DETECTION OF A POSSIBLE PRECURSOR OF IMMUNOGLOBULIN LIGHT CHAIN IN MOPC 41A PLASMACYTOMA CELLS

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1. Introduction

A new aspect of immunoglobulin (Ig) synthesis has emerged from recent experiments employing Ig light chain messenger RNAs from mouse plasmacytomas in cell-free protein synthesizing systems. Ig light chain mRNAs from at least six different plasmacytomas, including those synthesizing either κ or λ2 light chains, direct the cell-free synthesis of a polypeptide variously estimated to be 740–4700 daltons larger than the corresponding light chain [1–6]. These larger cell-free products, designated here as P chains [1], possess extra amino acid residues preceding the normal amino-terminus of the light chain [1,4]. It seems unlikely that P chains are merely artifacts of cell-free translation since other eukaryotic mRNAs are faithfully translated by the same cell-free systems [7–9]. Furthermore, three different cell-free systems all synthesize a P chain of the same size in response to an individual κ or λ2 light chain mRNA ([6] and manuscript in preparation). It has been suggested that P chain is a precursor to Ig light chain [1–3], but no precursor–product relationship has yet been demonstrated nor has an intracellular precursor polypeptide been found.

We report here that MOPC 41A plasmacytoma cells in the presence of an inhibitor of proteolytic enzymes synthesize a polypeptide which appears to be equivalent to P chain made in cell-free systems.

2. Experimental

The protease inhibitors used (from Calbiochem) were TLCK (*N*-α-tosyl-L-lysyl chloromethane, hydrochloride), a trypsin inhibitor [10], TPCK (tosyl-L-phenylalanyl-chloromethane), a chymotrypsin inhibitor [10], and TAME (*p*-toluene sulphonyl-L-arginine methyl ester, hydrochloride) [11]. Stock solutions (in ethanol) were respectively 25 mg/ml, 5 mg/ml, and 44 mg/ml.

The murine plasmacytoma lines used in this study and their secreted immunoglobulins are (for ref. see [6]): MOPC 41A, light chain (κ); MOPC 315, IgA (λ2); P3K, IgG1 (κ); HPC-108, IgA (κ); and MPC 11, IgG2b (κ) [12]. MOPC 41A was maintained as a subcutaneous solid tumour in BALB/c mice; before each experiment a small tumour (1–2 g) was dispersed into a cell suspension, damaged cells were removed [13] and the cells cultured for about 20 hr at $4–6 \times 10^5$ per ml in DME (Dulbecco's modified Eagle's medium from GIBCO) supplemented with 20% heat-inactivated horse serum (Laboratory Services, Sydney). The other lines were maintained in cell culture in DME supplemented with 10% heat-inactivated foetal calf serum (Commonwealth Serum Laboratories, Melbourne).

Prior to labelling, cells were washed in methionine-free DME (C.S.L., Melbourne), resuspended at $2–10 \times 10^6$ per ml in the same medium, and dispensed into plastic tubes (0.9 ml/tube). Up to 20 μl protease inhibitor was added to appropriate tubes; the final concentrations tested were TLCK, 50–250 μg/ml; TPCK, 10–100 μg/ml; TAME, 875 μg/ml. After 2 min at 37°C, [^{35}S] methionine (Amersham 25-40 mCi/μmol) was added to a final concentration of 1.4–2.0 μM and the tubes were incubated for up to 30 min at 37°C. The tubes were then chilled rapidly and 0.5 ml crushed frozen buffer (150 mM NaCl, 1.5 mM $Mg(CH_3COO)_2$, 10 mM Tris–Cl, pH 7.2) was added to each. All subsequent procedures were at 0–4°C. The cells were

sedimented and resuspended at 4×10^6 per ml in lysis buffer (0.5% Nonidet P-40, 250 μg/ml TLCK, 50 μg/ml TPCK, 150 mM NaCl, 1.5 mM $Mg(CH_3COO)_2$, 10 mM Tris–Cl, pH 7.2); five min later nuclei were removed by sedimentation (1400 *g*, 3.5 min) and the post-nuclear supernatant was used for subsequent analyses.

