CONFORMATION DEPENDENCE OF A MONOClonAL ANTIBODY DEFINED EPITOPE ON FREE HUMAN KAPPA CHAINS

ROBERT L. RAISON* and HEATHER A. BOUX†
Clinical Immunology Research Centre, University of Sydney, Sydney, N.S.W. 2006, Australia

(First received 20 April 1985; accepted in revised form 12 July 1985)

Abstract—Chemically and enzymatically modified kappa chains were tested by inhibition radioimmunoassay for their ability to block the binding of antibody K-1-21 with native kappa chains. Complete reduction and carboxymethylation of intrachain disulphide bonds destroyed the free kappa-chain epitope, a result confirmed by Western blotting of unreduced and reduced kappa monomers and dimers. Purified V kappa fragments failed to block the homologous interaction while inhibition was obtained with a pepsin digest yielding predominantly the C kappa region. Dimeric kappa chains were less effective than monomers in the inhibition assay, although HPLC analysis of immune complexes demonstrated the binding of two antibody molecules per molecule of dimer. Thus, the epitope on free kappa chains recognized by K-1-21 is dependent upon conformational integrity of the C kappa domain, the decreased binding activity of dimeric chains possibly being due to minor conformational changes induced by C-domain interactions.

INTRODUCTIONS

Epitopes expressed by immunoglobulin molecules have been used at the cellular level to distinguish B-lymphocytes in general (Vitetta et al., 1971) and individual clones of B-cells in particular, based on idiotypic determinants (Krolick et al., 1979). We have recently reported the expression of a cell surface marker, KMA, on B-cells obtained from patients with kappa B-cell malignancies such as lymphoma, myeloma and macroglobulinaemia (Boux et al., 1983). KMA is detected by a murine monoclonal antibody, K-1-21, exhibiting specificity for free human kappa chains, and has been identified as non-heavy-chain-associated kappa chains expressed on the cell surface predominantly in the disulphide-bonded dimer form (Boux et al., 1984; Goodnow and Raison, 1985). This antibody also reacts with a component, STA, expressed in the cytoplasm of Sezary T-cells (Walket et al., 1985). Characterization of the STA molecule indicates that it is not kappa light chains. In order to resolve this apparent conflict of reactivities of K-1-21 we have examined the nature of the epitope recognized by this antibody on human kappa chains. The results demonstrate the dependence of this epitope on the conservation of the C kappa domain conformation, thus suggesting that the reactivity observed in T-cells may be attributed to a molecule possessing an Ig domain like conformation.

MATERIALS AND METHODS

MAb†

K-1-21, an IgG1 MAb, was derived from a fusion between NS-1 and spleen cells from mice immunized with human kappa BJP NAP. Production and purification of K-1-21 have been previously described (Boux et al., 1983).

Purification of BJP

Kappa and lambda BJPs were purified from urine of myeloma patients by ammonium sulphate precipitation at 75% saturation followed by gel filtration on AcA-44 (LKB Produkter AB, Stockholm, Sweden). Monomers and dimers of VOR kappa BJP were prepared in this way and purity verified by SDS-PAGE under non-reducing and reducing conditions.

Enzymatic and chemical modification of light chains

Peptic digestion of purified kappa chains to yield V_l and C_l fragments was carried out according to Seon et al. (1972).

Reduction and alkylation of kappa chains using dithiothreitol and iodoacetic acid was performed in either 0.5 M Tris-HCl, pH 8.2, to yield partially reduced chains or in 6 M guanidine-HCl to achieve complete disulphide-bond reduction (Fleischman et al., 1962).

Electrophoresis

SDS-PAGE was carried out in 12.5% acrylamide gels according to Laemmli (1970). Samples were
suspended in SDS sample buffer in the presence or absence of 4 mg/ml dithiothreitol and boiled for 3 min prior to application to the gel.

Immunoblotting of light chains separated on SDS-PAGE was performed by the method of Towbin et al. (1979). Nitrocellulose strips were blocked with BSA, reacted with antibody K-1-21 (1 μg/ml in PBS containing 0.1% BSA) then developed with alkaline phosphatase conjugated sheep anti-mouse Ig (New England Nuclear). Reactive bands were visualized with naphthol ASBI phosphate and fast red TR salt (Sigma Chemical Co., St. Louis, MO).

**SPRIA**

This was performed in PVC microtitre trays (Cooke Laboratories, Dynatech Corp., Alexandria, VA) according to Elleman and Raison (1981). Wells were coated with antigen at a concn of 100 μg/ml and antibody binding was detected by the addition of 125I-sheep anti-mouse Ig.

**Inhibition radioimmunoassay**

K-1-21 antibody was titrated against VOR kappa monomers in SPRIA to determine the optimal concn for use in the inhibition assay. A concn yielding 70% maximal binding was chosen, thus ensuring that K-1-21 was non-saturating in the subsequent inhibition assay. Samples for analysis in the inhibition assay were diluted in 0.1% BSA, 0.02% NaN3 in PBS, pH 7.2, mixed 1:1 (v/v) with diluted K-1-21 and incubated for 1 hr at 37°C in microtitre wells pre-blocked with 1% BSA. Triplicate samples were removed and K-1-21 binding activity assessed by SPRIA as described above.

