Polymerization of Human Immunoglobulin M†

Charles E. Wilde III‡,* and Marian E. Koshland*

ABSTRACT: The repolymerization of human IgM following mild reductive cleavage was studied as a model for intracellular polymer assembly. Repolymerization was found to require the presence of J chain and a disulfide exchanging system which could be furnished either intrinsically by the use of the monofunctional thiol mercaptoethylamine or extrinsically by the inclusion of a protein–mercaptan mixed disulfide, and/or a disulfide exchanging enzyme. The degree of repolymerization was dependent on the extent of monomer reduction and the product covalently incorporated one J chain per five monomer units. Disulfide exchanging enzyme probably served as a source of mixed disulfides rather than as an enzymatic catalyst of the reaction. These results are discussed in terms of a tentative mechanism for IgM polymerization.

Two classes of immunoglobulins, IgA and IgM, exist as disulfide bonded polymers of the basic four-chain (H_2L_2) immunoglobulin molecule. In addition to heavy and light chains, J chain is included in the polymeric structure in a ratio of one J per polymer (Halpern & Koshland, 1970, 1973; Chapuis & Koshland, 1974). This protein is attached through disulfide bonds to the cysteine residues in the penultimate positions of the polymer heavy chains (Mestecky & Schrohenloher, 1974; Mestecky et al., 1974). In the case of polymeric IgA, evidence indicates that J chain bridges the α chains of adjacent subunits whereas direct bonding between the penultimate cysteines of the remaining α chains completes the polymeric structure (Chapuis & Koshland, 1975). In the case of IgM, a similar linkage has been inferred but not unequivocally demonstrated (Chapuis & Koshland, 1974; Wilde & Koshland, 1973). These structural data suggest that the J chain functions to mediate the polymerization of both classes of polymers.

Numerous studies have been directed toward clarifying the polymerization reaction. The approaches have generally involved reductive depolymerization and repolymerization through oxidation and/or disulfide exchange. Initial investigations using very mild reductive cleavage showed that the intermonomer disulfides in IgM are the most labile (Beale & Buttress, 1969; Beale & Feinstein, 1969; Morris & Inman, 1968). It was later shown that substantial depolymerization could be achieved without concomitant breakage of J-heavy disulfides; thus the role of J chain as an initiator of polymer assembly was suggested (Chapuis & Koshland, 1974).

Prior to the discovery of J chain, attempts to assemble immunoglobulin monomers were largely unsuccessful except in cases where carrier polymer was added (Askonas & Parkhouse, 1971; Parkhouse et al., 1970). Once the significance of J chain became apparent, reassembly studies indicated that the J polypeptide played an essential role in polymerization (Wilde & Koshland, 1972; Koshland & Wilde, 1974; Della Corte & Parkhouse, 1974). Removal of J chain from depolymerized IgM inhibited subsequent assembly (Wilde & Koshland, 1972). The addition of J chain to secreted mouse IgM monomers, together with an enzyme that catalyzes disulfide–sulfhydryl exchange, resulted in quantitative polymerization of the monomers to a size at least equivalent to tetramers (Della Corte & Parkhouse, 1974). The incorporation of J chain into these reassembled molecules was demonstrated, though less than stoichiometric amounts were found. Furthermore, the lack of hybrid polymer formation upon simultaneous asembly of IgM and IgA polymers indicated that the specificity for polymer size and composition resides in the respective heavy chains (Della Corte & Parkhouse, 1974).

The following report details investigations into the mechanisms and specificity of human IgM reassembly. These studies were designed to examine in greater detail the requirement for J chain and to determine the state of the sulfhydryls needed to promote polymerization. In addition, the need for a disulfide–sulfhydryl exchange system was examined.

Materials and Methods

Purification of IgM. Macroglobulinemic sera were generously provided by Dr. H. H. Fudenberg of the University of California at San Francisco. Pentameric IgM was isolated by four-fold euglobulin precipitation and gel filtration on Sepharose 6B as previously detailed (Chapuis and Koshland, 1974).

