FUCOSE INCORPORATION INTO OOCYTE-SYNTHESIZED RAT IMMUNOGLOBULINS

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1. Introduction

The translation of exogenous mRNA in *Xenopus laevis* oocytes is accompanied by extensive post-translational modification of synthesised polypeptides [1]. Synthesis of immunoglobulin heavy and light chains in microinjected oocytes is followed by their assembly into 7 S H_2L_2 molecules [2] such that specific antibody activity can be demonstrated [3].

In order to study further the post-translational modifications of oocyte-synthesised polypeptides the incorporation and distribution of L-fucose into immunoglobulins was determined.

2. Methods

RNA extraction was carried out using a modification of the phenol–alcohol method [2]. Poly(A) containing mRNA from the spleens of adult Sprague Dawley rats was purified on cellulose [4], dissolved in injection medium [5] and microinjected into batches of 35 oocytes. Each oocyte received 100 nl of a solution of RNA (0.5–1.0 mg/ml) and either [^{35}S]methionine (Radiochemical Centre, Amersham, Bucks, England; spec. act. 565 mCi/mmol) or L-[6-^{3}H]fucose (New England Nuclear; spec. act. 12.5 Ci/mmol). After incubation in L 15 medium [6] at 20°C for 24 h rat immunoglobulins in the supernatant of homogenised oocytes were immunoprecipitated using a rabbit anti-rat immunoglobulin antiserum followed by excess goat anti-rabbit immunoglobulin antiserum. Twice washed precipitates were alkylated or reduced and analysed by SDS–polyacrylamide gel electrophoresis on 5% gels [7] and cut into 2 mm slices.

Incorporation of methionine and fucose into immunoglobulins was determined in cultures of 1.5×10^6 rat spleen cells/ml in RPM 1640 (Gibco Bio-cult) supplemented with 10% v/v autologous rat serum. After 2 h at 37°C in the presence of 10 μCi/ml [^{35}S]methionine or 6 μCi/ml L-[6-^{3}H]fucose, washed cells were lysed with 1% v/v Synperonic NP9 (ICI) in PBS and rat immunoglobulins precipitated and analysed as before.

3. Results

Microinjection of oocytes with rat spleen mRNA resulted in the synthesis of immunoprecipitable rat immunoglobulins, amounting to 17% of total protein synthesis; on analysis by SDS–polyacrylamide gel electrophoresis (fig.1) we showed the presence of assembled 7 S H_2L_2 molecules as well as free heavy and light chains. Similarly, analysis of spleen cell synthesised immunoglobulins revealed the presence

Abbreviations: H_2L_2, Immunoglobulin molecules comprising 2 heavy and 2 light chains; PBS, Phosphate buffered saline, pH 7.2

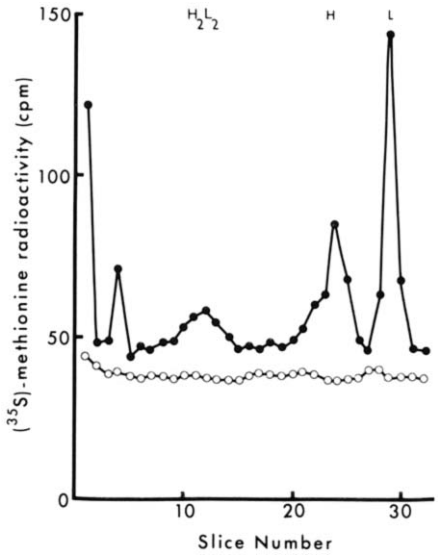

Fig.1. Translation of mRNA from rat spleens in *Xenopus laevis* oocytes. Oocytes (35) were injected with rat spleen mRNA and [^{35}S]methionine, as described in the Methods. Following incubation oocytes were homogenised, rat immunoglobulins precipitated and analysed by SDS–polyacrylamide gel electrophoresis. Marker proteins consisting of H_2L_2, H (heavy) and L (light) chains were run in parallel gells. Closed symbols: mRNA injected oocytes. Open symbols: control oocytes receiving no mRNA.

of 7 S μ- and γ-H_2L_2 together with free μ- and γ-heavy chains and -light chains (fig.2). Spleen cell immunoglobulin synthesis represented approximately 10% of total protein synthesised.

Immunoprecipitates of rat immunoglobulins synthesised in oocytes in the presence of L-[6-^{3}H]fucose were analysed by SDS–polyacrylamide gel electrophoresis of alkylated and reduced samples (fig.3). It

