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Sequence analysis of cloned cDNA encoding part of an immunoglobulin heavy chain

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ABSTRACT

The recombinant plasmid pH2l-l consists of mouse-derived complementary DNA (cDNA) in the E. coli plasmid pMB9. The mouse insertion has been completely sequenced, and encodes the CH3 domain and half the CH2 domain of the immunoglobulin γl heavy chain. The predicted amino acid sequence differs at several positions from that previously published for this protein. The pattern of codon usage resembles that in some other eukaryotic messenger RNAs. A computer program has been used to predict the optimum secondary structure for the mRNA encoding the CH3 domain and the inter-domain junction.

INTRODUCTION

With the development of elegant techniques (1-4) for cloning DNA complementary to eukaryotic messengers (cDNA), it has become possible to prepare large quantities of many such cDNAs for use both in sequence analysis, and in locating the same sequences in cellular DNA and RNA. The immunoglobulin heavy chain genes are of particular interest, since not only do they undergo the generation of diversity and joining of variable and constant regions which so far appear to be peculiar to immunoglobulins, but also they are members of a developmentally regulated multigene family, and the ancestral heavy chain gene apparently arose by tandem duplication of a still smaller genetic unit, the immunoglobulin domain.

A recombinant DNA plasmid, pH2l-l, has been constructed containing sequences from the heavy chain messenger of the IgGl-producing mouse myeloma MOPC21 (R. Wall, K. Toth, G. Paddock, R. Higuchi, and W. Salser, unpublished). Here we report the complete restriction map and DNA sequence of the mouse-derived insert in this cloned plasmid. It contains 459 nucleotides encoding the C-terminal 1½ domains of the γl constant region. Some characteristics of the coding sequence are discussed.

MATERIALS AND METHODS

Construction of pH21-1

Construction of a series of cDNA clones containing κ light chain mRNA sequences has been reported (5). The mRNA was from solid tumors of the IgG1-producing mouse myeloma MOPC21 (6). In these same experiments, cDNA clones containing heavy chain mRNA sequences were constructed by the same methods using the 16-17S fraction of the mRNA, in which the principal species is the heavy chain messenger (7). The cDNA was inserted into the EcoRI site of plasmid pMB9, by means of poly-dA and poly-dT "tails" on the respective 3' ends of insert and plasmid, and the recombinant plasmids were cloned in *E. coli* (R. Wall, K. Toth, G. Paddock, R. Higuchi, and W. Salser, unpublished). One clone gave a distinct peak of hybridization with 16-17 S MOPC21 mRNA (R. Wall and D. DeBorde, personal communication) and was designated pH21-1.

Restriction Analysis

Plasmid DNA was prepared as in refs. (8) and (9). EndoR.TaqI was prepared by S. Hendrich using an unpublished technique of M. Komaromy. It was used at 65°C in 10 mM HEPES pH 8.4, 6 mM $MgCl_2$, 6 mM β-mercaptoethanol , 25 mM $(NH_4)_2SO_4$, 100 μg ml^{-1} gelatin. Enzymes HaeIII and HincII were purchased from New England Biolabs, and AluI, HhaI and HinfI from Bethesda Research Laboratories; they were used as suggested by the suppliers.

Polyacrylamide gel electrophoresis of restriction fragments for preparative or analytical purposes was carried out in 20 x 40 cm gels made with 6% acrylamide, 0.2% methylene bisacrylamide, 12% glycerol, in running buffer. Running buffer was 50 mM Tris borate, 1 mM EDTA (TBE). For strand separation the gel consisted of 4% acrylamide plus 0.14% methylene bisacrylamide in the running buffer, which was 36 mM Tris base, 30 mM NaH_2PO_4, 1 mM EDTA. Samples for 6% gels were loaded in the restriction buffer, diluted if necessary, plus ¼ volume of dye solution (0.03% bromphenol blue plus xylene cyanol in 20% glycerol). Samples for strand separation were prepared in 90 μl of the same dye solution made up to 300 μl 0.3 M NaOH, and heated at 37°C for 3 minutes immediately before loading. After electrophoresis, DNA fragments were visualized by ethidium bromide staining and UV fluorescence, or, if end-labelled, by autoradiography. Elution of DNA fragments was carried out as in ref. (10), except that

incubation of the crushed gel in elution solution was for 2 days at 42°C.

DNA Sequence Analysis

Restriction fragments purified from digests of 150-300 µg of the 6-kb plasmid pH21-1 were treated with bacterial alkaline phosphatase (Worthington Biochemical, grade f) in 10 mM Tris-HCl pH 8.0, for 30 minutes at 37°C. This mixture was phenol-extracted thrice, ether-extracted twice, ethanol-precipitated, redissolved in 5 mM Tris pH 9.5, 0.1 mM spermidine, and 0.01 mM EDTA.Na_3, and then denatured by boiling. 5' end labelling with ^{32}P was carried out as in ref. (10), using Tris-HCl rather than sodium glycine as buffer. T4 polynucleotide kinase was purchased from PL Biochemicals, and $\gamma^{32}P$-ATP was made from $^{32}P_i$ (ICN Pharmaceuticals) (10). After ethanol precipitation, the labelled ends of the DNA were separated by strand separation, or by another restriction cleavage and electrophoretic separation of the fragments.

