IMMUNOGLOBULIN A BIOSYNTHESIS. INTRACELLULAR ACCUMULATION OF 7 S SUBUNITS

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1. Introduction

Assembly of murine IgA* has been studied in short term cultures of the mouse plasmacytome MOPC 5647. As previously shown with murine IgM [1] and another mouse IgA myeloma MOPC 315 [2], only the 7 S, four-chain structure (H_2L_2) can be detected inside the cells, although mostly oligomeric forms are secreted.

2. Methods

The mouse myeloma MOPC 5647 (from the collection of Dr. M. Potter), is maintained in this laboratory by subcutaneous transfer in Balb/c mice. Cell suspensions were incubated with radioactive leucine and the labelled intracellular and secreted myeloma protein were precipitated with rabbit anti-(mouse IgA) (raised in rabbits against the K-type IgA myeloma protein of MOPC 47A) as previously described [1]. The cells were lysed with phosphate-buffered saline containing 5 mM $MgCl_2$, 1% (w/v) Nonidet P40 (Shell Chemicals, Ltd., London, W.C.2) and 0.1 M iodoacetamide. Radioactive immune precipitates were reduced by dissolving precipitates in 2% (w/v) sodium dodecyl sulphate containing 0.025 M dithiothreitol and 0.3 M tris-HCl, pH 8.0; iodoacetamide was added to 0.1 M after 1 hr.

For analysis, the immune precipitates of radioactive myeloma protein were dissolved in 2% (w/v) sodium dodecyl sulphate and electrophoresed on 4.25% (w/v) acrylamide gels containing 0.1% (w/v) sodium dodecyl sulphate and 0.5 M urea [4]. The acrylamide gels, which contained ethylene diacrylate as the cross-linking reagent, were sliced into 1 mm slices, dissolved and counted [4]. Fractions are numbered from the top to the bottom of the gel. Due to the presence of sodium dodecyl sulphate, non-covalently bonded polypeptide complexes are dissociated and the electrophoretic separation obtained is primarily a function of molecular size [5]. The various species resolved by acrylamide gel electrophoresis were identified by comparison with marker proteins prepared from MOPC 315 and previously identified by sucrose gradient centrifugation [2] and electron microscopy [6]. Other radioactive markers (IgMs, IgG, $\mu\lambda$, μ, γ and K) were prepared from mouse myeloma tumours MOPC 104E (IgM) and Adj PC5 (IgG2a) as previously described [1, 7] and run on parallel gels.

3. Results

The kinetics of secretion of immunoglobulin by the cells in culture was similar to that previously described for other myeloma tumours [1, 8]. After three hours of incubation with radioactive leucine, the acid insoluble radioactivity in the extracellular medium was about 70% precipitable with rabbit antiserum to mouse IgA. The secreted immunoglobulin contained four molecular forms of IgA: the 7 S monomer, its dimer, trimer and tetramer (fig. 1a). In this, as in most mouse IgA proteins [2, 9] disulphide bonds link the heavy chains, but heavy light disulphide bonds are absent. In consequence, under the dissociating conditions that prevail upon electrophoresis in sodium dodecyl sulphate, light chain dissociates from

* Terms recommended by the committee on nomenclature of human immunoglobulins, Bull. World Health Organization 30 (1964) 447.

