J Chain is Covalently Bound to Both Monomer Subunits in Human Secretory IgA*

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Previous work has established that the secretory component (SC) in human secretory IgA is covalently linked to only one of the two IgA monomer subunits, but it has not been clear whether the J chain is covalently linked to one or both of these subunits. In view of the asymmetry in the disulfide bonding between SC and the IgA subunits, an arrangement which follows disulfide interchange, several models for the disulfide linkage of J chain and the bonds between IgA subunits were envisioned and investigated. When sIgA was gel filtered through Sephadex G-200 in acetic acid, a single major symmetrical peak eluted at the front. This material contained SC, α and L chains, and all of the J chain. The greater resolution afforded by polyacrylamide gel electrophoresis in detergent confirmed that human sIgA contains no major noncovalently linked components in the 150,000-200,000 molecular weight range. In another series of experiments the Fc monomer, which is not covalently attached to SC, isolated after treatment of sIgA with IgA protease and cyanogen bromide, was investigated to learn whether a chain COOH-terminal octapeptides could be released by reduction. The results were negative. The available data thus favor a model in which J chain is disulfide-bonded to both IgA monomer subunits in sIgA.

Secretory immunoglobulin A, the major immunoglobulin in external secretions, is composed of four different kinds of polypeptide chains: H (α) and L chains, J chain, and secretory component (Halpern and Koshland, 1973; Heremans, 1974; Lamm, 1976). The J chain probably plays an important role in the dimerization of the IgA produced by local plasma cells (Della Corte and Parkhouse, 1973). On the other hand, final assembly of the sIgA molecule takes place in the mucosal epithelium where SC is synthesized (Poger and Lamm, 1974; Brandtzæg, 1974; Brown et al., 1976). Although no covalent interaction between J chain and SC exists (Mestecky et al., 1974a), the J chain is believed to play a role in the binding of IgA to SC by imposing the optimal conformation (Brandtzæg, 1976). This is consistent with experiments showing that dimeric IgA, which contains J chain, but not monomeric IgA, which lacks J chain, binds to SC (Mach, 1970; Weicker and Underdown, 1975; Lindh and Björk, 1976; Murkofsky and Lamm, 1979). The covalent binding of SC to IgA in human sIgA is through two disulfide bridges between SC and one of the two IgA monomer subunits (Cunningham-Rundles and Lamm, 1975; Lindh and Björk, 1976; Underdown et al., 1977; Garcia-Pardo et al., 1979). The J chain is also linked to dimeric IgA by two disulfide bridges, and the particular half-cystine residues participating in this binding have been determined for both the J chain and the α chain (Mendez et al., 1973b; Mestecky et al., 1974b).

In polymeric and dimeric serum IgA, which lacks SC, the J chain covalently bridges two monomer subunits (Chapuis and Koshland, 1976; Hauptman and Tomasi, 1975), but the situation could be different in sIgA. Indeed, a model in which J chain is disulfide-bonded to only one subunit has been subsequently proposed (Mestecky, 1976). In view of the fact that the union of SC to J chain-containing dimeric IgA in the assembly of human sIgA involves disulfide interchange (Cunningham-Rundles and Lamm, 1975; Lindh and Björk, 1976; Murkofsky and Lamm, 1979), which could alter the pattern of J chain attachment, it was considered worthwhile to investigate directly in sIgA whether the two α chains which are covalently bound to the J chain are from the same or different IgA monomer subunits. In this report, we present evidence in support of a model in which the J chain is covalently linked to both of the IgA monomer subunits in sIgA.

MATERIALS AND METHODS

Isolation of sIgA and Fragments—Secretory IgA containing both subclasses of IgA was isolated from pooled human milk and colostrum as previously described (Lamm and Greenberg, 1972). In addition, two fragments of sIgA from the IgA1 subclass were also studied. These include the Fc monomer which is not covalently attached to SC, and a J chain-containing fragment. Their preparation has been described in detail (Garcia-Pardo et al., 1979). In brief, sIgA was digested with streptococcal IgA protease, filtered through Sepharose 6B, and absorbed with an anti-L chain immunosorbent to give the SC-(Fc)2 fragment from the IgA1 subclass. This fragment corresponds to sIgA without the Fab portions. The SC-(Fc)2-J fragment was cleaved with cyanogen bromide and gel filtered on Sephadex G-150 in 1 M acetic acid (Fig. 1, which is taken from Garcia-Pardo et al., 1979). Peak III contains the Fc monomer not linked to SC, and Peak IV contains the J chain linked to COOH-terminal octapeptides of two α chains.