Ig chains in post-nuclear supernatants (20–150 μl) were precipitated with antisera (40–150 μl); the final immunoprecipitation mixtures were adjusted to 500 μl with phosphate-buffered saline (120 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4) and contained 3% Triton X-100. After at least 3 hr at 2°C the immunoprecipitates were washed 2–3 times by centrifugation (8000 *g*, 20 min) in the same buffer, then dissolved in electrophoresis sample buffer [14]. The antisera used were prepared from rabbits immunized with purified HPC-108 κ chain (anti-κ serum) or with purified MOPC 315 IgA (λ2) (anti-IgA (λ2) serum).

Labelled polypeptides in post-nuclear supernatants or in immunoprecipitates were analyzed by electrophoresis on 15% polyacrylamide gels containing SDS [14] and the gels were stained, sliced, dried and autoradiographed for 1–4 weeks [15].

3. Results

In initial attempts to detect intracellular P chain, we labelled MOPC 41A cells with a pulse of [^{35}S] methionine (1–10 min) and used SDS gel electrophoresis [14] to analyze the size of newly synthesized polypeptides precipitable by an anti-κ serum. Although L chain was a very prominent product, we failed to detect any immunoprecipitable polypeptide the size of MOPC 41A P chain, which is 2000 daltons larger than the L chain ([6] and manuscript in preparation). This suggested that if intracellular P chain exists it must be very short lived.

Since any cellular mechanism for converting P chain to light chain would include at least one proteolytic event, the processing mechanism might be sensitive to inhibitors of proteolytic enzymes. Such agents have been used to reveal the existence of transient viral polypeptide precursors inside cells [16,17]. We therefore attempted to detect P chain in the plasmacytoma cells during a 30 min incubation with [^{35}S]methionine in the presence of three protease inhibitors.

The size of polypeptides synthesized by MOPC 41A cells in the presence or absence of the inhibitor TLCK can be seen in fig.1. Since MOPC 41A does not synthesize a heavy chain, the most prominent product in the post-nuclear supernatant from untreated cells is Ig light chain (L, gel 1), identified by its size, specific precipitation by anti-κ serum (L, gel 6), and comigration with secreted MOPC 41A κ chain. The untreated cells appeared to contain no immunoprecipitable polypeptide larger than L chain (gel 6), even after an autoradiographic exposure three times longer than that shown. The precise mobility expected for the P chain was determined by adding MOPC 41A P chain synthesized in a cell-free system [6] to other control samples prior to electrophoresis (P, gels 2 and 7). Cells labelled in the presence of 50, 125 or 250 μg/ml TLCK, however, yielded a new polypeptide (P, gels 3–5) which had exactly the same mobility as the reference MOPC 41A P chain and which was precipitable by anti-κ serum (gels 8–10). At the highest concentration of TLCK used, there was about twice as much of this polypeptide as there was light chain.

The new polypeptide was detected only when the inhibitor was present during the incorporation of [^{35}S] methionine; addition of TLCK at the time of cell lysis was not sufficient. The intracellular P chain has been detected in five experiments with TLCK, despite a substantial depression of total protein synthesis caused by the inhibitor (see also [17]. TLCK was the only inhibitor tested which consistently revealed the new polypeptide in MOPC 41A cells. With TAME, it was not detected; with TPCK the new polypeptide was found in one experiment but not in a second. In some experiments with TLCK, a faint band between P chain and L chain was seen (see fig.1 gel 4) but whether this is due to extraneous proteolysis or to a processing mechanism involving more than one proteolytic event is not known.

We have not yet detected any immunoprecipitable polypeptide with the mobility of a P chain in cells of the four other plasmacytoma lines tested. No new polypeptides were observed in P3K, MPC 11, or HPC-108 cells labelled in the presence of any of the inhibitors. MOPC 315 cells during a 30 min incubation in the presence of TLCK, TPCK or TAME synthesized a new polypeptide, about 900 daltons larger than MOPC 315 Ig light chain, which appears to be precipitable with anti-IgA (λ2) serum. However, this

