FRAGMENTATION AND REDUCTION OF PORCINE 19 S IMMUNOGLOBULIN M

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1. Introduction

Human 19 S immunoglobulin M (IgM) is converted by trypsin at 37°C to 3.7 S Fab_μ and 6.2 S $F(ab')_{2\mu}$ fragments [1,2] during which process the Fc_μ region is destroyed. However, digestion at 60°C gives Fab_μ and 11 S $(Fc)_{5\mu}$ fragments [3]. Treatment with pepsin at pH 4.5 causes extensive degradation of human IgM although Fab_μ and $F(ab')_{2\mu}$ fragments form in the early stages [4,5]. A quantitative conversion to 7 S subunits occurs when 1% solutions of IgM are reduced with 1 mM dithiothreitol (DTT) [6]. However, it has been shown that subunits begin to form in the presence of 0.05 mM DTT and at 0.125 mM DTT half of the IgM has been reduced to 7 S material. [7].

The present paper shows that porcine IgM is appreciably more resistant to proteolytic enzymes than is human IgM. Trypsin fails to produce Fab_μ or $F(ab')_{2\mu}$ fragments and pepsin digestion gives high yields of Fab_μ and $(Fc)_{5\mu}$ fragments. The possibility of conformational differences is assessed. It is also shown that porcine IgM requires more than ten times the molarity of DTT than does human IgM in order to form 7 S subunits. This is due to non covalent interactions holding the 19 S molecule together after most of the interchain disulphide bridges have been reduced. The J chain does not appear to be essential for these non covalent interactions.

2. Methods

Porcine 19 S IgM was prepared from normal serum as previously described [8]. Digestions with trypsin were carried out on 1% protein solutions in 0.1 M NH_4HCO_3, pH 8.5 or 0.2 M NaCl, 0.1 M Tris–HCl, 0.01 M $CaCl_2$, pH 8.5 at 37° or 60°C for 3, 6, 12 and 24 hr using a protein:enzyme ratio of 50:1 and 25:1. Digestions with pepsin were performed on 1% protein solutions in 0.2 M NaCl, 0.1 M sodium acetate, pH 4.0, 4.6 and 5.0 at 37°C for 1, 3, 6, 12 and 24 hr using a protein:enzyme ratio of 100:1. Digestion products were studied by means of immunoelectrophoresis and double agar gel diffusion against specific antisera; by sedimentation on an analytical ultracentrifuge and by SDS-polyacrylamide gel electrophoresis [2,8].

Reductions with DTT were carried out at 20°C for 1 hr under nitrogen using 1% solutions of IgM in 0.2 M NaCl, 0.1 M Tris–HCl, pH 8.0. A 3-fold molar excess of iodoacetic acid was then added and the reaction mixture left for 1 hr at 20°C. The reduction products were examined by sedimentation and by SDS-polyacrylamide gel electrophoresis. The release of J chain was studied by means of alkaline urea-disc gel electrophoresis [8].

3. Results and discussion

Immunoelectrophoresis (fig. 1) failed to detect any difference between trypsin treated and untreated IgM. Sedimentation studies (fig. 2) showed a single component having $s^o_{20,w}$ = 17.8 S compared with 18.8 S obtained for the original IgM. Also there appeared to be a difference in diffusion properties. These results were reproducible irrespective of the various digestion conditions used. The failure to produce Fab_μ or $F(ab')_{2\mu}$ fragments was particularly

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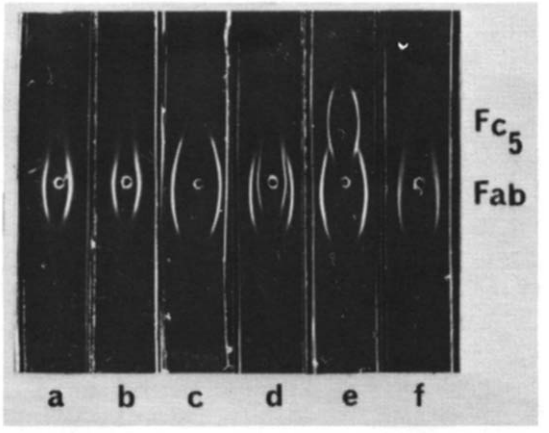

