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ANTIGEN BINDING AND COMPLEMENT FIXING ACTIVITY OF IgM MOLECULES REASSOCIATED IN THE PRESENCE AND ABSENCE OF J CHAINS

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SUMMARY

Monoclonal IgM with anti-Pr_2 activity was purified from the serum of a patient with chronic cold agglutinin disease. After reduction with 20 mM dithiothreitol IgM molecules and J chains were separated. The subunits were reoxidized in the presence and in the absence of J chains. In both mixtures cold agglutinin activity against the Pr_2 antigen was restored to the titre of the native IgM. Complement fixation as measured by the C1 fixation and transfer test was recovered in the reassociated materials no matter whether J chains were present or not. It was concluded that the presence of J chains was not required for antigen binding and complement fixing activity of IgM antibodies.

INTRODUCTION

Polymeric immunoglobulins contain a polypeptide distinct from their heavy and light chains (Halpern & Koshland, 1970; Mestecky, Zikan & Butler, 1971; Kownatzki, 1971). It was felt to be responsible for linking the monomers and was termed 'J' chain (Halpern & Koshland, 1970). The presence of a J chain was not required for the reassociation of IgM subunits obtained by reduction with dithiothreitol (DTT) (Kownatzki, 1973a). However, it was shown to modify the reassembly, since in the presence of J chains only 19S pentamers were formed, but more than five 7S subunits were reassembled in its absence (Kownatzki, 1973a). The data suggested that J chains exert a controlling function on the reassembly of IgM subunits.

In the present study two biological activities of the reassociated IgM molecules were examined. The specific binding to antigen was chosen as a property of the Fab part of the molecule and the ability to fix the complement component C1 was studied as a property of the Fc part (Plaut, Cohen & Tomasi, 1972). The studies, performed on the cold agglutinin L.Th. with specificity for the Pr_2 antigen of human erythrocytes (Roelcke, Uhlenbruck & Bauer, 1969; Roelcke & Uhlenbruck, 1970), indicated that the presence of J chains was not required for reestablishing either of the two functions.

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MATERIALS AND METHODS

Cold agglutinin L.Th.

The patient L.Th. with chronic cold agglutinin disease had in his serum a high titre cold agglutinin. It was of the IgM class, had κ-type light chains (Roelcke & Jungfer, 1970). and was specific for the Pr_2 antigen which has been characterized (Roelcke *et al.*, 1969; Roelcke & Uhlenbruck, 1970).

The monoclonal IgM protein was isolated from the serum and purified by a combination of Pevikon block electrophoresis (Müller-Eberhard, 1960) and gel filtration on Sephadex G-200. The purified protein did not contain any contaminants detectable by immunoelectrophoresis and Ouchterlony analysis.

Cold agglutinin activity

Cold agglutinin activity was tested for on microtitre plates (Cooke Engineering Company) at 0°C using group O Rhesus-negative human erythrocytes and phosphate-buffered saline (PBS) at pH 7·2 as diluent. The criteria for Pr activity was the elimination of agglutination by treatment of the erythrocytes with neuraminidase (RDE) (Roelcke & Dorow, 1968).

C1 fixation and transfer test

The C1 fixation and transfer test (C1 FT test) was performed according to Borsos & Rapp (1965) with minor modifications. Human group O Rhesus-negative red cells, 0·1 ml, containing $1{\cdot}5 \times 10^8$ cells per ml, were incubated with 0·1 ml purified L.Th. IgM or reassociated L.Th. IgM in a subagglutinating concentration at 0°C for 15 min. The cells were centrifuged and resuspended in 0·2 ml of a buffer containing two parts of Veronal-buffered saline (VBS), $\mu = 0{\cdot}15$ (Kabat & Mayer, 1961) and three parts of 5% glucose to give an ionic strength of $\mu = 0{\cdot}065$ (Rapp & Borsos, 1970). This buffer will be referred to as VBS-glucose. Purified guinea-pig C1, 0·1 ml, containing 4×10^{12} effective molecules per ml were added and incubated for 20 min at 0°C. The cells were washed, resuspended in 5 ml VBS-glucose, transferred to another tube, washed five times in VBS-glucose and resuspended in 1·5 ml VBS, $\mu = 0{\cdot}15$. For the transfer step 0·2 ml of this suspension at twofold dilutions from 1:150 to 1:1200 were added to 0·2 ml of sheep erythrocytes having amboceptor and the complement component C4 attached (EAC4) and containing $1{\cdot}5 \times 10^8$ cells/ml (prepared according to the method of Borsos & Rapp, 1967).

Miscellaneous

Protein concentrations were measured by the Folin-phenol method according to Lowry *et al.* (1951). Protein solutions were concentrated by negative pressure filtration through collodion bags obtained from Sartorius Membranfilter GmbH, Göttingen, Germany.