**High-pressure liquid chromatography**

This was carried out using a Waters Liquid Chromatograph incorporating a model 6000A pump, U6K injector and 414 absorbance monitor (Millipore-Waters, Sydney, Australia). Separations of antibody and soluble antibody–antigen complexes were performed in 20 mM triethanolamine, 100 mM NaCl, pH 7.2, using a Waters I-250 and an LKB-TSK-3000 (LKB Produkter AB) column linked in series. Proteins eluted at a flow rate of 0.5 ml/min were monitored at 280 nm. Molecular-wt standardization of the columns was performed using thyroglobulin (669,000), ferritin (440,000), catalase (232,000) and aldolase (158,000) (Pharmacia Fine Chemicals, Uppsala, Sweden).

**RESULTS**

**Specificity of K-1-21 for free kappa light chains**

Antibody K-1-21, an IgG1, murine Ab is produced by a hybridoma cell line derived from spleen cells stimulated with the human kappa BJ3 NAP (Boux et al., 1983). After initial identification and cloning based on reactivity with NAP and VOR kappa chains, the specificity of this antibody for free kappa chains was confirmed by SPRIA using a number of human serum- and urine-derived proteins as immobilized antigens (Boux et al., 1983).

**Chemical and enzymic modification of kappa chains**

The nature of the K-1-21-specific epitope was examined using an inhibition assay to assess the effect of chemical and enzymic modification on the expression of the determinant on kappa chains. Reduction and carboxymethylation in a non-denaturing buffer had no effect on epitope expression, but, when carried out under denaturing conditions, this treatment completely destroyed the K-1-21 epitope (Fig. 1a). Oxidation with 20 mM periodic acid did not diminish the reactivity of the kappa chains (Fig. 1a). Figure 1b demonstrates the effect of pepsin digestion of kappa chains at 55 and 37°C, yielding predominantly Cg and Vg fragments, respectively (Seon et al.,...
Table 1. Reactivity of V kappa (V\textsubscript{k}) peptides with K-1-21\textsuperscript{a}

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Designation</th>
<th>Concentration ((\mu\text{g/ml}))</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>VOR\textsuperscript{a}</td>
<td>Intact light chain</td>
<td>10</td>
<td>32.4</td>
</tr>
<tr>
<td>FOR\textsuperscript{a}</td>
<td>V\textsubscript{c}</td>
<td>75</td>
<td>0</td>
</tr>
<tr>
<td>BR\textsuperscript{a}</td>
<td>V\textsubscript{c}</td>
<td>750</td>
<td>0</td>
</tr>
<tr>
<td>Te\textsuperscript{a}</td>
<td>V\textsubscript{c}</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Determined by inhibition immunoassay.

\textsuperscript{b}Generous gift of Dr K. Dorrington.

1972). Proteolytic cleavage with pepsin at 37°C greatly reduced the epitope expression while digestion at 56°C had little effect. Thus, at inhibitor concns of 100 \(\mu\text{g/ml}\), the crude V\textsubscript{L} preparation gave a value of 13% inhibition, while the crude C\textsubscript{L} preparation exhibited 80% inhibitory activity (Fig. 1b). The epitope was therefore retained in the digestion regime which yielded C\textsubscript{L} fragments. Furthermore, purified V\textsubscript{L} fragments from kappa chains FOR, BR and Te, used at concns equal to and greater than those used for the VOR digests, failed to inhibit the binding of K-1-21 to intact kappa chains (Table 1).

**Epitope expression on dimers and monomers of VOR kappa chains**

The reactivity of purified monomers and dimers of VOR kappa chains with K-1-21 was determined by inhibition analysis (Fig. 2). Using monomeric VOR bound to the PVC tray, 50% inhibition was obtained with monomer at a concn of 13 \(\mu\text{g/ml}\), whereas 27 \(\mu\text{g/ml}\) dimer was required to achieve the same level of inhibition.

In order to assess the number of epitopes expressed on dimers of kappa chain, the mol. wt of K-1-21–dimer immune complexes was determined and compared with that of the K-1-21–monomer complex. Figure 3 shows the HPLC elution profiles of K-1-21 alone (a), K-1-21 incubated with monomer (b), and K-1-21 incubated with dimeric kappa chain (c). Reference to a standard curve of mol. wt vs retention time revealed the apparent mol. wt of antibody–dimer complexes to be \(\sim 400,000\) and that of the antibody–monomer complex to be \(\sim 180,000\).

**Conformation dependence of the K-1-21-defined epitope**

Purified dimers and monomers of VOR were separated by SDS-PAGE under non-reducing and reducing conditions and the protein bands electrophoretically transferred to nitrocellulose. The nitrocellulose sheets were either stained for protein or specifically developed by incubation with K-1-21 followed by alkaline phosphatase conjugated second antibody (Fig. 4). Both dimers and monomers electrophoresed under non-reducing conditions were capable of binding with antibody, but samples run under reducing conditions were not visualized in the antibody blot.

