r protein synthesized when the inhibitor was not added (fig.2). The wheat germ translation of both early and term human placental mRNA without the inhibitor has also been demonstrated by other investigators who obtained proteins of M_r lower than 30000 [13].

It is interesting that although the inhibition resulted in a 70–80% increase in total protein synthesis the proportion of pre-hPL and pre-hCG was not significantly affected (table 1). In that these are both relatively low-M_r proteins the results do not conflict with the fact that the inhibitor has a greater effect on the synthesis of high-M_r proteins [5]. In vitro synthesis of dog pancreas preamylase ($M_r \simeq 55000$) is increased more than 10-fold by the inhibitor, whereas only a 20% increase is observed for serine protease ($M_r \simeq 26000$) synthesis [5].

Human placenta has been suggested as the site of origin of many pregnancy specific proteins [see review 14]. The improved translational system in the present studies would provide a better way of studying the biosynthesis of other human placental proteins, in addition to hPL and hCG, such as PP5 ($M_r \simeq 36000$), SP_1-glycoprotein ($M_r \simeq 90000$) and PAPP-A ($M_r \simeq 750000$) [15].

ACKNOWLEDGEMENTS

We are grateful to Drs P. Blackburn and H.F. Lodish for kindly providing us the placental ribonuclease inhibitor and the wheat germ, respectively. The secretarial assistance of Ms U. Sajjaharutai is gratefully acknowledged. T.K. is a recipient of Grant RF-8031, The Rockefeller Foundation, New York. W.H. is supported by a fellowship from The University Bureau-UDC programme.

REFERENCES

[1] Daniels-McQueen, S., McWilliams, D., Birken, S., Canfield, R., Landefeld, T. and Boime, I. (1978) J. Biol. Chem. 253, 7109–7114.
[2] Chatterjee, M., Baliga, S. and Munro, H.N. (1976) J. Biol. Chem. 251, 2945–2951.
[3] Fiddes, J.C. and Goodman, H.M. (1979) Nature 281, 351–356.
[4] Boime, I., McWilliams, D., Szczesna, E. and Camel, M. (1976) J. Biol. Chem. 251, 820–825.
[5] Scheele, G. and Blackburn, P. (1979) Proc. Natl. Acad. Sci. USA 76, 4898–4902.
[6] Laga, E.M., Driscoll, S. and Munro, H.N. (1973) Biol. Neonate 23, 260–283.
[7] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
[8] Roberts, D.E. and Paterson, B.M. (1973) Proc. Natl. Acad. Sci. USA 70, 2330–2334.
[9] Mans, R.J. and Novelli, G.D. (1961) Arch. Biochem. Biophys. 94, 48–53.
[10] Laemmli, U.K. (1970) Nature 227, 680–685.
[11] Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83–88.
[12] Kessler, S.W. (1975) J. Immunol. 115, 1617–1624.
[13] Eichler, D.C., Tatar, T.F. and Lasater, L.S. (1981) Biochem. Biophys. Res. Commun. 101, 396–403.
[14] Mous, J., Peeters, B. and Rombouts, W. (1980) FEBS Lett. 122, 105–108.
[15] Klopper, A. (1980) Placenta 1, 77–89.