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Purification of J-Chain

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J-Chain의 순수분리에 관한 연구

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摘 要

免疫抗體分子의 基本 構造成分인 H-chain, L-chain외에 重合抗體分子 속에서만 발견되는 이른바, J-chain의 構造와 機能을 밝히기 위한 前段階로 J-chain의 純粹分離를 試圖하였다.

우선 多發性骨髓腫 患者의 血淸으로부터 重合型 IgA를 純粹分離한후, 이를 환원시켜 L-J-chain 混合物을 얻은 다음 3가지 方法, 1) 제조용 디스크 전기영동법 2) 脫鹽 透析法, 3) 이온—교환 크로마토그라피법으로 J-chain을 순수분리할 수 있었다. 위 3가지 방법으로 분리한 J-chain의 物理化學的 및 生化學的 성상은 동일하였으며, 사용한 세가지 방법 중 脫鹽 透析法이 가장 간편하고 효과적인 方法임을 알았다.

INTRODUCTION

In addition to two basic types of polypeptide chains, heavy (H) and light (L) in common immunoglobulin molecules, a third unique glycopeptide referred to as secretory component (SC) is associated with the IgA in various external secretions (SIgA) (Tomasi *et al.*, 1965; South *et al.*, 1966; Hong *et al.*, 1966; Hanson and Johansson, 1967; Tomasi and Bienenstock, 1968; Newcomb *et al.*, 1968; Shim *et al.*, 1969; Chung and Kang, 1973). Furthermore, a fourth type of polypeptide chain, so-called J-chain has been described in polymeric forms of human and rabbit IgA (Halpern and Koshland, 1970). Later, it was also found in human IgM (Mestecky *et al.*, 1971; Kang *et al.*, 1974) and even in leopard shark 19S immunoglobulin (Klaus *et al.*, 1971) and in canine polymeric IgA (Kehoe *et al.*, 1972), while it was not present in monomeric 7S IgA, IgG, IgD, or IgE (Nieder-

Abbreviations: Nomenclatures for human immunoglobulins recommended by WHO (Bull. World Health Org. 30(1964) were used).

meier *et al.*, 1972). In view of the findings of its absence in monomeric immunoglobulins and its presence in all polymeric forms of immunoglobulins examined so far, it has been suggested that J-chain plays an important structural function in assembly of IgG-like monomeric units in polymeric immunoglobulin formation (Halpern and Koshland, 1970; Morrison and Koshland, 1972; Kang and Shim, 1972; Chung and Kang, 1973; Mendez *et al.*, 1973; Wilde and Koshland, 1973).

Various methods have been used for isolation and characterization of J-chain. Morrison and Koshland (1972) have used immunoadsorbents to isolate human J-chain from IgA and IgM, while Mestecky *et al.* (1971) used chromatography on DEAE-Sephadex with 8 M urea. In both cases, the investigators separated the L- and J-chain fractions from the H-chain before subsequently isolating the J-chain.

The present study was performed in an attempt to exploit an efficient method for the isolation of the J-chain from polymeric IgA molecules. To this end several techniques of isolation procedures were employed.

MATERIALS AND METHODS

Isolation of Polymeric Serum IgA

Serum was obtained from patients with multiple myeloma and kept frozen until use. In the sera employed approximately 80% of the protein was in the polymeric form. Polymeric serum IgA was isolated from sera by the following procedures: A 16% (w/v) Na_2SO_4 precipitate of serum was redissolved and dialyzed against phosphate buffer (0.1 M) of pH 8.0. It was then chromatographed on BioGel P-200 (2.5 X 100 cm) equilibrated with the same buffer. The void volume contained the majority of the polymeric IgA, but was contaminated with α2-macroglobulin. The IgA+α2-macroglobulin was passed through a rabbit anti-α2-macroglobulin immunoadsorbent column. The resulting preparation was free of contamination as judged by double immunodiffusion on Ouchterlony plates and immunoelectrophoresis employing anti-whole human serum.

Dissociation of IgA into its Subunits

Reduction of IgA was carried out in Tris-HCl buffer, (0.1 M) pH 8.6 with 0.01 M dithiothreitol for 2 hours at 37°C under N_2 gas. Alkylation was done by the addition of a 10% molar excess of recrystallized iodoacetamide for 15 minutes at room temperature in a darkened test tube. After dialysis against 0.1 M NH_4HCO_3, the preparations were lyophilized.