Purification of J Chain. The J chain used in reassembly studies was isolated by preparative polyacrylamide gel electrophoresis. Myeloma IgA at 9.2 mg/mL was reduced with 20 mM dithioerythritol (DTE)[1] in the presence of 10 M urea, 0.1 M Tris-HCl, pH 8.0, and 2 mM EDTA for two hours at 45 °C. The protein sulfhydryl groups thus generated were converted to mixed disulfides by the addition of 1.0 M cystamine (2,2′-dithiobis(ethylamine)) (Sigma) for two hours at 45 °C. Completeness of mixed disulfide formation was assured by making the mixture 20 mM in Diamide (diazine dicarboxylic acid) (Calbiochem). The J chain was then isolated by preparative polyacrylamide gel electrophoresis (Wilde and Koshland, 1973). Purity of the J chain preparation was determined by amino acid analysis and polyacrylamide gel electrophoresis using alkaline urea and sodium dodecyl sulfate systems (Morrison and Koshland, 1972). No free sulfhydryls were detected following reaction with dithiobisnitrobenzoic acid or iodoacetamide.

Assay of Disulfide Exchanging Enzyme. A sample of partially purified disulfide exchanging enzyme was kindly supplied

† From the Department of Bacteriology and Immunology, University of California at Berkeley, Berkeley, California 94720. *Received August 10, 1977; revised manuscript received April 28, 1978.* Supported by Research Grant AI 07079 from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service.

‡ Present address: Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, Ind. 46202.

[1] Abbreviations used: RNase, ribonuclease; DTE, dithioerythritol.

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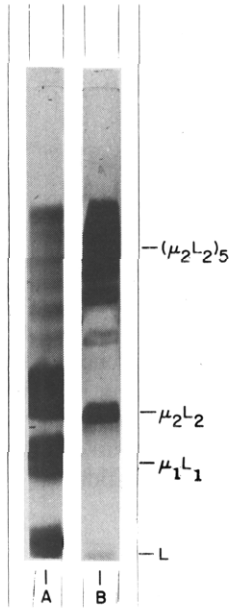

FIGURE 1: Electrophoretic separation of IgM reduced for 1 h at room temperature with 15 mM cysteamine prior to (A) and following (B) removal of the reducing agent by passage over Sephadex G-25. Gels were 2% polyacrylamide containing 1% sodium dodecyl sulfate and 0.5% agarose.

by Dr. Robert Freedman of the University of Kent at Canterbury. The enzyme had been partially purified from an acetone powder of beef liver microsomes by ammonium sulfate fractionation and chromatography on Sephadex CM-50 (DeLorenzo et al., 1966). The lyophilized enzyme preparation was readily soluble in physiological buffers; however, only 20% by weight of the material was precipitable by 10% trichloroacetic acid, as determined by quantitative amino acid analysis. This precipitable material had an amino acid composition in good agreement with that reported for the purified enzyme (DeLorenzo et al., 1966). All quantitations of enzyme were based upon the amount of Cl_3CCOOH precipitable material.

The disulfide exchanging enzyme was assayed by observing the rate of conversion of randomly reoxidized ribonuclease (RNase) (Worthington Biochemicals) to active RNase (DeLorenzo et al., 1966; Fuchs et al., 1967). The amount of active RNase was determined by measuring the rate of cleavage of the cyclic phosphate bond of cyclic cytidine 3′,5′-monophosphate and comparing that rate with the rate produced by a solution of native RNase at known concentration (Croom et al., 1960). One milligram of the enzyme preparation catalyzed the regeneration of 1.02 nmol of RNase per min.