For sequencing we used four of the base-specific cleavage reactions of Maxam and Gilbert (10), entitled G>A, A>C, T+C, and C. A fifth reaction cleaving at A+G was performed as follows (A. Maxam, personal communication). End-labelled DNA and 1 µg carrier DNA were made up to 30 µl in 17 mM sodium citrate pH 4.0 and heated for 10 minutes at 90°C. 2 µl of 1 M NaOH was added and the mixture sealed in a capillary and heated for a further 30 minutes at 90°C. 20 µl of urea-dye mixture (10) was then added and the sample was ready for loading on a ladder gel.

Ladder gels (20% acrylamide, 0.7% methylene bisacrylamide, 7 M urea in TBE) were made, loaded and run as in ref. (10). In our later runs we used thin gels (11), of thickness 0.32 mm instead of the regular 1.6 mm, and found considerably improved resolution of bands. Ladder gels were autoradiographed at -70°C on Cronex 4 X-ray film with Dupont Hi-plus intensification screens.

Secondary Structure Prediction

The most stable secondary structure for the RNA represented by the sequence was predicted using the computer program of Studnicka et al. (12). This program will examine a large number of possible regions of base pairing to find that combination of regions which forms the most stable structure. The program begins by cataloguing all possible regions of 2 or more consecutive base pairs. There were 5231 regions in this "primary region catalogue" for the sequence considered here. It would be prohibitively expensive to consider all of these regions in a single computation cycle.

Therefore we rank the regions and carry out the computation in several cycles. The ranking uses a weighting function which is the sum of the energy of the region itself, divided by the square root of its length, and the energy of the best "local structure" which can be obtained by combining the region with all neighboring regions which are separated from it by less than 10 nucleotides on either strand (W. Salser and L. Nagy, in preparation). Where two primary regions would overlap, a "branch migration" procedure is used to determine the most stable non-overlapping combination of parts of the two primary regions.

The 150 regions with the most favorable weighting factors were chosen for the first cycle and the 100 most stable combinations of these regions were computed, the energies being calculated using the rules given in ref. 13. All these structures shared certain features which permitted us to break up the computation into three smaller jobs for the second cycle. In this second cycle all regions down to a weighting factor of 39 were considered (the equivalent of the top 900 of the original 5231 regions). The alternative structures for the second cycle were in turn examined for common features; these allowed us to subdivide the sequence into eight jobs for the final cycle, in which all regions of two or more base pairs were considered. In theory it would be possible to improve the structure slightly by considering single G-C pairs. For example, according to our base-pairing rules (13), the structure 5' CUUC-GU
3' ĠȦ-ĠUĊȦ
is more stable than the computed structure 5' CUUCGU
3' ĠȦGUĊȦ
by 2 kcal. This additional refinement of the structure was not performed.

Biosafety Precautions

P3 physical containment was used throughout for growth of transformed bacteria. The initial isolation of pH21-1 had been carried out in *E. coli* χ1849, an EK1 host, in compliance with the Asilomar Guidelines in effect at that time. When the NIH Guidelines (14) were issued, pH21-1 was transferred to *E. coli* χ1776, an EK2 host, and all subsequent experiments were conducted in accordance with those Guidelines.

RESULTS

Restriction Analysis

Various restriction enzymes were tested in parallel digests of pH21-1 and pMB9 DNA. Since the mouse sequence was inserted at the single EcoRI site in pMB9, it was expected that comparison of the digests would show one band unique to pMB9 which was replaced by one or more bands unique to pH21-1. This was found to be the case, and all the enzymes tested indicated an inserted segment of about 560 bp in length.

Mapping was helped by the observation that each pH21-1 digest exhibited one pair of submolar bands not shown by pMB9. Consistent values for the size of the insert could only be deduced if the doublet was considered to represent a single restriction fragment for each enzyme. It probably resulted from an approx. 44-bp deletion at a single site in a minor population of the plasmid DNA, since on preparative gels the larger band was homogeneous, while the smaller band was seen to be only one of up to seven equally spaced bands, the others being faint (Figure 1). Since no such heterogeneity is seen in pMB9, deletions of various sizes probably occurred in the mouse insert or the A.T joints. There is no repetitive sequence in the mouse insert which could account for this (see below), and the restriction fragment affected always contained the left-hand A.T joint, which therefore could be the site of the deletions.

It was possible to locate some of the restriction sites in the insert

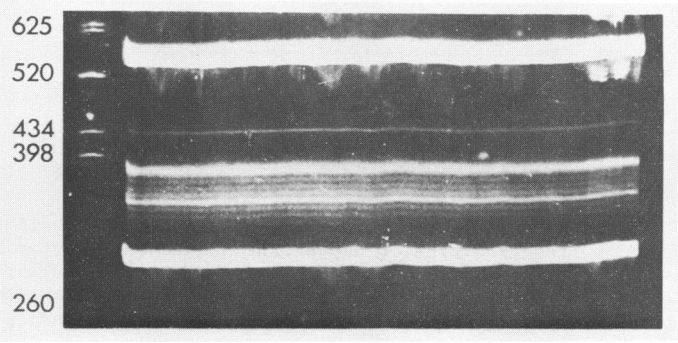

Figure 1. Preparative gel of TaqI-digested pH21-1 (300 µg), showing heterogeneity in one band. The uppermost and brightest member of the set is approximately 372 bp in length. The prominent doublets above and below the set are fragments from the pMB9 parts of the plasmid. The side lane contains HaeIII-digested pMB9, with fragment sizes marked in basepairs.

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