The immunoglobulin preparations were dissolved in 4 M guanidine hydrochloride (ultra pure, Mann Research Lab, Orangeburg, N.Y.) and chromatographed on an amberized column (2.5×110 cm) of BioGel P-200 equilibrated with the same sol-

vent. Under these conditions, a fraction containing J- and L-chains (as determined by disc electrophoresis) was completely resolved from the H-chains. This fraction was dialyzed against 0.1 M NH_4HCO_3 and freeze-dried until use.

Electrophoresis

Analytical polyacrylamide disc gel electrophoresis was performed by the method of Ornstein and Davis (1964) using 5% monomer concentration in the lower gel. The protein bands were stained with Coomassie brilliant blue after fixing proteins in 12.5% TCA for 1—2 hours and destained in 7% acetic acid.

The same system used for analytical disc electrophoresis was also employed for preparative disc electrophoresis (apparatus from Shandon Co., London). The separation gel was 10 cm in height. The effluent buffer was the same as that in the gel diluted to the same ionic strength (0.06 M Tris-HCl, pH 9.3). The flow rate was maintained at 25 m*l*/hour with a peristaltic pump and 50 drop fractions were collected. The sample was applied directly to the separating gel in 2 m*l* of phosphate buffer, pH 6.7, with 10% sucrose. Bromphenol blue was added as a marker. Electrophoresis was carried out at 25 to 30 mA (600 volts) at 10°C for 20 hours.

Ion Exchange Chromatography

A mixture of L- and J-chains obtained by chromatography on a BioGel P-200 column was applied to DEAE-cellulose column (2×12 cm) equilibrated with 0.045 M Tris-glycine buffer, pH 8.9, containing 4 M urea and 0.05 M NaCl. The first elution was performed with starting buffer, which enabled us to isolate L-chain from J-chain. With increasing NaCl concentration to 0.6 M in the same buffer, consequently J-chain was eluted out completely from the column.

Other Techniques

Immunoadsorbents were prepared according to the methods of Cuatrecasas (1968) using antisera prepared against human α2-macroglobulin or the papain Fab fragment derived from IgG (Cohn fraction II).

Analytical ultracentrifugation was performed in a Spinco model E ultracentrifuge equipped with Schlieren and absorption optics.

Double immunodiffusion test in agar gel was performed by the method of Ouchterlony (1958). Immunoelectrophoresis was carried out according to the method as described by Scheidegger (1955).

RESULTS AND DISCUSSION

Purification of Polymeric Serum IgA

The final IgA preparation obtained from a rabbit anti-α2-macroglobulin imm-

unoadsorbent column showed a single precipitin arc in immunoelectrophoresis against both rabbit anti-whole serum and anti-IgA antiserum (Fig. 1. A). However, in analytical ultracentrifugation there appeared three different molecular species which were a dimer fraction sedimenting at 11S, a trimer fraction sedimenting at 13S, and a tetramer fraction sedimenting at 16S (Fig. 1. B). These fractions represented stable covalently-linked structures since no change in their relative proportions could be detected when the isolated IgA samples were made