**DISCUSSION**

Epitopes expressed on immunoglobulins and immunoglobulin polypeptide chains fall into several
Fig. 4. SDS-PAGE and immunoblotting of VOR kappa chain monomers (m) and dimers (d). Gels were run under non-reducing (A and C) or reducing conditions (B and D). Proteins transferred to nitrocellulose were either stained with Amido Black (A and B) or immunoblotted with K-1-21 followed by alkaline phosphatase conjugated sheep anti-mouse Ig (C and D).

categories. Idiotypic determinants are expressed in the variable regions of antibodies produced by a single clone of B-cells and of a given antigen specificity (Oudin, 1966; Kunkel et al., 1963). Allotypic determinants, found on antibodies with unrelated antigen specificities, are comprised of residues expressed in either the framework region of \( V_H \) (Mage et al., 1976), or in the constant region of light or heavy chains (Oudin, 1960; Dubiski, 1969). Serological distinction of murine \( V_k \) regions into serogroups is based upon sequence differences residing largely in the third framework region (Julius et al., 1981). Recently, a series of MAbs reacting with free kappa chains, but not with IgG-, IgM- or IgA-associated kappa chains have been described (Ling et al., 1983). The extent to which these various epitopes are governed by conformational vs primary sequence parameters is poorly understood.

The evidence presented in this paper indicates that the K-1-21 epitope expressed on free human kappa chains is determined by the conformation of the polypeptide. The finding that reduction under denaturing conditions completely destroys the determinant (Figs 1a and 4) demonstrates the requirement
for intra-chain disulphide-bond integrity, implying that the domain structure is essential for expression of this epitope. The failure of purified V kappa fragments to bind K-1-21 (Table 1), together with the retention of antibody binding activity in a pepsin digest (Fig. 1b) previously shown to yield predominantly C kappa domains, localises the K-1-21-defined epitope to the C kappa domain. The lack of expression of this epitope on light chains covalently associated with early chains suggests that such interchain interaction results in either an alteration of the conformation of C kappa or in steric blocking of the antigenic determinant.

The association between constant-region domains is very close for C1–C1H pairs in Fab fragments (Poljak et al., 1973; Segal et al., 1974; Matsushima et al., 1978) and intact immunoglobulin (Colman et al., 1976; Silverton et al., 1977) and involves transinteraction of the four beta sheets of the homology units (Amzel and Poljak, 1979). However, as such interactions also occur between C1 pairs of the light-chain dimer Mcg (Schiffer et al., 1973; Edmundson et al., 1975) and K-1-21 binds to a disulphide-bonded dimer of VOR (Figs 2 and 4) it would appear that domain–domain interaction is not responsible for the restriction of this epitope to non-heavy-chain-associated kappa chains.

A particularly interesting aspect of the M eq structure is the demonstrated conformational differences between the monomer and dimer forms of the lambda light chain. The angle made by the V L and C L axes in the monomer is 70° and is the same for one light chain in the dimer. However, this angle increases to 110° in the other member of the light-chain pair of the dimer (Schiffer et al., 1973; Edmundson et al., 1975). With this in mind we determined the number of K-1-21-reactive epitopes expressed on the VOR dimer. HPLC analysis of dimer–K-1-21 complexes (Fig. 3) revealed an apparent mol. wt of ~400,000 which is consistent with an overall composition of two IgG molecules binding per molecule of VOR dimer. Although this result argues against the loss of one of the K-1-21 epitopes due to possibilities in the V L–C L angle in one chain of the dimer, binding inhibition studies revealed a reduced capacity of dimer to inhibit monomer–K-1-21 interaction when compared to the monomer (Fig. 2). This suggests that K-1-21 binds with reduced affinity to the kappa chain dimer, perhaps due to conformational changes in one or both of the epitopes present in the dimer.

The conformational dependence of this epitope may explain our recent finding in human T-cells of a molecule exhibiting cross-reactivity with the K-1-21 antibody (Walker et al., 1985). It is likely that this represents the detection of a polypeptide in T-cells with an Ig domain like conformation. Such a molecule may represent a new member of the "Ig superfamily" said to include immunoglobulin, class I and class II histocompatibility antigens, and the antigen receptor on T-cells (Williams, 1984).

Other unexpected cross-reactivities of MAbs have been reported including that of an anti-Thy-1.2 with actin and vimentin (Dales et al., 1983; Dulbecco et al., 1981). Such reactivities may indeed become commonplace in view of the findings that MAbs raised against native proteins are in general conformation-specific (Westhof et al., 1984).

Acknowledgements—We are indebted to Dr K. Dorrington, University of Toronto, for his generous gift of V, fragments FOR, Br and Te. We thank Margaret Pritchard for the careful typing of the manuscript, Polly J for preparation of diagrams and Christopher Goodnow for stimulating discussion and constructive comment.

REFERENCES