Reassembly. Polymer reassembly was examined according to the following general protocol; specific conditions are given in the Results section. One-milliliter aliquots of IgM polymer at 10 mg/mL were reduced with a variety of reducing agents, then separated from the reducing agent on a 1 × 12 cm column of Sephadex G-25 Fine equilibrated with 0.02 M Tris-HCl (pH 7.5), 0.15 M NaCl. Aliquots containing 50–100 μg were placed in 6 × 50 mm tubes containing various combinations of disulfide exchanging enzyme, J chain or RNase in various forms and/or buffer (0.02 M Tris-HCl (pH 7.5), 0.15 M NaCl). In some instances, the disulfide exchanging enzyme was activated by including 1 mM 2-mercaptoethanol in the reassembly mixtures. The tubes were capped with Parafilm and incubated at 37 °C for 1 h. Any residual sulfhydryls were subsequently alkylated by treatment with 0.2 μmol of iodoacetamide for 30 min at room temperature. In addition, aliquots of the reduced mixture were alkylated both before and after passage over Sephadex G-25. All reassembly was conducted at a constant IgM concentration of 5 mg/mL determined spectrophotometrically following elution from Sephadex G-25. RNase with mixed disulfides was produced under conditions identical with those used for reduction and cystamine treatment of IgA.

Alternatively, reduced IgM preparations were reoxidized during dialysis against 0.1 M Tris-HCl, pH 8.0 or 0.1 M sodium acetate, pH 5.5. Reoxidation was conducted at IgM concentrations ranging from 0.2 to 20 mg/mL.

Assay of Reassembled Products. Analysis of reaction products was conducted by electrophoresis on 2% polyacrylamide gels containing 0.5% agarose (Dingman & Peacock, 1968; Peacock & Dingman, 1968), 1% sodium dodecyl sulfate and 0.1 M Tris-HCl, pH 8.0 (Shapiro et al., 1967). A standard amount of 50 μg of IgM was applied per gel. Following electrophoresis, the gels were scanned at 278 nm in a Zeiss PMQ II spectrophotometer equipped with a linear transporter. The gels were subsequently stained in 0.25% Coomassie Brilliant Blue R-250 in methanol:acetic acid:water (45:9:46). Quantitation of peak areas from the gel scans was made manually with a planimeter.

Products formed by reoxidation of IgM during dialysis were examined by velocity ultracentrifugation in dialysis buffer and in 6 M guanidinium chloride. Sedimentation was conducted at a constant temperature of 20 °C; samples were centrifuged at 52 640 rpm in the Spinco Model E ultracentrifuge using the AnD rotor. Double sector cells were utilized and each sample was compared with native IgM. Following reoxidation, polymerized material was separated from residual monomers by gel filtration on Sephadex G-200. The polymerized material was analyzed for covalently and noncovalently bound J chain by electrophoresis of unreduced and completely reduced and alkylated samples on alkaline urea–polyacrylamide gels.

Determination of Stoichiometry. Reassembled samples were electrophoresed on multiple 3.5% polyacrylamide gels containing 1% sodium dodecyl sulfate. Under these conditions, the pentamer fraction was localized in the top few millimeters of the gels. One gel was stained for protein localization, while the remaining gels were frozen and sliced. The extraction of the protein from the gel slices and the determination of J chain stoichiometry by reduction and radioalkylation were performed as described by Chapuis & Koshland (1974).

Results

Reassembly Following Reduction with a Monofunctional Reducing Agent. Treatment of human IgM with the monofunctional reducing agent cysteamine (mercaptoethylamine) at 15 mM produced extensive dissociation of the pentamers and yielded species with mobilities similar to μ_2L_2 monomers, μ_1L_1 half-monomers, and free μ and L chains (Figure 1A). Small amounts of products with mobilities greater than monomer were observed. Under these reducing conditions, more than 90% of the J chain was released as determined by alkylation with iodo[^{3}H]acetic acid and quantitation on alkaline urea–polyacrylamide gels. When the reducing agent was removed by passage of the reduced IgM over Sephadex G-25, some spontaneous reassembly occurred. The major products formed represented pentamers, tetramers and trimers, with some residual monomers (Figure 1B). Quantitation of the spontaneous reassembly indicated that greater than 80% of the monomers and all the half-monomers were repolymerized to higher forms. The extent of polymer formation was not altered by inclusion of the disulfide exchanging enzyme, low molecular weight disulfides and/or by continued incubation at 37 °C.