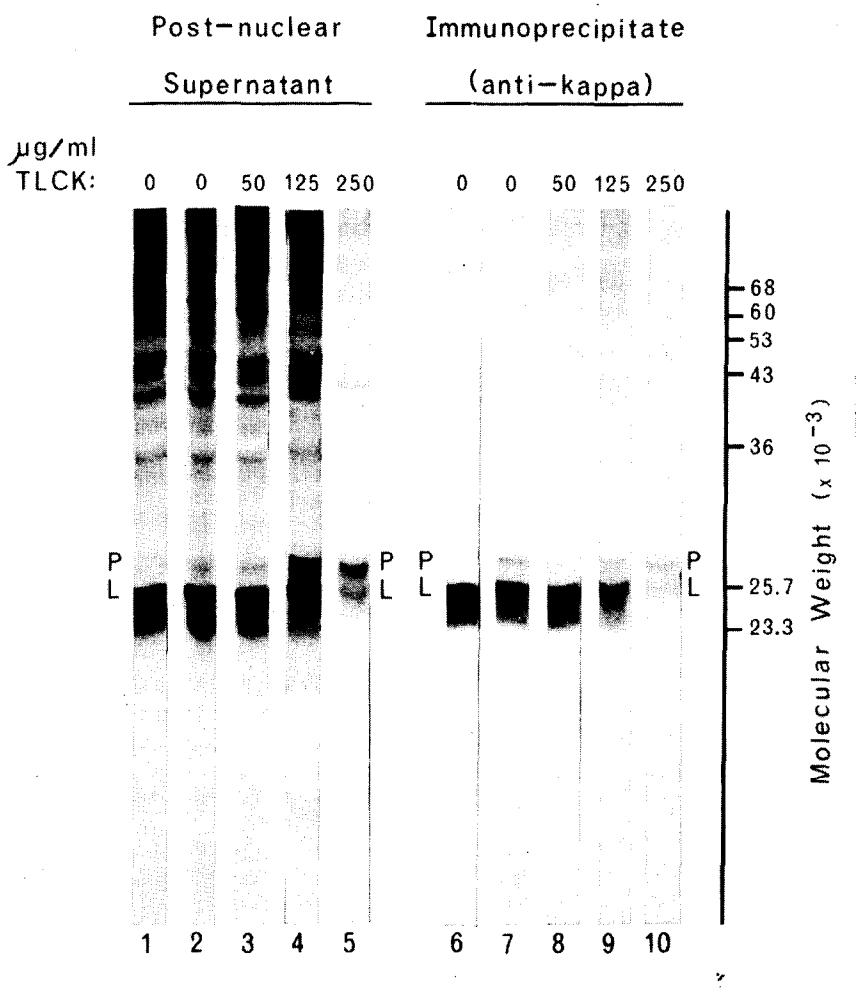

Fig.1. Electrophoretic analysis on SDS acrylamide gels of polypeptides synthesized by MOPC 41A cells in the presence or absence of TLCK. The cells were incubated for 30 min with [^{35}S]methionine and the indicated concentrations of TLCK. MOPC 41A P chain synthesized in a wheat germ cell-free system [6] was added to samples from untreated cells in gels 2 and 7. To compensate for the reduced total protein synthesis in the TLCK-treated cultures, up to twice as much post nuclear supernatant from treated cultures was used for analysis. The molecular weight scale is based upon the mobilities of a set of ^{125}I-labelled polypeptides of known mol. wt run on a parallel gel [6].

product was smaller than the MOPC 315 P chain, which has an apparent mol. wt of 2200 greater than MOPC 315 light chain ([6] and manuscript in preparation).

4. Discussion

We have shown here that MOPC 41A plasmacytoma cells in the presence of the proteolytic inhibitor TLCK synthesize a new polypeptide serologically related to Ig light chain. This polypeptide is exactly the same size as the MOPC 41A P chain synthesized in three different cell-free translating systems programmed with MOPC 41A light chain mRNA ([3,6] and manuscript in preparation). This demonstration of an intracellular P chain supports the argument that the polypeptide formed in the cell-free systems is not an artifact and considerably strengthens the case that P chain is a biosynthetic precursor to the light chain.

The effect of TLCK presumably is due to its ability to inhibit some trypsin-like processing activity, although we have no direct evidence for this. The absence of detectable P chain in pulse-labelled MOPC 41A cells not treated with TLCK may indicate that the extra amino acid residues in P chain are normally excised either before or very shortly after synthesis of the chain has been completed.