Fig. 1. Immunoelectrophoresis with anti-μ serum: a) 19 S IgM; b) 24 hr trypsin digest of IgM; c) 6 hr trypsin digest of 7 S subunits; d) 1 hr pepsin digest of IgM; e) 12 hr pepsin digest of IgM; f) 3 hr pepsin digest of 7 S subunits.

noticeable and in marked contrast to the results obtained with human IgM [1,2]. However, 7 S subunits obtained by 5 mM DTT reduction of porcine IgM were rapidly degraded to Fab_{μ} fragments by trypsin (fig. 1) and the Fc_{μ} region was destroyed. Hence these subunits behaved in a similar manner to subunits from human IgM [1,2]. It therefore appears that sites for tryptic cleavage only become accessible when the porcine 19 S polymer is reduced. This could be due to large side chains, particularly carbohydrate, masking sites in the polymer or to conformational changes during the formation of 7 S subunits. The finding of consistent differences in $s^{o}_{20,w}$ values and diffusion properties between trypsin treated and untreated porcine IgM suggests that a minor structural alteration has occurred. SDS polyacrylamide gel electrophoresis of samples reduced and alkylated in 6 M guanidine revealed no significant difference between the peptide chains. A possible explanation for these results is that a small section might have been removed from the C-terminal of the μ chain and/or that the J chain has been degraded. Indeed no J chain has been detected in 24 hr trypsin-treated IgM.

It has already been reported that digestion of porcine IgM with pepsin at pH 4.6 for 18 hr produces high yields of Fab_{μ} and $(Fc)_{5\mu}$ fragments [8]. It has now been found that the reaction is completed at 12 hr (figs. 1 and 2) and that no significant degradation of the $(Fc)_{5\mu}$ fragment occurs when the digestion is continued to 24 hr. Examination of digestion products after 1 and 3 hr failed to detect any $F(ab')_{2\mu}$ fragment (figs. 1 and 2). These results are in marked contrast to those obtained for human IgM which is extensively degraded to small fragments and peptides by pepsin although Fab_{μ} and $F(ab')_{2\mu}$ fragments can be found in the early stages of digestion [4,5]. Subnits obtained from porcine IgM were rapidly degraded to Fab_{μ} fragment by pepsin and the Fc_{μ} region was destroyed (fig. 1). Hence these subunits behaved in a similar manner to those obtained from human IgM. Again it was only in the polymeric state that porcine IgM behaved so differently from human IgM particularly as regards the Fc_{μ} region. Indeed when the porcine $(Fc)_{5\mu}$ fragment was reduced to

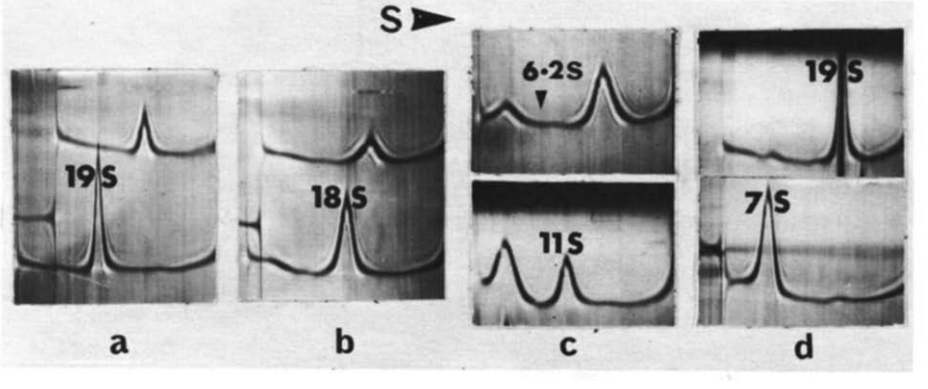

Fig. 2. Sedimentation at 60 000 rpm of a) IgM, 0.11% and 0.28%, after 16 min; b) 24 hr trypsin digest, 0.19% and 0.53%, after 20 min; c) 1 hr pepsin digest (top) and 12 hr pepsin digest (bottom) after 32 min; d) IgM reduced with 0.5 mM DTT (top) and 2 mM DTT (bottom) after 24 min.