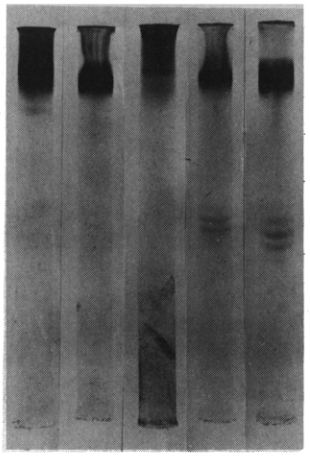

FIGURE 2: Electrophoretic separation on alkaline urea–polyacrylamide gels of IgM reduced with 15 mM cysteamine and reassembled at 20 mg/mL by dialysis against 0.1 M sodium acetate, pH 5.5. From left to right: aggregated material formed upon reoxidation in the absence of J chain; aggregated material formed in the absence of J chain followed by complete reduction and alkylation; aggregated material formed in the presence of J chain; aggregated material formed in the present of J chain followed by complete reduction and alkylation; completely reduced and alkylated native IgM.

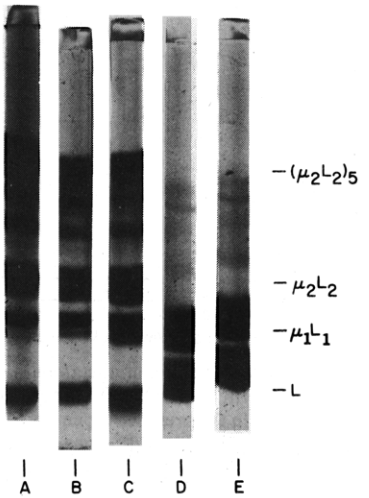

FIGURE 3: Electrophoretic separations of IgM following reduction with various levels of DTE and reassembly with 2.5 mg/mL of disulfide exchanging enzyme. DTE concentrations were: (A) 0.5 mM; (B) 0.75 mM; (C) 1.0 mM; (D) 5.0 mM; (E) 10.0 mM. Gels were 2% polyacrylamide containing 1% sodium dodecyl sulfate and 0.5% agarose.

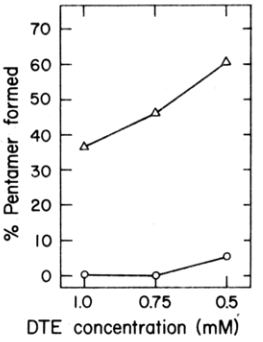

FIGURE 4: The relationship between the amount of pentamer formed during reassembly in the presence (△) and absence (○) of 2.5 mg/mL of disulfide exchanging enzyme and the amount of DTE used for the initial reduction of the IgM.

Partial reassembly could also be effected by slow removal of the cysteamine during the extended dialysis. By this procedure, up to 40% of the monomers could be reformed into pentamers that were stable in 6 M guanidinium chloride and, thus, represented disulfide bonded products. This slow assembly was maximally efficient at high protein concentrations (20 mg/mL) and at pH 5.5. Following isolation by gel filtration, the reassembled material appeared to contain a normal amount of J chain as judged by the intensity of staining of the fast anodal bands characteristic of J chain on alkaline urea–polyacrylamide gels. The covalent incorporation of J chain was indicated by the necessity for reduction of the reassembled product in order to observe the characteristic J chain bands (Figure 2).

The dependence of the polymerization reaction on J chain was studied by removing the J chain from reduced IgM. IgM was reduced with 20 mM cysteamine and passed over a column of Sephadex G-200 equilibrated with 20 mM cysteamine, 0.02 M Tris-HCl (pH 8.0), 0.15 M NaCl. The resulting monomers were virtually free of J chain as judged by alkaline polyacrylamide gel electrophoresis and by lack of reactivity with anti-J-chain antiserum. These monomers could not be subsequently reassembled by oxidation during prolonged dialysis under optimal conditions. Analysis by velocity ultracentrifugation revealed the presence of some noncovalently bonded aggregates which dissociated upon equilibration with 6 M guanidinium chloride.