COOH-terminal Cyanogen Bromide Peptides of α and J Chains—The Fc monomer fragment, M, = 51,000, which is not linked to J and the J chain-containing fragment, M, = 15,000 (Peaks III and IV, respectively, from Fig. 1) were individually reduced with 0.01 M dithiothreitol in 0.3 M Tris-chloride, pH 8.2, and alkylated with 0.22 M iodoacetic acid in water and ending with starting solution diluted with 2 volumes of acetonitrile was used. The effluent was monitored at 210 nm with 0.1 absorbance units at full scale. Amino acid analysis was done with a Durrum D500 amino acid analyzer.
Determination of whether J chain in sIgA is also bound to the other IgA monomer subunits, whereas in the next three models disulfide bonds join J chain to both IgA subunits.

**RESULTS**

Intact sIgA (30 mg) containing both subclasses, was dissolved in 2 ml of 1 M acetic acid and applied to a Sephadex G-200 column in 1 M acetic acid (Fig. 2). Fractions I and II were pooled as shown, lyophilized, and 0.6–0.8 mg samples were analyzed by urea-PAGE (Fig. 3). This electrophoretic system allows the identification of J chain, which is the fastest moving protein clearly distinct from the other components (Koshland, 1975). By this method, J chain was found only in fraction I. Further characterization of fractions I and II from the G-200 column was achieved by SDS-PAGE (Fig. 4). Under these conditions, fraction I after reduction contained three bands of M, = 70,000 (SC), 52,000 (α), and 23,000 (L and J); fraction II contained a component of M, = 44,000, L dimers from the IgA2 subclass (Grey et al., 1968), which upon reduction gave a M, = 23,000 band.

When unreduced sIgA was subjected to SDS-PAGE, no distinct bands in the M, = 150,000–200,000 range were observed (Fig. 5), which confirms that no large noncovalently bound monomer subunits exist.

The J chain-containing cyanogen bromide fragment of sIgA of the IgA1 subclass (2 mg) was reduced, radioactively alkylated, and filtered through Sephadex G-25 (Fig. 6). Tubes 41 and 52 were separately lyophilized and subjected to high performance liquid chromatography. In both cases (not shown), single peaks eluted near the middle of the gradient with the Tube 41 material eluting slightly later. Tube 41 peptide had the following amino acid composition: Cys(cm), 0.4; Asp, 1.0; Thr, 1.2; Glu, 1.0; Gly, 1.0; Ala, 1.0; Val, 0.9; Leu, 0.9; Tyr, 0.8. This corresponds to the COOH-terminal 13-residue cyanogen bromide peptide of J chain (Mole et al., 1977). Tube 52 peptide had the following amino acid composition: Cys(cm), 0.4; Asp, 1.0; Thr, 1.2; Glu, 1.0; Gly, 1.0; Ala, 1.0; Val, 0.9; Tyr, 0.8. This corresponds to the COOH-terminal cyanogen bromide octapeptide of the α chain (Kratzin et al., 1975; Liu et al., 1976).

The Fc monomer (IgA1 subclass) not covalently linked to SC (5 mg) was treated in the same manner as the J chain-containing fragment. In this case, COOH-terminal octapeptide from the chain was undetectable even though multiple tubes from the appropriate region of the G-25 column were pooled and examined.

**DISCUSSION**

Secretory IgA contains two four-chain IgA monomer subunits. In human sIgA, Underdown et al. (1977) and Garcia-Pardo et al. (1979) demonstrated that SC is covalently bound to only one of them. The purpose of the present study was to determine whether J chain in sIgA is also bound to only one or, instead, to both IgA monomer subunits, a point which has been controversial (Mestecky, 1976). In Fig. 7, six different models for sIgA are considered. In the first three, J chain is covalently bound to only one of the IgA subunits, whereas in the next three models disulfide bonds join J chain to both subunits.

The first series of experiments was performed on intact sIgA, M, = 400,000, which was filtered through a Sephadex G-200 column under dissociating conditions to determine whether the two IgA subunits are covalently or noncovalently linked. If the two subunits are not covalently linked (Fig. 7, Model 1), there are two possibilities. In one, J chain is linked to one monomer and SC to the other, as illustrated. In this case, two major dissociable components would exist, one containing an IgA monomer and SC (M, = 220,000) and the second composed of the other IgA monomer and the J chain (M, = 165,000). In the other noncovalent possibility (not shown), J chain is linked to the same monomer subunit as SC; in this case two major dissociable components would also exist, one containing the IgA monomer bound to SC and to J chain (M, = 235,000) and the second corresponding to the other IgA monomer (M, = 150,000). However, after gel filtra-
The above-discussed experiments indicate that the IgA monomer subunits are covalently linked, but do not permit the conclusion that such linkage is through the J chain. Previous work with IgA protease and cyanogen bromide cleavage (Underdown et al., 1977) indicates that intersubunit disulfide bridges do not interconnect both Fc monomers NH2-terminally to the methionines near the COOH termini of the α chains. Thus, Model 2 of Fig. 7 can be excluded along with a variation in which J chains is disulfide bonded to the same subunit as SC.