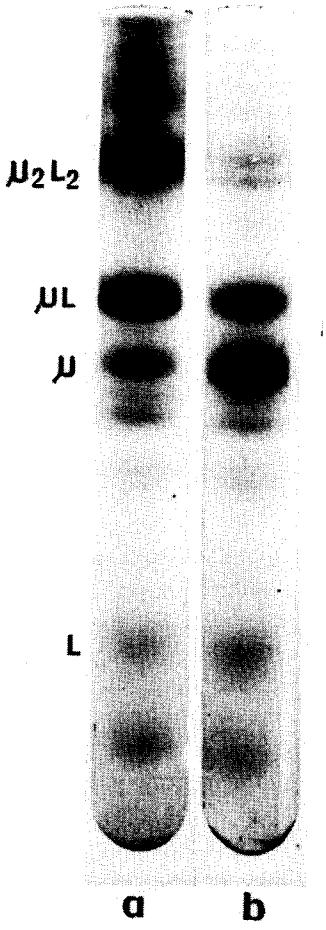

Fig. 3. SDS polyacrylamide gel electrophoresis of reduced samples of porcine IgM: a) 0.5 mM DTT; b) 2.0 mM DTT.

monomers the material could be completely digested by pepsin to small fragments and peptides. However, when monomers were reoxidised back to polymers [8] resistance to pepsin was regained.

Reduction of 1% solutions of porcine 19 S IgM with DTT failed to produce 7 S subunits until the reducing agent was in excess of 0.5 mM and conversion to subunits was not complete until 2 mM DTT was reached (fig. 2). However, SDS-polyacrylamide gel electrophoresis of 0.5 mM DTT reduced and alkylated samples (fig. 3) caused extensive dissociation. The major component consisted of μL half subunits and there were much smaller amounts of μ chain, L chain and $\mu_2 L_2$ subnits. Traces of higher molecular weight components were also present but no 19 S material could be detected. This result indicated that extensive reduction of interchain disulphide bridges had occurred and that the 19 S material was held together largely by non covalent bonds. These results are considerably different to those obtained for human IgM [7] which begins to form subunits in the presence of 0.05 mM DTT and undergoes 50% conversion to subunits with 0.125 mM DTT. A similar degree of subunit formation is also obtained by treating human IgM with 20 mM mercaptoethylamine and the 19 S material remaining after this reduction dissociates in 4 M guanidine [9]. These dissociation products appeared to be subunit monomers and dimers and it was suggested that J chain might link two subunits together to form a special dimer that induced non covalent bonding between other subunits [9]. The results obtained above for 0.5 mM DTT reduced and alkylated porcine IgM, which is entirely 19 S in aqueous solution, show that it can be dissociated almost entirely to components that are smaller than subunit monomers and dimers. Also, alkaline urea-disc gel electrophoresis of reduced and alkylated samples of porcine IgM shows that 25–30% of the J chain is released by 0.5 mM DTT (fig. 4). Since at this stage of reduction the sample

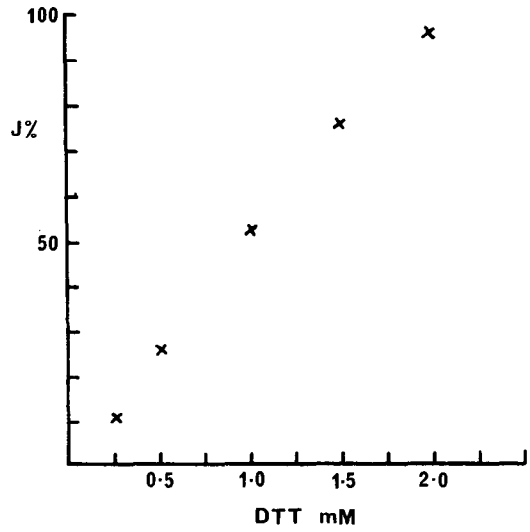

Fig. 4. Densitometric measurement of J chain released from porcine IgM by DTT. Release by 20 mM DTT in 8 M urea was taken as 100% [8].

is entirely 19 S in aqueous solution J chain cannot be an essential requirement for non covalent bonding between subunits. Further studies are in progress to elucidate the nature of the non covalent bonding and why it is eliminated by higher molarities of DTT.

Porcine IgM therefore differs appreciably from human IgM as regards its behaviour towards proteolytic enzymes and reduction by DTT. It appears likely that stronger interactions operate between porcine subunits than between human subunits. This might lead to a tighter packing in the 19 S polymer and thereby make proteolytic sites more inaccessible.

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