Reassembly Following Reduction with a Bifunctional Reducing Agent. Reduction of human IgM with the bifunctional reducing agent dithioerythritol (DTE) produced extensive dissociation of the pentamers to monomers and half-monomers. The effective levels of DTE were found to range from 0.5 to 2.0 mM, 10- to 100-fold lower than the effective levels of cysteamine. At 0.5 mM DTE, small but significant amounts (6%) of polymer species remained following reduction; above 0.5 mM, no detectable species larger than monomers remained. In addition, all the J chain was released by this treatment as determined by radioalkylation and electrophoresis on alkaline urea–polyacrylamide gels.

Spontaneous repolymerization was not observed following removal of the DTE by gel filtration over Sephadex G-25. However, this process may be dependent on the time of incubation and on the presence of small amounts of divalent metal ions (Eskeland, 1977), and thus the amount formed spontaneously could vary.

Incubation of DTE-reduced IgM with 2.5 mg/mL of disulfide exchanging enzyme resulted in substantial repolymerization (Figure 3). The extent of pentamer formation ranged from 20% for IgM reduced at 2.0 mM DTE up to 61% for IgM reduced at 0.5 mM DTE (Figure 4). When reduction was carried out with concentrations greater than 2.0 mM DTE, no pentamer assembly could be achieved. Somewhat lower pentamer yields were obtained when the disulfide exchanging enzyme was included at 0.5 mg/mL. However, under no conditions was the amount of pentamer assembly augmented by activation of the enzyme with 1.0 mM 2-mercaptoethanol, nor could increased yields be obtained using incubation periods longer than one hour.

The contrast between the capacities of cysteamine- and DTE-reduced IgM preparations for spontaneous reassembly suggested that assembly occurred by disulfide interchange rather than by oxidation. This hypothesis was tested by including in the reassembly mixtures proteins in either mixed disulfide or reduced forms. J chain with cysteamine-blocked sulfhydryls was isolated by preparative electrophoresis from

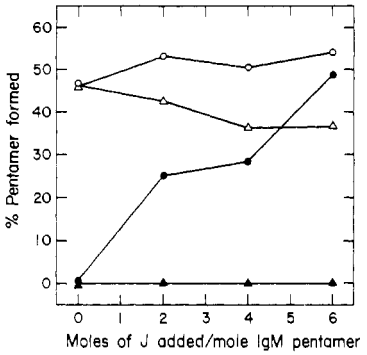

FIGURE 5: The effect of added J chain on the amount of pentamer assembly. IgM was reduced with 0.75 mM DTE, separated from the reducing agent, and incubated in the presence (open symbols) and absence (closed symbols) of 2.5 mg/mL of disulfide exchanging enzyme. J chain was added during the incubation in either cysteamine-blocked (○ and ●) or reduced (△ and ▲) forms. The amounts of J chain added were in addition to the 1 mol equiv of J chain present in the original IgM. The amount of pentamer was calculated from planimetric measurements of the 278-nm scans of unstained gels and expressed as a percent of total A_{278} measurable on each gel.

myeloma IgA following complete reduction and reaction with cystamine. The addition of cysteamine-blocked J chain in amounts ranging from 2 to 6 mol per mol of DTE-reduced IgM resulted in an increase in the amount of pentamer formation (Figure 5). This effect was most marked in the absence of disulfide exchanging enzyme, where the addition of 6 mol of cysteamine-blocked J chain per mol of IgM resulted in pentamer assembly comparable to that obtained with 2.5 mg/mL of enzyme (59%). In the presence of enzyme, the stimulatory effect was less marked and amounts greater than 2 mol of J chain added per mol of IgM did not further increase pentamer formation (Figure 6).

The need for a disulfide exchanging system was further indicated by the results obtained when reduced J chain was added to the reassembly mixtures. Cysteamine-blocked J chain was reduced with 10 mM DTE to remove the blocking agent, freed from reducing agent on Sephadex G-25 and added to DTE-reduced IgM. Over a range of 0 to 6 mol of reduced J added per mol of IgM, no pentamer assembly was found to occur in the absence of disulfide exchanging enzyme (Figure 5). When enzyme was included at 2.5 mg/mL, reduced J chain had an inhibitory effect.