Ultracentrifugation was carried out in a Spinco Model E analytical ultracentrifuge using Schlieren optics. Preparative electrophoresis was performed according to Müller-Eberhard (1960).

RESULTS

Reduction and reassociation

The purified L.Th. IgM was reduced with 20 mM dithiothreitol (DTT) for 1 hr at room temperature in a buffer containing 0·1 M Tris, 0·1 M NaCl, pH 8·0. IgM molecules and J

chains in this preparation were separated on a column of Sephadex G-200 in 0·1 M Tris, 0·5 M NaCl, pH 8·0, containing 1 mM DTT. The separation was identical with that obtained previously with other IgM proteins (Kownatzki, 1973a). The purity of the pools was established by acrylamide gel electrophoresis as described by Kownatzki (1973a). The IgM molecule fraction did not contain any J chain in amounts detectable by this method, but the J chain fraction was slightly contaminated with IgM subunits.

One half of the IgM subunit pool was mixed with one half of the J chains. The other half of IgM subunits was mixed with a corresponding amount of buffer. To make all SH groups available the mixtures were again reduced with 20 mM DTT. Reassociation was allowed to take place in the course of dialysis against PBS at pH 7·2 over 24 hr.

The reassociated preparations of L.Th. IgM were examined by analytical ultracentrifugation as demonstrated in Fig. 1. The pattern showed the characteristic features seen previously with other IgM proteins reassociated with and without J chains (Kownatzki,

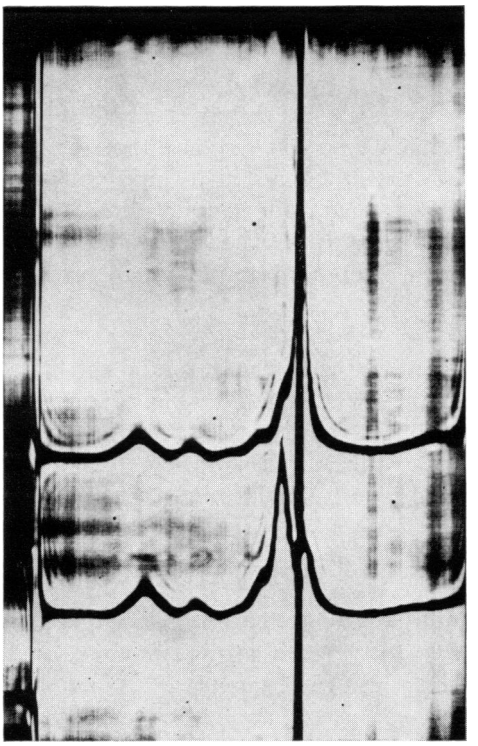

FIG. 1. Ultracentrifugal pattern of LTh. IgM reassociated in the absence (top) and presence (bottom) of J chains after 24 hr of dialysis against PBS. Sedimentation proceeds from left to right. Picture was taken 40 min after reaching 59,780 rpm.

1973a). While IgM subunits reassociated in the presence of J chains (bottom of Fig. 1) yielding products with an S rate of 16·5 (uncorrected at a protein concentration of 7·0 mg/ml), those reassembled in its absence (top of Fig. 1) were larger, with an S rate of 18·7.

Cold agglutinin activity of reassociated molecules

Preparations of (i) native L.Th. IgM, (ii) L.Th. IgM reduced with 20 mM DTT and alkylated with excess iodoacetamide, (iii) L.Th. IgM reassociated in the presence of J chain and (iv) L.Th. IgM reassociated in the absence of J chain were brought to equal concentration and tested for cold agglutinin activity. As can be seen from Table 1 the cold agglutinin

TABLE 1. Agglutination titres of the cold agglutinin L.Th. against native and RDE (neuraminidase)-treated human erythrocytes

L.Th. IgM	Titre against group O Rhesus negative RBC	Titre against RDE-treated RBC
(i) *Native	1024	0
(ii) Reduced and alkylated	0	0
(iii) Reassociated with J chain	1024	0
(iv) Reassociated without J chain	1024	0

* All cold agglutinin preparations at 7·0 mg/ml.

activity was completely destroyed by reduction and alkylation. Following reassociation it was restored and even reached the original titre. No difference between the two reassociated preparations was observed and even agglutination titres were identical. None of the preparations showed any agglutinating activity at 37°C. No agglutination was observed, when the erythrocytes were treated with neuraminidase, indicating that it was anti-Pr activity which was restored in the reassociated molecules. There was no agglutination by a Waldenström macroglobulin of unknown antibody activity reassociated in the same fashion.

C1 FT test with reassociated cold agglutinin

The results of a C1 fixation and transfer test on the native and reassociated L.Th. IgM are recorded in Table 2. As is evident from this table, not only native IgM, but also preparations of reassociated IgM subunits were capable of fixing C1. IgM molecules reassociated in the absence of J chains appeared to fix C1 slightly less efficiently. The difference was so small that it could not be caused by a contamination of the J chain-free preparation with J chains.