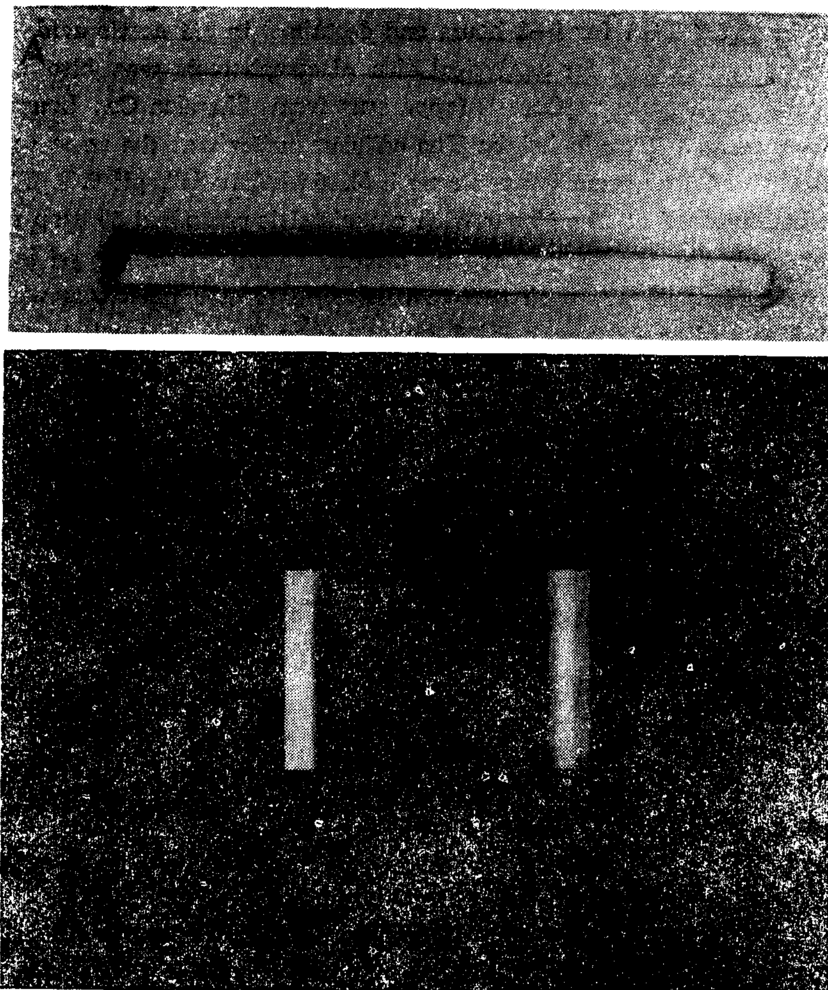

Fig. 1. (A) Immunoelectrophoretic pattern of purified IgA. Anode is at the right side and Amido black 10B stained. Upper trough: rabbit anti-human serum (Hyland); lower trough: rabbit anti-serum IgA (Behringwerke). The preparation showed a single precipitin arc indicating that the isolated IgA is pure.
(B) Schlieren pattern obtained by analytical ultracentrifugation of IgA preparation. Photograph was taken 176 min after reaching a speed of 53,640 rpm. The temperature of the rotor was 20°C and phase plate angle was 60 degrees.

in 4 M guanidine hydrochloride and reexamined in the analytical ultracentrifuge. The finding raised the possiblity that trimers and tetramers were being generated by disulfide exchange during the course of storage or purification. To test this possibility, we isolated several IgA preparations from fresh serum of multiple myeloma patients under conditions minimizing exchange. When the polymer distribution of these samples were determined, again similar amounts of trimer and tetramer molecules were found. Thus, if any disulfide exchange occurred, the reaction took place before the collection of the serum.

Isolation of J-chain

L-chain **fractions** were obtained by chromatography of reduced and alkylated IgA on BioGel P-200 in 4 M guanidine hydrochloride. J-chain was separated from L-chains by three different methods.

In previous report it was found that urea was not needed in disc electrophoresis to separate J-chain from L-chain. This has recently been reported by Klaus *et al.* (1971). Therefore, preparative electrophoresis could be used in the absence of urea. The results with an L-J fraction from IgA are shown in Fig. 2.

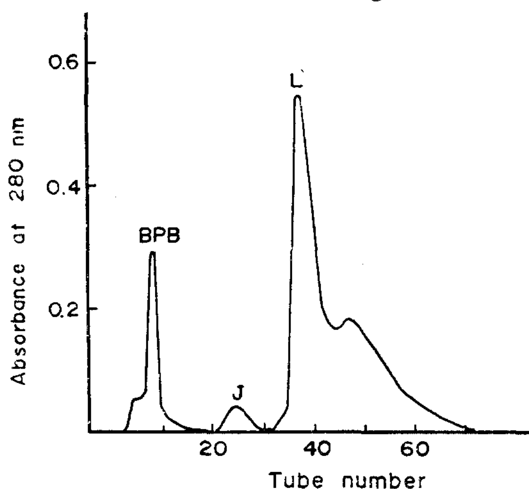

Fig. 2. Preparative disc electrophoresis elution pattern of J-L-chain mixture obtained from IgA myeloma protein. Bromphenol blue (BPB) was used as marker (see text).

A second method was dialysis against deionized water, which proved to be the simplest and the most satisfactory. Under these conditions J-chain precipitated, whereas the L-chain and a small amount of J-chain remained in the supernatant. The precipitated J-chain could then be redissolved in aqueous buffer for further study. In a typical experiment, a mixture of J- and L-chain (10 to 20 mg/m*l*) was dialyzed for 2 days at 4°C against distilled water. The yield was greater than

95% since little or no J-chain could be detected in the supernatant, by disc gel electrophoresis.

The third technique was the use of an ion exchange chromatography in 4 M urea. After applying the L-J fractions to DEAE-cellulose column in 0.045 M Tris-glycine, pH 8.9, containing 4 M urea and 0.05 M NaCl, stepwise elution was performed with increasing the concentration of NaCl in the same buffer. Two peaks were obtained (Fig. 3). The first one, which was eluted in the starting buffer, contained L-chain, while the second peak eluted by 0.6 M NaCl was identified to be the J-chain by disc gel electrophoresis.