The specificity of added J chain for enhancing the reassembly process was examined by comparing the amount of pentamer formation in the presence of added J chain to that induced in the presence of added RNase. Cysteamine-blocked RNase was prepared and added to IgM reduced with 0.75 mM DTE; from 0 to 6 mol of RNase was added per mol of IgM. Pentamer assembly was stimulated by cysteamine-blocked RNase to a degree comparable to that seen for cysteamine-blocked J chain (up to 56%), and the addition of RNase in a reduced form again inhibited the assembly process. These studies indicated that the presence of a disulfide exchange capability in the form of a mixed protein–cysteamine disulfide was a sufficient stimulatory factor for effecting pentamer assembly.

Although the pentamers formed during the reassembly process exhibited the correct mobility during electrophoresis, it was not clear whether they contained a normal stoichiometric amount of J chain; this question was answered by isolation and characterization of the reformed pentamer products. IgM was reduced with 0.75 mM DTE, thereby freeing all the covalently bound J chain. After removal of the DTE on Sephadex G-25,

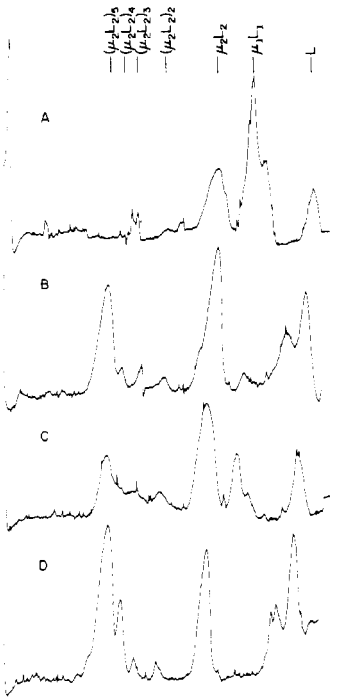

FIGURE 6: Electrophoretic separations of IgM following reduction with DTE and reassembly. (A) IgM reduced with 0.75 mM DTE and alkylated; (B) IgM reduced with 0.75 mM DTE followed by removal of the reducing agent and incubation with 2.5 mg/mL of disulfide exchanging enzyme; (C) IgM reduced with 0.75 mM DTE followed by removal of the reducing agent and incubation with a two-fold excess of cysteamine-blocked J chain; (D) IgM reduced with 0.75 mM DTE followed by removal of the reducing agent and incubation with 2.5 mg/mL of disulfide exchanging enzyme and a twofold excess of cysteamine-blocked J chain. Gels were 2% polyacrylamide containing 1% sodium dodecyl sulfate and 0.5% agarose; following electrophoresis, the gels were scanned for absorbance at 278 nm prior to staining.

TABLE I: Stoichiometry of J Chain in Reassembled IgM Pentamer.

	chain	slices	total ^{3}H cpm	μmol of chain[a]	μmol of chain per pentamer[b]
A.	μ	1–14	16 125	4.19×10^{-4}	9.7
	L	15–25	6 160	4.48×10^{-4}	10.3
	J	47–58	678	4.11×10^{-5}	0.95
B.	μ	1–16	20 805	5.40×10^{-4}	10.1
	L	17–28	7 221	5.25×10^{-4}	9.9
	J	60–70	831	5.04×10^{-5}	0.95

[a] Micromoles of chain calculated from the known specific activity of the iodo[^{3}H]acetic acid and the known cysteine contents of 14, 5, and 6 for μ, L, and J chain, respectively. [b] Normalized to 10.0 μ_1L_1 pairs per pentamer.

