AMINO ACID COMPOSITION OF THE \( \mu \) CHAINS OF IgM OF NORMAL SERUM, MONOCLONAL CRYOGLOBULIN AND WALDENSTRÖM MACROGLOBULIN

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The purified IgM of normal human serum, monoclonal cryoglobulin and Waldenström macroglobulin were reduced, alkylated and subjected to Sephadex G-100 filtration. The \( \mu \) chains separated were hydrolysed and analysed for amino acid content. In both monoclonal proteins the content of hydroxyproline in \( \mu \) chains was lower than in the IgM of normal serum. The \( \mu \) chain of monoclonal cryoglobulin contained less serine, methionine and tyrosine, whereas \( \mu \) chains of Waldenström macroglobulin showed a lower level of cystine as compared with the IgM of normal serum. On the other hand, the content of valine and basic amino acids was higher in both monoclonal proteins.

Heavy chains of IgM contain a variable (V) region of the 110 - 120 N-terminal amino acid residues, and a constant (C) region representing the rest of the chain. It is generally accepted that the amino acid sequence of the V-region may be characteristic for each specific antibody, whereas the composition and sequence of the C-region is probably nearly the same for all molecules of the IgM class (Wang & Fudenberg, 1975).

Monoclonal proliferation of the cells responsible for IgM synthesis in cryoglobulinemia or Waldenström macroglobulinemia leads to the overproduction of IgM molecules which usually show altered properties. Although these disturbances may be caused by changes in carbohydrate moiety or secretion of J chain (Brandtzaeg, 1975), one should consider first of all differences in the primary structure of the polypeptide chain (Wang & Fudenberg, 1975; Frangione et al., 1976).
The lack of hydroxyproline in monoclonal cryoglobulin was reported previously (Tomaszewski et al., 1977). The subject of the present study was to determine the amino acid composition of the whole μ chains separated from IgM of normal human serum, monoclonal cryoglobulin and Waldenström macroglobulin.

**MATERIAL AND METHODS**

Monoclonal cryoglobulin was obtained from the serum of the ANT patient, as described previously (Tomaszewski et al., 1977). Waldenström macroglobulin from ZUK patient and IgM from normal human serum were isolated by precipitation of euglobulin followed by repeated Sephadex G-200 chromatography (Tomaszewski, 1971). Purity of the isolated IgM was checked by means of polyacrylamide-gel electrophoresis according to Davies (1964) and by immuno-electrophoresis (Scheidegger, 1955), using poly- and mono-specific anti-μ sera from Hyland Laboratories (Costa Mesa, Calif., U.S.A.).

The reduction and alkylation of pure IgM from normal subjects and from the ANT and ZUK patients were carried out as described by Azuma et al. (1975). The solution containing 5 mg of protein/ml was dialysed against 0.2 M-Tris/HCl buffer, pH 8.2, containing 0.15 M-NaCl, and flushed with nitrogen for 15 min. The reduction was carried out in the presence of 10 mM-dithiothreitol for 1 h at room temperature. The released sulphhydryl groups were alkylated for 45 min in an ice-bath by iodoacetic acid added in 25% molar excess. To separate heavy and light chains, the solution was dialysed against several changes of 1 M-propionic acid at 4°C and subjected to gel filtration on the Sephadex G-100 column (2.6 × 50 cm), using 1 M-propionic acid as the eluent. Fractions containing heavy chains of IgM were pooled and concentrated in the Visking tubing in the presence of dry Dextran to about 5 mg of protein/ml.

Hydrolysis of pure μ chains was carried out under nitrogen in sealed glass tubes in 6 M-HCl at 110°C for 16 and 24 h. The hydrolysate was evaporated under vacuum, dissolved in 0.2 M-acetate buffer, pH 2.2, and submitted to chromatography on a one-column Jeol JLC-3BC2 amino acid analyser. Because of the limited amount of the material available, we were unable to run a parallel oxidative hydrolysis with periodic acid. However, the hydrolysis under our experimental conditions permitted to obtain sufficiently accurate results (Tomaszewski et al., 1979). The content of threonine and serine was calculated by extrapolation to zero time of hydrolysis. The hydroxyproline content was determined in a part of the hydrolysate by the modified Prockop-Udenfriend method (Tomaszewski & Hanzlik, 1971).

*Other chemicals.* Sephadex and Dextran were Pharmacia (Uppsala, Sweden) products. Amino acid calibration standard, iodoacetic acid and propionic acid were purchased from B.D.H. Chemicals Ltd. (Poole, England) and
dithiothreitol from Calbiochem (Lucerne, Switzerland). Other reagents, of an analytical grade, were obtained from P.O.Ch. (Gliwice, Poland) and were used without further purification.

RESULTS AND DISCUSSION

The purified preparation of IgM isolated from pooled normal human sera, serum from the ANT patient with cryoglobulinemia and the ZUK patient with Waldenström macroglobulinemia, were homogeneous in polyacrylamide-gel electrophoresis and on immunoelectrophoretic examination with polyvalent anti-human serum. Similarly, a single precipitation line against goat anti-μ serum was obtained. Using the anti-λ and anti-κ sera, only the κ-type of the light chains was detected in the ANT cryoglobulin, whereas both types of λ-chains were observed in IgM from normal human serum and from serum of the ZUK patient.

When the purified preparations of IgM were subjected to reduction and alkylation followed by Sephadex G-100 separation, the elution profiles were similar (Fig. 1). As demonstrated by polyacrylamide-gel electrophoresis (Fig. 2), and immunoelectrophoresis (Fig. 3), the first fraction represents chains without any detectable contamination with other proteins.