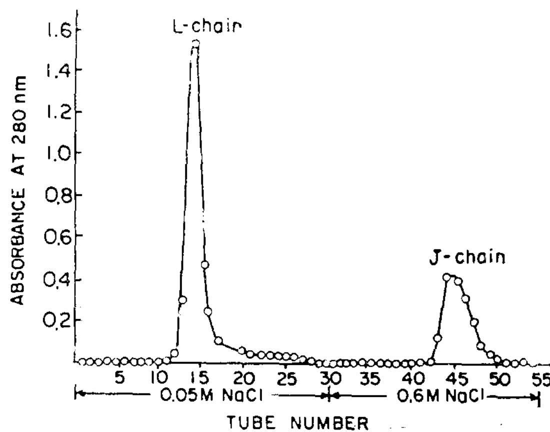

Fig. 3. Isolation of J-chain from J-L-chain mixture on a DEAE-cellulose column equilibrated with 0.045 M Tris-glycine buffer, pH 8.9, containing 4 M urea and 0.05 M NaCl. Fractions 1—30 were eluted with the starting buffer and fractions 12—17 contained L-chain. Fractions 31—55 were eluted with the same starting buffer containing 0.6 M NaCl and fractions 43—50 contained J-chain.

The disc gel electrophoretic patterns of J-chain obtained from IgA by the methods of preparative disc electrophoresis, water precipitation and ion exchange chromatography were shown in Fig. 4. The three J-chain preparations were appeared to be very similar in mobility and band-patterns. Sometimes the isolated J-chain by ion exchange chromatography and that of preparative polyacrylamide gel electrophoresis showed more than three bands. Considering the cysteine-rich content of these components, they might be produced by possible disulfide interchange during the process of purifications, and it consequently resulted in an appearance of several forms of polymeric J-chain. Another possibility of multi-bands on polyacrylamide gel could be illustrated to the different contents of sialic acids in J-chain molecule. Unpublished evidence supporting the posturation has been obtained by treating the J-chain with neuraminidase.

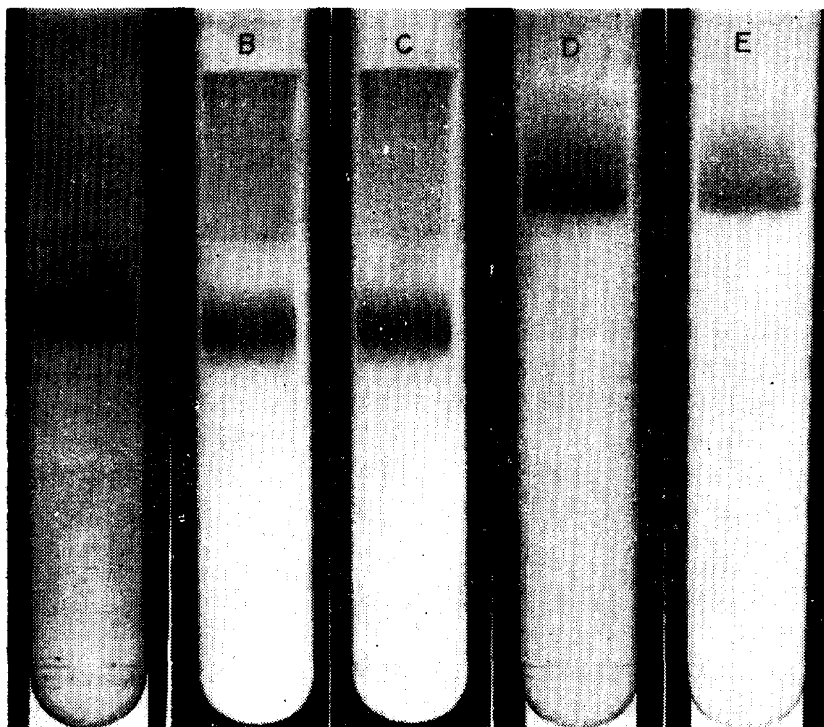

Fig. 4. Disc gel electrophoresis of J-chain of IgA obtained by preparative disc electrophoresis (A), by water precipitation (B), and by ion exchange chromatography (C). These are compared with the L-chain obtained from the supernatant of the J-chain precipitation (D) and that obtained from ion exchange chromatography (E) as shown in Fig. 3.

SUMMMARY

J-chain was purified from human serum IgA by three different methods which involve preparative disc electrophoresis, ion exchange chromatography and water precipitation. It was found that the simplest and satisfactory method was precipitation in water. All the J-chain preparations obtained from the above different procedures showed similar electrophoretic mobility and band-patterns.

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