the IgM was reassembled in the presence of 2.5 mg/mL of disulfide exchanging enzyme and a sixfold molar excess of added cysteamine-blocked J chain. Pentamer formation (54%) was confirmed on 2% polyacrylamide gels. Replicate aliquots were then electrophoresed on 3.5% polyacrylamide gels containing sodium dodecyl sulfate. Under these conditions, the pentamer species was confined to the top few millimeters of gel. Several gels were sliced, the pentamer containing fractions were pooled, and the protein was extracted. After treatment of this extract to remove residual detergent (Chapuis & Koshland, 1975), the protein was completely reduced and alkylated with iodo[^{3}H]acetic acid. The amounts of ^{3}H in the μ, L, and J chains were determined following electrophoresis on alkaline urea polyacrylamide gels. Table I indicates that,

for two determinations, 0.95 mol of J chain was incorporated for each 10 mol of μ and L chains. These results indicated that the pentamer assembled from completely depolymerized IgM in the presence of a sixfold molar excess of J chain and disulfide exchanging enzyme contained the appropriate stoichiometric amount of J chain.

Discussion

The results of these studies indicate that human IgM can be reductively dissociated and repolymerized with reasonable efficiency. Under appropriate conditions, up to 70% of the reduced material was repolymerized to a form indistinguishable from native pentamers on the basis of electrophoretic mobility and J chain content. The efficiency of reassembly was found to be dependent on the presence of J chain, the degree of reductive cleavage of the monomers and the presence of a disulfide exchanging system.

The requirement for J chain was demonstrated by the lack of stable polymerization in the absence of J chain and by the incorporation of stoichiometric amounts of J into reformed pentamer products. These results support the work of Della Corte & Parkhouse (1974), who demonstrated a requirement for J chain during enzymatic assembly of murine IgM and IgA myeloma proteins. Reports of polymerization in the absence of J chain may reflect nonspecific aggregation similar to that seen in the present study (Kownatzki, 1973; Kownatzki & Drescher, 1973; Eskeland, 1974, 1977). Previous studies that indicated polymerization in the absence of J chain have not demonstrated the covalent nature of the reassembled products. Despite this, Eskeland (1974) and Kownatzki (1973) have suggested that polymers reformed in the presence of J chain were distinct from those formed without J chain and were more similar to native 19S IgM. While polymerization in the absence of J chain cannot be excluded by the present work, the efficiency of formation and the homogeneity of the IgM pentamers assembled in the presence of J chain suggest that this process may reflect polymerization in vivo.

The efficiency of reassembly of reduced IgM was markedly affected by the nature and concentration of the reducing agent utilized. As the thiol concentration was increased, the amount of reformed pentamer decreased, despite the nearly quantitative conversion of half-monomers to monomers at all reducing agent concentrations. These results indicate that monomer status is an insufficient condition for incorporation into pentamers and suggest that the maintenance of a particular disulfide bond(s) within the monomer is essential for reassembly.

Assembly of reduced IgM is effected by a process of disulfide exchange. This interchange can be instituted either by an enzyme which has been shown to facilitate disulfide interchange (DeLorenzo et al., 1966; Fuchs et al., 1967) or by a protein–mercaptan mixed disulfide. The inclusion of either of these agents is both necessary and sufficient for the reassembly of DTE-reduced IgM. Although specific catalysis by the disulfide exchanging enzyme cannot be ruled out, it seems likely that the enzyme preparation may be serving instead as a source of mixed disulfides. This conclusion is supported by two lines of evidence: (1) reactivation of randomly reoxidized RNase requires activation of the disulfide exchanging enzyme (DeLorenzo et al., 1966), while reassembly of IgM does not; (2) two proteins, RNase and J chain, which have no known enzymatic activity for disulfide exchange, can equally well promote IgM reassembly if they are included as protein–mercaptan mixed disulfides but are inhibitory when included as reduced forms.

The differentiation between specific enzymatic catalysis and nonspecific disulfide exchange promoted by the disulfide exchanging enzyme would require a more extensive kinetic analysis than that undertaken in this study. While specific catalysis of disulfide exchange by the beef liver enzyme seems unlikely, one report has suggested that a distinct disulfide exchanging activity exists in cells of lymphoid origin (Delamette et al., 1975). This activity is either absent or inhibited in unstimulated spleen cells, but appears following stimulation with Concanavalin A or antigen, and efficiently catalyzes the polymerization of IgM monomers. It was suggested that this activity was distinct from that of the liver enzyme; as such, this activity may mediate specific catalysis of IgM polymerization in contrast to the nonenzymatic assembly observed in the present study.