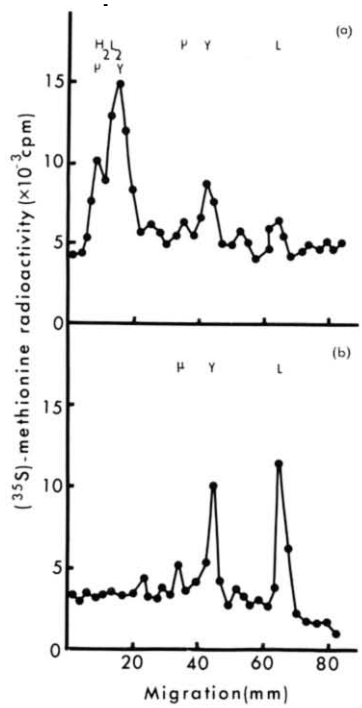

Fig.2

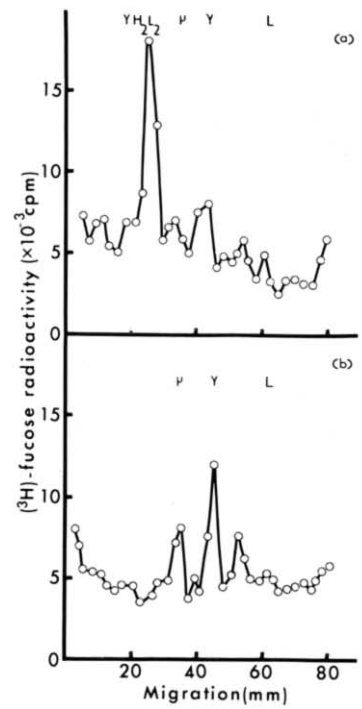

Fig.3

Fig.2. Spleen cell distribution of [^{35}S]methionine labelled immunoglobulins. Immunoprecipitated immunoglobulins from spleen cells cultured in the presence of [^{35}S]methionine (10 μCi/ml) were (a) alkylated or (b) reduced and analysed by SDS–polyacrylamide gel electrophoresis. Marker proteins run in parallel gels were: μ-H_2L_2, γ-H_2L_2, μ- and γ-heavy chains and -light chains.

Fig.3. Distribution of [^{3}H]fucose labelled rat immunoglobulins synthesised in oocytes. Material immunoprecipitated from oocytes was (a) alkylated or (b) reduced and analysed by SDS–polyacrylamide gel electrophoresis. Marker proteins are as described in fig.2.

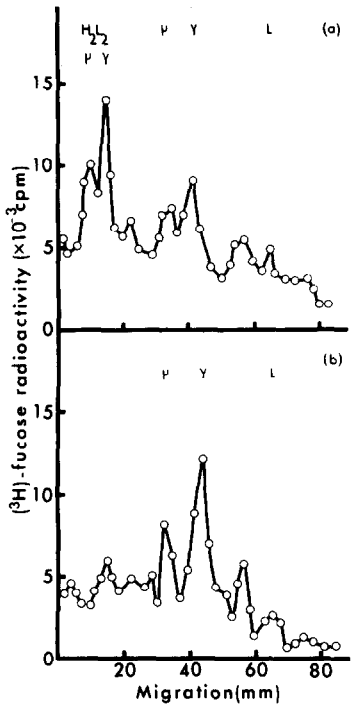

Fig.4. Spleen cell distribution of [^{3}H]fucose labelled immunoglobulins. Immunoprecipitates from spleen cells cultured with L-[6-^{3}H]fucose (6 μCi/ml) were (a) alkylated or (b) reduced and analysed by SDS–polyacrylamide gel electrophoresis. Marker proteins are as described in fig.2.

can be seen that in the oocyte fucose is incorporated into immunoglobulin. The major peak of fucose radioactivity in the alkylated sample (fig.3a) corresponds to assembled H_2L_2 molecules. Analysis of material reduced with 2-mercaptoethanol (fig.3b) indicated the presence of fucose predominantly associated with μ- and γ-heavy chain types. Incorporation of fucose into light chains was not detected.

Electrophoretic analysis of immunoglobulins labelled by spleen cells in vitro with L-[6-^{3}H]fucose (fig.4) indicates the presence of fucose in 7 S IgC and IgM molecules as well as in free μ- and γ-heavy chains. The distribution of fucose in reduced in vitro synthesised immunoglobulins (fig.4b) indicates a restriction of fucose to heavy chain types only.

A peak of fucose containing material, migrating between γ- and light chains, equivalent to approximately 30 000 mol. wt, was observed in both spleen cell and oocyte-synthesised immunoprecipitated material. This appears to have properties similar to the immunoglobulin binding protein previously reported [8].

4. Discussion

In order to determine the extent of post translational modification of immunoglobulins synthesised in *Xenopus* oocytes the incorporation of carbohydrate was studied. In myeloma cells the addition of carbohydrate to immunoglobulins is a stepwise process; mannose and glucosamine are added early, while galactose and fucose are added late in the passage of immunoglobulin molecules through the endoplasmic reticulum-golgi system [9]. Therefore, fucose incorporation into immunoglobulins represents a late modification to the newly translated molecule.

Rat immunoglobulins synthesised in *Xenopus* oocytes were found to incorporate L-[6-^{3}H]fucose radioactivity. Because of the low rate of conversion of L-fucose to other sugars [9] it may be assumed that the bulk of radioactivity is incorporated as fucose. The distribution of fucose in oocyte-synthesised immunoglobulins was similar to that found in cultured spleen cells in that the presence of label was restricted to the heavy chains. Similar restriction of fucose to heavy chains was reported for MOPC 21 [10] and MOPC 104E [9] immunoglobulins. The presence of fucose in oocyte-synthesised immunoglobulins does not necessarily mean that entire oligosaccharide sequences have been correctly synthesised in a manner similar to normal immunoglobulin carbohydrate addition. However, the restriction of fucose to heavy chains in oocytes parallels the distribution of fucose in spleen cell-synthesized immunoglobulins indicating extensive and faithful post-translational modification of proteins synthesised in response to injection of exogenous mRNA.

While the signal for carbohydrate attachment is probably the presence of asparagine residues in the primary sequences of heavy chains the specificity and control of the sequence of sugars attached in the oocytes is unknown although probably related to the cellular content of glycosyl transferase enzymes. Whether the appropriate enzymes are normally present in the oocyte or whether their synthesis is induced by the injection of mRNA and subsequent synthesis of immunoglobulins is unknown.

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