Model 3 illustrates an arrangement in which J chain is intrasubunit, yet the two subunits are disulfide bonded between the COOH-terminal ends of the subunit attached to SC and the interior of the FC of the other subunit, the one to which J chain is attached (Mestecky, 1976). The second series of experiments was directed to this model by investigating the Fc monomer which is not covalently attached to SC (Peak III, Fig. 1). This fragment was isolated after cleavages with IgA protease, which splits at the hinge region, and cyanogen bromide, which splits the α chain at the two methionines near the COOH terminus. Under the conditions used to prepare this fragment, no disulfide bridges are split. Thus, the Fc monomer derived from the righthand subunit of Model 3 would lack the COOH-terminal octapeptides of its own α chains because of the cyanogen bromide cleavage, but would still be disulfide bonded to the COOH-terminal octapeptides released by cyanogen bromide from the other subunit on the left, the one to which SC is linked. However, no α-chain COOH-terminal octapeptide was released by reduction of the Fc monomer lacking SC. This negative result is not likely to be a question of yield or technique since α-chain COOH-terminal octapeptide was easily recovered from the J chain-containing cyanogen bromide fragment (Peak IV, Fig. 1). Thus, Model 3 is excluded.

In Models 4–6 of Fig. 7, the J chain is intersubunit. Model 4 is excluded for the same reason as Model 2, namely the lack of an intersubunit bridge linking the interiors of both Fc monomers (Underdown et al., 1977). Model 5 is unlikely for two reasons. First, if each IgA subunit has one of its two COOH-terminal penultimate half-cystines in intrachain disulfide linkage, the opposite internal half-cystines on the other α chains (marked by X’s in Model 5) should be present as cysteine residues, i.e., in reduced form, or as mixed disulfides.
However, studies on both rabbit and human sIgA suggest that insufficient sulphydryl groups and mixed disulfides are present in the Fc regions to accommodate Model 5 (Lindh and Björk, 1976; Elliott et al., 1980). Second, Model 5 predicts that the Fc monomer not attached to SC (Peak III, Fig. 1) should upon reduction release an α-chain COOH-terminal octapeptide, the one containing the penultimate half-cystine residue which is not attached to J chain. As discussed above, no α-chain COOH-terminal octapeptide was recovered from this Fc monomer.

Thus, Model 6 of Fig. 7 is left as the one model which is consistent with all the data, at least for the IgA1 subclass. Only one major peak was observed after G-200 gel filtration of sIgA in acetic acid (and there were no distinct penetrating bands of high molecular weight in SDS-5% PAGE of unreduced sIgA). The major G-200 peak was symmetrical, eluted at the void volume, and contained J chain by urea-PAGE. A minor second peak (fraction II) was also observed in this gel filtration; this did not contain J chain. When these gel filtration fractions were further analyzed by SDS-PAGE, fraction I was shown to be composed of sIgA as demonstrated by the three components SC, α, and L (plus J) chains released by reduction, and fraction II was shown to be composed of dimers of L chain from the IgA2 subclass (Grey et al., 1968).

In Model 6, the two IgA monomers are held together covalently through the J chain and by an interchain disulfide bridge involving the penultimate half-cystine residues at the COOH termini of the other two α chains. The SC is bound to only one of the IgA subunits, as was previously demonstrated (Underdown et al., 1977; García-Pardo et al., 1979), possibly to the same half-cystine residue in each α chain, and perhaps to residue position 311 since the functions of the half-cystines are known (Mendez et al., 1973a; Kratzin et al., 1975; Liu et al., 1976). If this is so, the corresponding half-cystines in the other IgA subunit could be joined together to form an extra intrasubunit interchain disulfide bridge.

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Fig. 7. Six models (not drawn to scale) of human sIgA to illustrate different possible arrangements of intersubunit, interchain, and some intrachain disulfide bridges. Disulfide bridges are designated by thin lines. The sIgA molecule contains two four-chain monomer subunits composed of α and L chains, an SC chain covalently linked to one of the subunits, and a J chain. The wavy line indicates where IgA protease cleaves. Open circles indicate invariant methionine residues, located 9 and 40 residue positions from the COOH termini of the α chains (Kratzin et al., 1975; Liu et al., 1976) and 14 residue positions from the COOH-terminus of the J chain (Mole et al., 1977); SC contains no methionines (Lamm and Greenberg, 1972). In Model 5, x designates half-cystines that should be present in reduced form or as mixed disulfides. In the first three models, J is intrasubunit, and in the second three, intersubunit. Variations of the first two models would have J chain linked to the same subunit as SC. The various models are discussed in the text, which indicates why Model 6 is favored. Whether both disulfides from SC go to two different α chains, as shown, or to only one α chain is not known. The most COOH-terminal inter-a chain disulfide bridge in the IgA monomer subunit not attached to SC in Models 1, 2, 4, 5, and 6 is speculative.

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