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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

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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 (Walker *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 IgG_1 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

*Author to whom correspondence should be addressed.
†Present address: Connaught Research Institute, 1755 Steeles Avenue, West Willowdale, Ontario, Canada.
‡Abbreviations: MAb, monoclonal antibody; BJP: Bence-Jones protein; SDS–PAGE, polyacrylamide gel electrophoresis in sodium dodecyl sulphate containing buffer; V_L, variable region of light chain; C_L, constant region of light chain; BSA, bovine serum albumin; SPRIA, solid-phase radioimmunoassay; PBS, 0.01 *M* phosphate, 0.15 *M* NaCl, pH 7.2.

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 ^{125}I-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% NaN_3 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 preblocked 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 441 absorbance monitor (Millipore–Waters, Sydney, Australia). Separations of antibody and soluble antibody–antigen complexes were performed in 20 m*M* triethanolamine, 100 m*M* 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 IgG_1 murine Ab is produced by a hybridoma cell line derived from spleen cells stimulated with the human *kappa* BJP 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).

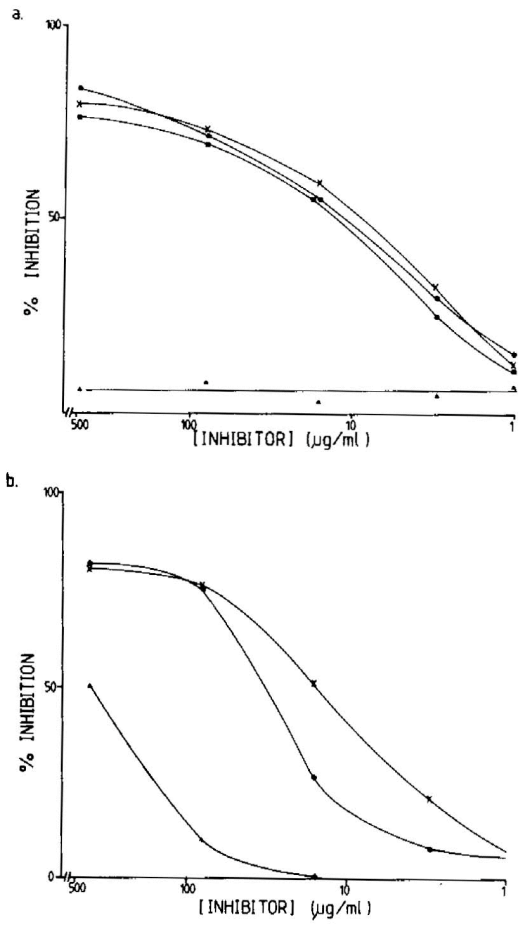

Fig. 1. Inhibition of binding of K-1-21 to VOR *kappa* chains by *kappa* chains subjected to the following treatments: (a) untreated (×——×), 20 m*M* periodic acid (●——●), reduction and alkylation in non-denaturing buffer (■——■), and reduction and alkylation in denaturing buffer (▲——▲). (b) Untreated (×——×), pepsin digestion at 55°C (●——●), and pepsin digestion at 37°C (▲——▲).

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 m*M* 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 C_L and V_L fragments, respectively (Seon *et al.*,

Table 1. Reactivity of V *kappa* (V_κ) peptides with K-1-21[a]

Inhibitor			
Designation	Fragment	Concentration (μg/ml)	% Inhibition
VOR	Intact light chain	10	32.4
		100	83.8
FOR[b]	V_κ	75	0
		750	0
Br[b]	V_κ	60	0
		600	0
Te[b]	V_κ	20	0
		200	0

[a]Determined by inhibition immunoassay.
[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 μg/ml, the crude V_L preparation gave a value of 13% inhibition, while the crude C_L preparation exhibited 80% inhibitory activity (Fig. 1b). The epitope was therefore retained in the digestion regime which yielded C_L fragments. Furthermore, purified V_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 μg/ml, whereas 27 μg/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 ~400,000 and that of the antibody–monomer complex to be ~180,000.

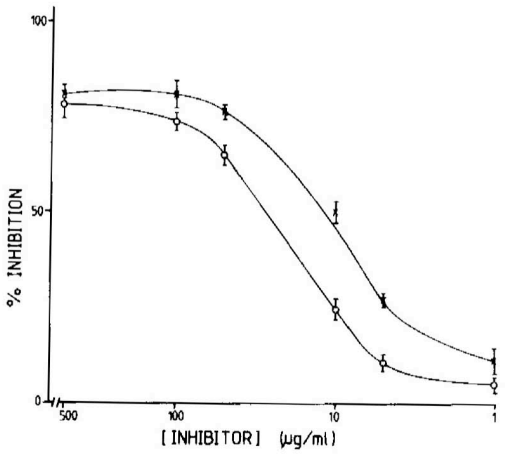

Fig. 2. Inhibition of binding of K-1-21 to VOR *kappa* chains with purified monomers (× —— ×) or dimers (○——○) of *kappa* chains.

