# AMINO ACID SEQUENCE AT THE N-TERMINAL END OF A COLD AGGLUTININ KAPPA CHAIN

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Received 10 October 1968

Most structural studies on the immunoglobulins have been performed on the unique proteins found in the serum and urine of patients with myeloma and Waldenström's disease because these proteins are usually present in large amounts, and are chemically and immunologically sufficiently homogeneous to be considered monoclonal. However, an antibody activity has not been shown for the vast majority of these immunoglobulin proteins. In contrast, most antibody preparations are present in small amounts and are too heterogeneous to allow detailed structural study. To establish a correlation between antibody structure and specificity, it would be helpful to study a group of antibodies which were chemically homogeneous and which had similar specificity, in other words, a group of monoclonal antibodies.

The cold agglutinins found in the chronic cold agglutinin disease are known to be unusual antibodies. They are often present in serum in large amounts, are sufficiently homogeneous to appear as single narrow bands in the beta-gamma region [1,2] on cellulose acetate electrophoresis [3], and have almost exclusively light chains of the Kappa type [4]. Recent studies [5] of the alkaline urea starch gel electrophoresis of a series of cold agglutinin light chains showed these to be comparable to Bence-Jones light chains in their degree of homogeneity [6]. This report provides evidence based on amino acid sequence analysis that these agglutinins are monoclonal antibodies.

The anti-I cold agglutinin from a patient (*Ste*) with chronic cold agglutinin disease was purified from the serum by absorption onto red cell stroma at 0–2°C and elution at 37°C, followed by Sephadex G-200 Gel filtration as described elsewhere [5]. The preparation was shown to be electrophoretically homogeneous on cellulose acetate [3] and to react only with anti-Mu and anti-Kappa type sera by Ouchterlony [7] and immunoelectrophoretic [8] analysis. The purified cold agglutinin was reduced with 0.005 M dithiothreitol (Cleland's reagent), and alkylated with a 10% excess of iodoacetamide by the methods of Miller and Metzger [9]. The heavy (Mu) and light (Kappa) chains were separated by Sephadex G-100 gel filtration (1 N acetic acid) by the method of Fleischman et al. [10]. Of the recovered protein, 75% was heavy chain and 25% was light chain. Alkaline urea starch gel electrophoresis [6] of the cold agglutinin Kappa chain showed two strong and two weak bands (fig. 1).

The N-terminal sequence was determined by the phenylisothiocyanate technique [11]. Ten mg of lyophilized Kappa chain were subjected to 22 cycles in the protein sequenator [12] and the phenylthiodantoin derivatives were identified by thin layer chromatography [12] except for the derivative of arginine, where a Sakaguchi reaction on paper was used. Only one amino acid was observed in each position.

It has recently been shown by Niall and Edman [13] that Kappa-chains fall into either of two major subclasses depending on the sequence at the N-terminus. Sequences of the two prototypes $Kappa_{Smi}$ and $Kappa_{Tra}$ * are shown in table 1. Very similar conclusions have been reached independently by Smithies

* In accordance with the suggestions of the World Health Organisation [14] Niall and Edman [13] provisionally designated the new subclasses by abbreviations of the patients' names. These designations will be retained until agreement is reached on the kind, number and designations of the subclasses.