The lack of demonstrable intracellular P chain in four other plasmacytoma cell lines is somewhat surprising, although there are many possible explanations, such as differences between cell lines in inhibitor uptake, in non-specific proteolysis after lysis, or in sensitivity of processing enzymes to inhibition. Moreover, MOPC 41A may have been particularly favorable for these studies since 30% of the protein synthesized by the cells of this tumour line is Ig light chain compared to 2–4% in the four other cell lines (A. Harris, unpublished results).

Several other proteins are known to be synthesized as larger precursors, including collagen [18], rat serum albumin [19], and some hormones (eg. insulin and parathyroid hormone) [20]. The cell-free translation product of parathyroid hormone mRNA is even larger than the known prohormone and is apparently a very transient molecule [21], like Ig P chain. Perhaps all proteins made on membrane-bound polysomes are normally synthesized as larger polypeptides, which are then reduced in size during or after transport across the microsomal membrane. Recent experiments with HeLa cells grown in the presence of TPCK [22] suggest that transient larger precursors may occur during the biosynthesis of many proteins.

The function of the extra residues on Ig P chain is still obscure (see [1,23,24]). They could be involved in ensuring translation of Ig light chain mRNA on membrane-bound polysomes, in assembling intracellular Ig, or in transporting newly synthesized polypeptide across the microsomal membrane.

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References

[1] Milstein, C., Brownlee, G., Harrison, T. and Mathews, M. (1972) Nature New Biol. 239, 117–120.
[2] Swan, D., Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. 69, 1967–1971.
[3] Mach, B., Faust, C. and Vassalli, P. (1973) Proc. Natl. Acad. Sci. 70, 451–455.
[4] Schechter, I. (1973) Proc. Natl. Acad. Sci. 70, 2256–2260.
[5] Tonegawa, S. and Baldi, I. (1973) Biochem. Biophys. Res. Com. 51, 81–87.
[6] Schmeckpeper, B., Cory, S. and Adams, J. (1974) Molec. Biol. Reports 1, 355–363.
[7] Berns, A., Strous, G. and Bloemendal, H. (1972) Nature New Biol. 236, 7–9.
[8] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. 69, 1408–1412.
[9] Efron, D. and Marcus, A. (1973) FEBS Lett. 33, 23–27.
[10] Shaw, E. (1970) Physiol. Rev. 50, 244–296.
[11] Kassell, B. and Laskowski, M. (1956) J. Biol. Chem. 219, 203–210.
[12] Laskov, R. and Scharff, M. D. (1970) J. Exp. Med. 131, 515–541.
[13] von Boehmer, H. and Shortman, K. (1973) J. Immunol. Methods 2, 293–301.
[14] Laemmli, U. (1970) Nature 227, 680–685.
[15] Fairbanks, G., Levinthal, C. and Reeder, R. H. (1965) Biochem. Biophys. Res. Comm. 20, 393–399.
[16] Korant, B. (1972) J. Virol. 10, 751–759.
[17] Summers, D., Shaw, E., Stewart, M. and Maizel, J. (1972) J. Virol. 10, 880–884.
[18] Bornstein, P. (1974) Ann. Rev. Biochem. 43, 567–603.
[19] Urban, J., Inglis, A. S., Edwards, K. and Schreiber, G. (1974) Biochem. Biophys. Res. Comm. 61, 444–451.
[20] Tager, H. S. and Steiner, D. F. (1974) Ann. Rev. Biochem. 43, 509–538.
[21] Kemper, B., Habener, J. F., Mulligan, R. C., Potts, J. T. and Rich, A. (1974) Proc. Natl. Acad. Sci. 71, 3731–3735.
[22] Taber, R., Wertheimer, R. and Golrick, J. (1973) J. Molec. Biol. 80, 367–372.
[23] Harrison, T. M., Brownlee, G. G. and Milstein, C. (1974) Europ. J. Biochem. 47, 613–620.
[24] Baglioni, C. and Liberti, P. (1974) Molec. Biol. Reports 1, 329–335.