DISCUSSION

The presence of a J chain in polymeric, but not in monomeric IgA myeloma proteins led Halpern & Koshland (1970) to suggest that the component may function by linking the monomers. This hypothesis seemed to be supported by a number of findings. J chains were reported to be attached to the Fc part of the IgM molecules (Mestecky, Kulhavy & Kraus, 1971), which also carries the site of intersubunit bonds (Beale & Feinstein, 1969). J chains synthesized in IgA-producing mouse plasmocytoma cells attached to the dimeric IgA at or close to the time of assembly of the monomers (Halpern & Coffman, 1972). On reoxidation, of a Waldenström macroglobulin reduced with 0·015 M mercaptoethylamine, IgM subunits, which contained only trace amounts of J chains, did not reassociate to polymers (Wilde & Koshland, 1972).

The hypothesis was challenged by the finding that IgM subunits did reassociate to larger polymers in the absence of J chains (Kownatzki, 1973a). The majority of these polymers

TABLE 2. Results of C1 FT test (according to Rapp & Borsos, 1970)

L.Th. IgM native				
Dilution	1 : 150	1 : 300	1 : 600	1 : 1200
OD 412 nm	0·40	0·30	0·19	0·08
Lysis (%)	70·1	52·6	33·3	14·0
Number of $SAC\overline{14}$/cell corrected for non-specific uptake of C1	0·886	0·578	0·316	0·150
Average number of $C\overline{1}$/cell: 167.				

L.Th. IgM reassociated in presence of J chains				
Dilution	1 : 150	1 : 300	1 : 600	1 : 1200
OD 412 nm	0·37	0·27	0·19	0·10
Lysis (%)	64·9	47·4	33·3	17·5
Number of $SAC\overline{14}$/cell corrected for non-specific uptake of C1	0·608	0·489	0·329	0·192
Average number of $C\overline{1}$/cell: 165.				

L.Th. IgM reassociated in absence of J chains				
Dilution	1 : 150	1 : 300	1 : 600	1 : 1200
OD 412 nm	0·34	0·25	0·15	0·06
Lysis (%)	59·6	43·9	26·3	11·5
Number of $SAC\overline{14}$/cell corrected for non-specific uptake of C1	0·515	0·441	0·236	0·122
Average number of $C\overline{1}$/cell: 122.				

were larger than 19S, whereas with J chains present mainly 19S pentamers were formed (Kownatzki, 1973a). It was suggested that J chains have a controlling function on the reassembly of IgM subunits (Kownatzki, 1973a).

The present study confirmed and extended these findings. Again the polymers formed in the absence of J chains sedimented faster than those formed in its presence. Two properties of the native IgM molecule were sought in the reassociated materials, the specific combination with antigen, residing in the Fab part of the molecule, and the ability to fix the complement component C1, a property of the Fc part (Plaut, Cohen & Tomasi, 1972).

Recovery of cold agglutinin activity following reoxidation of reduced IgM cold agglutinins has been described (Harboe & Deverill, 1964; Harboe, Solheim & Deverill, 1969). The titre found by Harboe & Deverill was considerably lower than in the native protein (Harboe & Deverill, 1964). In the experiments reported here agglutination titres were identical in the native cold agglutinin and in the two preparations reassociated in the presence and absence of J chains. These results indicate that J chains were not required for restoring the antibody activity in the reassociated IgM molecules.

The ability of IgM antibodies to activate the complement sequence is destroyed by reduction with thiols (Murray, O'Connor & Goan, 1965; Stollar & Sandberg, 1966). The data presented here indicate that it reappears on reoxidation. The presence of a J chain

was not required for restoring the complement-fixing activity. Neither does it seem to be directly involved in the site of C1 fixation, nor does it exert an influence on this site indirectly. The amounts of C1 fixed and transferred in the C1 FT test were quite similar in the three preparations, so that an effect of a small J chain contamination in the J chain-free preparation could be excluded. It remains an open question whether the slightly less efficient C1 fixation in the cold agglutinin reassociated without J chains was due to a non-optimal assembly. For complete function it may be desirable to assemble five and no more subunits in the IgM molecule (Kownatzki, 1973a).

The biological significance of J chains remains unknown. They do not appear to be the linking agent as was suggested by Halpern & Koshland (1970). J chains were not required for restoring antibody activity and complement fixation after reduction and reoxidation of an IgM cold agglutinin. Recent evidence suggested that J chains modified the reassembly of IgM subunits by preventing the association of more than five monomers (Kownatzki, 1973a). There is also suggestive evidence that IgM molecules possessing a J chain are more resistant to reductive cleavage than molecules lacking the J chain (Kownatzki, 1973b). A stabilizing function of J chains on IgM pentamers is reminiscent of the stabilizing effect of the secretory component on IgA2 molecules (Jerry, Kunkel & Adams, 1972).

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