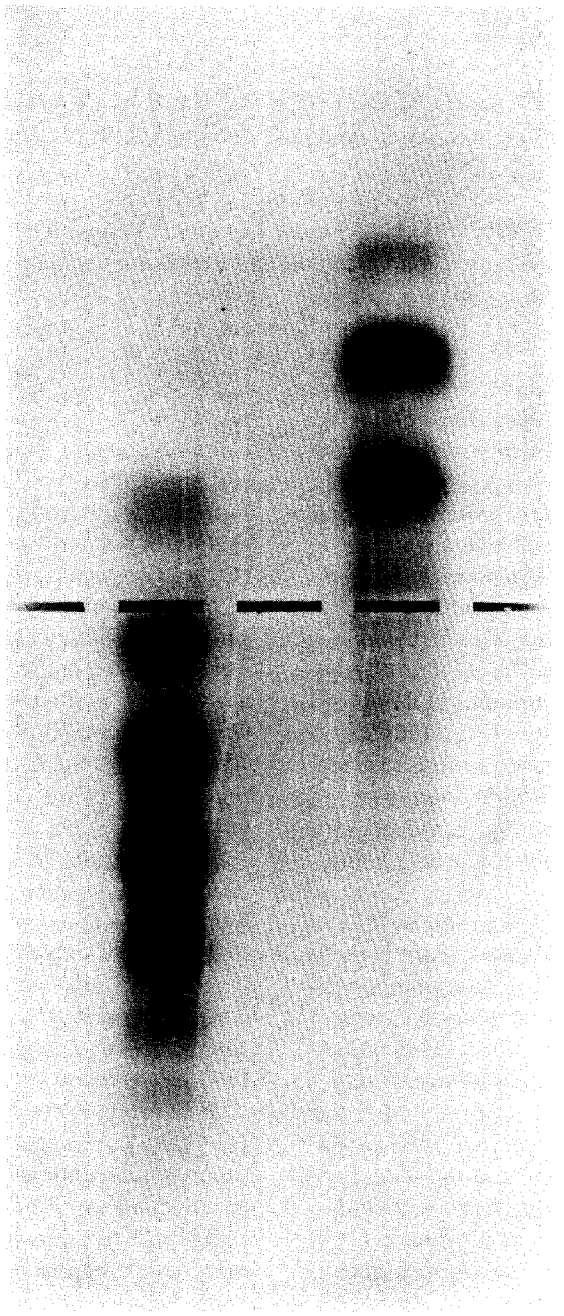

Fig. 1. Alkaline urea starch gel electrophoresis of normal light chains (left) and the light chains of cold agglutinin *Ste* (right). Anode to the bottom.

Table 1
N-terminal sequences of a cold agglutinin Kappa chain and of the two major subclasses of Kappa-chains.

| *Chain* | *Amino acid sequence* 1 10 20 |
|---|---|
| $Kappa_{Ste}$ | Glu-Ile-Val-Leu-Thr-Gln-Ser-Pro-Gly-Thr-Leu-Ser-Leu-Ser-Pro-Gly-Glu-Arg-Ala-Ala-Leu-Ser- |
| $Kappa_{Smi}$ * | Glu-Ile-Val-Leu-Thr-Gln-Ser-Pro-Ala-Thr-Leu-Ser-Leu-Ser-Pro-Gly-Glu-Arg-Ala-Thr-Leu-Ser- |
| $Kappa_{Tra}$ * | Asp-Ile-Gln-Met-Thr-Gln-Ser-Pro-Ser-Ser-Leu-Ser-Ala-Ser-Val-Gly-Asp-Arg-Val-Thr-Ile-Thr- |

* The sequence of the subclasses $Kappa_{Smi}$ and $Kappa_{Tra}$ is taken from the work of Niall and Edman [13].

[15]. Milstein [16] later presented evidence for a third subclass. The latter is obviously a minor group, since it is not observed in the sequence analysis of Kappa-chains from pooled IgG [13] **. The cold agglutinin Kappa chain clearly falls into subclass $Kappa_{Smi}$, from which it differs only in positions 9 and 20. The replacement of alanine for glycine in position 9 is frequently observed. However, the replacement of threonine for alanine in position 20 is exceptional, since this position is common to both subclasses, and has not been observed to vary before. This is convincing evidence (a) that the peptide chain has a unique amino acid sequence, and (b) that the cold agglutinin preparation itself was not detectably contaminated by the normal immunoglobulins.

The evidence presented here gives strong support to the earlier assumption that the antibodies found in chronic cold agglutinin disease are monoclonal in origin.

We thank Miss Susan Francis and Mrs. Elizabeth Minasian for technical assistance. The investigation was supported by a grant from the Australian Research Grants Committee (P.E.), and one of us (A.C.) was a recipient of a U.S. Public Health Service Fellowship (1-F2-A1-8963-01) from the National Institutes of Allergy and Infectious Diseases.

* It seems likely that, as more sequence material is collected, additional minor groups will be identified. In fact, a sequence has already been found, which does not fit into either of the two major groups, or the minor third group (P.Edman, unpublished).

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