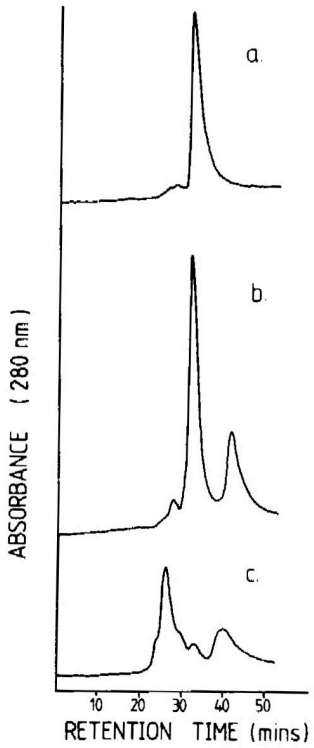

Fig. 3. HPLC elution profiles of: (a) K-1-21 antibody, (b) complexes of K-1-21 and monomeric *kappa* chains, and (c) complexes of K-1-21 and dimeric *kappa* chains.

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

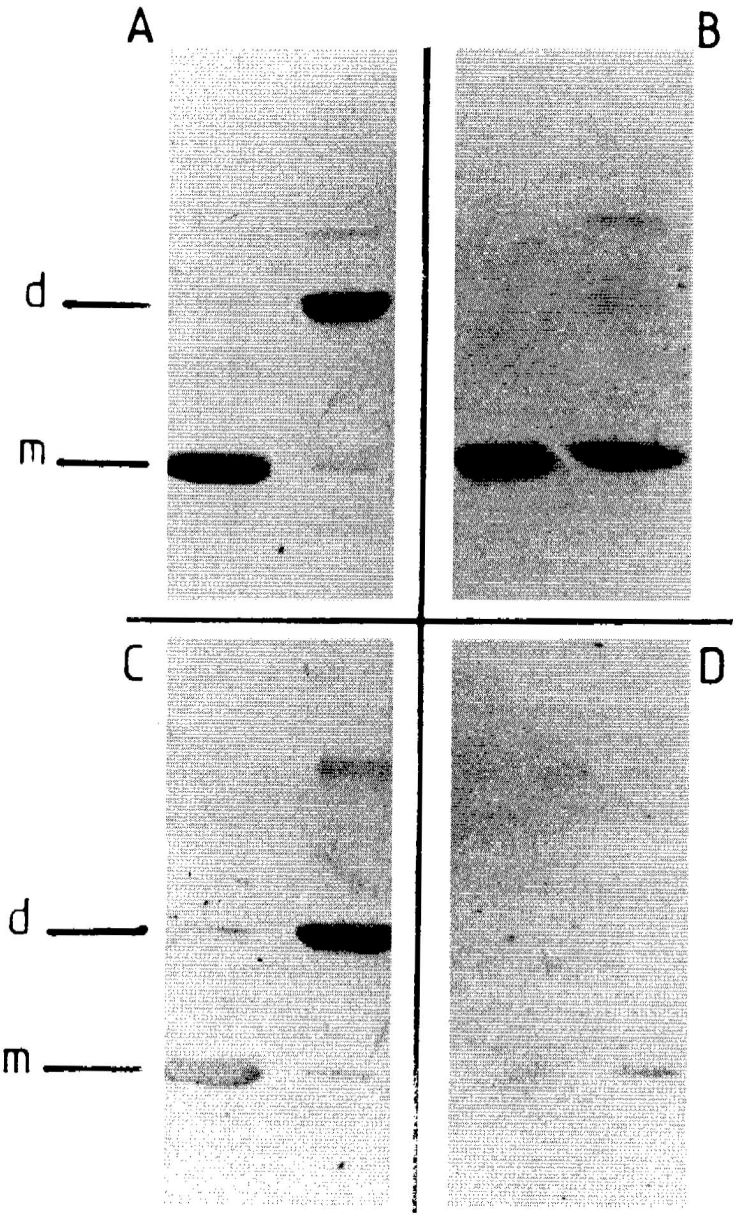

Fig. 4. SDS–PAGE and immunoblotting of VOR *kappa* chain monomers (m) and dimers (d). Gels were run under non-reducing (A and C) or reducing conditions (B and D). Proteins transferred to nitrocellulose were either stained with Amido Black (A and B) or immunoblotted with K-1-21 followed by alkaline phosphatase 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 *kappa* 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 inter-chain 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 C_L–C_{H1} 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 trans-interaction of the four beta sheets of the homology units (Amzel and Poljak, 1979). However, as such interactions also occur between C_L 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 Mcg 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 possibly to differences 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).

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