The results of amino acid analysis of the hydrolysate of the purified μ chains together with the earlier literature data, are presented in Table 1.

![Fig. 1. Sephadex G-100 gel filtration of reduced and alkylated IgM. Eluent: 1 M-propionic acid. I. Heavy (μ) chains; II. light chains; III. unidentified residual polypeptides; IgM of normal serum (●); cryoglobulin ANT (○); macroglobulin ZUK (▲).](image-url)
Fig. 2. Polyacrylamide-gel electrophoresis of: A, purified IgM; B, IgM after reduction and alkylation; C, fraction 1 from Sephadex G-100 filtration of reduced and alkylated IgM.

As compared with the \( \mu \) chains of IgM from normal serum, which represent a population of polymorphic molecules, amino acid composition of the \( \mu \) chains of our two monoclonal IgM species show some significant differences. In addition to a lowered content of hydroxyproline, ANT cryoglobulin showed less serine, methionine and tyrosine, whereas the content of valine and basic amino acids was increased. In ZUK macroglobulin a decrease in the content of hydroxyproline and cystine, and an increase in the content of valine and basic amino acids was also evident.

Little is known about the amino acid composition of \( \mu \) chains of IgM from normal human serum. Comparison of our results and amino acid composition of \( \mu \) chains of monoclonal IgM presented by others (Table 1) does not show any significant differences characteristic for Waldenström macroglobulin or monoclonal cryoglobulin IgM. The lowered content of glutamic acid, methionine and tyrosine in the \( \mu \) chain of cryoglobulin was observed by Zinneman et al. (1973). Some individual differences in three monoclonal cryoglobulin IgM were found by Wang et al. (1974). A lowered content of cystine and an increased content of basic amino acids in the

Fig. 3. Immunoelectrophoretogram of the purified \( \mu \) chains (F-I) against anti-\( \mu \) goat serum (A-\( \mu \)), anti-human serum (AHS), anti-\( \gamma \) (A-\( \gamma \)), and anti-\( \lambda \) light chains (A-\( \lambda \)).
Table 1

Comparison of amino acid composition of μ chains of IgM from normal human serum, monoclonal cryoglobulin IgM and Waldenström macroglobulin

Amino acids were expressed as residues/1000, tryptophan was omitted from the calculation.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Normal IgM</th>
<th>Cryoglobulin</th>
<th>Waldenström macroglobulin</th>
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<tr>
<td></td>
<td>ANT</td>
<td>MCE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>BOL&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Zinneman et al. (1973). <sup>b</sup> Wang et al. (1974). <sup>c</sup> Habeeb et al. (1970).

μ chain of Waldenström macroglobulin was observed by Habeeb et al. (1970), but Wang et al. (1974) did not find any significant differences in monoclonal macroglobulins they studied. Taking into account individual variability, it seems impossible to state which of these differences may be responsible for the altered physico-chemical properties of IgM in cryoglobulinemia or macroglobulinemia. It seems that in pathological conditions the amino acid composition of heavy chains depends on the type of the proliferated cell clone, whereas differences in the physico-chemical properties may be caused by slight changes in the amino acid sequence of the μ and/or the light chain, responsible for the conformational status of the molecule.

The differences in the content of hydroxyproline seem to be of special interest. Contrary to our previous results (Tomaszewski, 1973; Tomaszewski & Kowalewski, 1975) on correlation between the IgM level and serum protein hydroxyproline, the content of this amino acid in both monoclonal IgM and their μ chains was low. However, in our previous study the IgM level did not exceed 600 mg/100 ml of serum, whereas in the present investigation the observed concentration of IgM was very high: in the
ANT patient 1.6 g/100 ml and in the ZUK patient, 6.0 g/100 ml. The lack of hydroxyproline in these cases could be due to several reasons. First, some populations of IgM molecules may contain no hydroxyproline, and these could be accidently investigated. Secondly, if formation of the IgM hydroxyproline is due to the post-translational modification of the protein molecule, in the case of IgM overproduction, the activity of the enzymatic system responsible for hydroxylation of proline residues could be insufficient. Thirdly, if hydroxyproline is a constituent of other polypeptide chains closely connected with μ chain of IgM (Rybarska et al., 1975), the amount of this protein could be too small to bind with all possible sites of the μ chains. This last possibility, with respect to Cl-q, a known hydroxyproline-containing protein, is now under investigation.

REFERENCES

SKŁAD AMINO KWASOWY LÀńCUCHÓW ìgM SUROWICY PRAWIDŁOWEJ, MONOKLONALNYCH KRIOGLOBULIN I MAKROGLOBULIN WALDENSTRÔMA

Streszczenie

Oczyszczone IgM prawidłowej surowicy ludzkiej, monoklonalne krioglobuliny IgM i makroglobuliny Waldenstrôma poddano redukcji, alkilacji i filtracji na Sefadeksie G-100. Oznaczono skład aminokwasowy otrzymanych łańcuchów ciężkich. W łańcuchach ì białek monoklonalnych stwierdzono istotnie niższą zawartość hydroksyproliny. W porównaniu z IgM surowicy prawidłowej, łańcuchy ciężkie krioglobulin zawierały mniej seryny, metioniny i tyrozyny, natomiast makroglobuliny Waldenstrôma mniej cystyny. W białkach monoklonalnych zawartość waliny i aminokwasów zasadowych była wyższa.

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