The nonenzymatic nature of in vitro IgM assembly is further supported by the finding that exogenous mediators are not required for the reassembly of cysteamine-reduced IgM. Reduction of IgM with this monofunctional reducing agent intrinsically produces a thiol–disulfide equilibrium (Cecil & McPhee, 1959) which can undergo spontaneous exchange; this results in the spontaneous reassembly of IgM pentamers following the removal of the excess free reducing agent. In contrast, reduction with DTE required the addition of a disulfide–sulfhydryl exchange system. The requirement for an exogenous source of mixed disulfides may be the result of the bifunctional nature and tendency toward internal cyclization of DTE (Cleland, 1964). Thus treatment of IgM with DTE produces only free thiol groups, which by themselves cannot undergo a disulfide exchange.

The results reported in the present study are compatible with a proposed model for assembly of immunoglobulin polymers involving disulfide exchange. This model would suggest that a particular intramonomer disulfide bond is generated during reductive depolymerization or exists in naturally synthesized monomers. An initial exchange is promoted by the interaction of J chain with one or two monomers. The remaining intramonomer disulfide bond undergoes exchange with the homologous bond on another monomer, thus generating the intermonomer disulfide. The polymer is constructed by sequential exchanges until sufficient units have been linked to allow closure of the polymer.

The suggestion that an intramonomer disulfide exists between cysteines destined to form the intermonomer bonds is consistent with the observations that the reassembly process is sensitive to excess reduction, potentially destroying this bond in the monomers, and that the presence of monomeric units is an insufficient criterion for polymer assembly even in the presence of an ancillary disulfide exchanging system. The location of the proposed intramonomer disulfide cannot be specified. The cysteine at position 575 in the human μ chain forms a disulfide bond with the J chain (Mestecky & Schrohenloher, 1974) and, therefore, may contribute to this proposed disulfide. In human IgA, J chain is disulfide bonded to the cysteines in homologous positions on one α chain each of adjacent monomers (Mestecky et al., 1974; Chapuis & Koshland, 1975), whereas the remaining homologous cysteines form direct intermonomer disulfides. The high sequence homology between the terminal 18 residues of the α and μ chains (Abel & Grey, 1967; Chuang et al., 1973), and the ability of J chain to polymerize either class of monomers, suggests by analogy that the penultimate cysteines of the μ chains also contribute to direct intermonomer disulfides. Attempts to localize the intermonomer disulfides in human IgM have placed them at Cys-414 in the μ chain (Beale & Buttress, 1969; Beale & Feinstein, 1969; Putnam et al., 1973); since these determinations have been made following mild reduction of IgM pen-

tamers, it is possible that this reductive depolymerization generated an intramonomer disulfide at position 575 and liberated the cysteines at position 414.

The rearrangement of disulfide bonds as proposed above suggests a mechanism for explaining the shift in IgM expression that accompanies B cell activation. Upon antigenic or mitogenic stimulation, the B cell is known to switch from the deposition of IgM monomers in the membrane to the secretion of pentamers. Recent evidence indicates that this switch appears to be coupled with the initiation of J chain synthesis. First, J chain was found to be absent in precursor B cells, but could be detected following mitogenic stimulation, just prior to pentamer secretion (Mestecky et al., 1977; Mather & Koshland, 1977). Second, the in vitro polymerization described in this and previous reports has demonstrated that the J chain may serve as a nucleating agent, initiating IgM pentamer assembly by a process of disulfide exchange. Thus it is possible that this exchange is coupled with a conformational alteration, which converts a hydrophobic form of IgM adapted for membrane incorporation into a more hydrophilic form suitable for secretion. The finding that J chain is synthesized in cells secreting nonpolymerized immunoglobulins (Brandtzaeg, 1974; Kaji & Parkhouse, 1975; Mestecky et al., 1977) suggests that the regulation of J chain production may be obligatory only for the initiation of polymeric immunoglobulin secretion, and that its continued synthesis following a class switch may be superfluous.

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