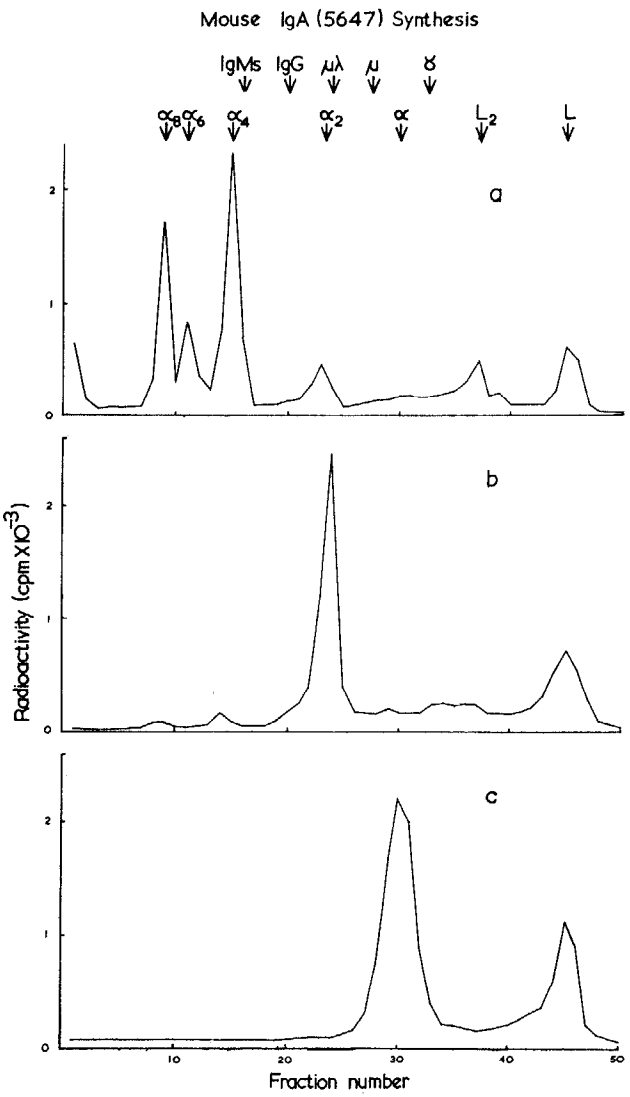

Fig. 1. Labelling of mouse myeloma 5647 tumour cells in vitro. Analysis by polyacrylamide gel electrophoresis. Cell suspensions were prepared at 2×10^7 cells/ml, incubated with 4,5-^{3}H-leucine (50 μCi/ml; 1 μCi/mmole) for 3½ hr and the intracellular and extracellular immunoglobulin determinants were co-precipitated with a specific rabbit anti-(mouse IgA) raised against the K-type mouse IgA myeloma MOPC 47A. Radioactive markers (IgMs, IgC, μλ, μ, γ, K) were prepared from mouse myoeloma tumours MOPC 104E (IgM) and Adj PC5 (IgG) as previously described [1, 7], and run on parallel gels.
a) Extracellular (secreted) IgA determinants;
b) intracellular IgA determinants;
c) extracellular (secreted) IgA determinants after reduction and alkylation.

heavy chain. The secreted IgA is therefore resolved into oligomeric forms of heavy chain (α_2, α_4, α_6, α_8), corresponding to monomer, dimer, trimer and tetramer, and light chain. The light chain associated with the secreted material is seen to consist of dimer as well as monomer. The position in the gels of single heavy and light chains was determined by reduction of the total secreted IgA with 0.025 M dithioerithritol (fig. 1c). The radioactivity ratio of total heavy to total light chain before and after reduction was the same, and thus MOPC 5647 represents another Balb/c IgA protein lacking the inter-heavy-light chain bond.

The pattern of polymerization is rather constant and characteristic for each IgA tumour [9, 10]. By comparison with MOPC 315 [2], the MOPC 5647 myeloma secretes relatively more trimer and tetramer and relatively less monomer. By contrast, the intracellular myeloma protein is similar in the two tumour lines, there being mostly monomer and only traces of oligomer even with labelling periods as long as 3½ hr (fig. 1b). The light chain exists largely in the monomeric form inside the cell, non-covalently associated with the heavy chain dimers. The complex sediments in the 7 S position on sucrose gradient centrifugation. Dimerization of the light chain can occur extracellularly [2] but there was no evidence for extracellular polymerization of heavy chains.

4. Discussion

It has previously been shown that the IgM molecule is formed by polymerization of 7 S subunits (IgMs) just before, or at the time of secretion [1]. The IgMs accumulates within the cell in the absence of detectable amounts of the fully assembled polymer. In contrast to the behaviour of artificially prepared subunits [11], isolated intracellular IgMs does not spontaneously polymerize; sulphydryl groups responsible for covalent bonds between the subunits appear to be blocked within the cell [12]. It now seems likely that a similar mechanism operates for IgA biosynthesis, and possibly for other secreted proteins composed of repeating subunits. Presumably the polymerization mechanism is controlled by unblocking of cysteine. The characteristic and stable degree of polymerization of IgA secreted by a given tumour [9, 10] may be related to the primary structure of the monomer form.

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