Binding of 2,4-Dinitrophenyl Compounds and Other Small Molecules to a Crystalline λ-Type Bence-Jones Dimer†

A. B. Edmundson,* K. R. Ely, R. L. Girling,# E. E. Abola, M. Schiffer,‡ F. A. Westholm, M. D. Fausch, and H. F. Deutsch§

ABSTRACT: Dinitrophenyl compounds, ε-dansyllysine, colchicine, 1,10-phenanthroline, methadone, morphine, meperidine, 5-acetyluracil, caffeine, theophylline, menadione (vitamin K_3), triacetin, and other compounds, were bound to crystals of a Bence-Jones dimer, an immunoglobulin consisting of two light chains with identical amino acid sequences but different conformations. The locations of the binding regions were determined by crystallographic techniques and related to the three-dimensional structure of the dimer. The combination of the two light chains resembles an Fab (antigen-binding) fragment, with one monomer (1) playing the structural role of the heavy chain. The solvent channel between the amino-terminal regions ("domains") of the light chains begins at the tip of the dimer as a large conical cavity. At the base of the cavity the solvent passes through an opening into a pocket. Binding of the small molecules listed above occurs in two sites (A and B) in the main cavity and one site (C) in the pocket. Site A is located near the cavity entrance, which is ringed with aromatic side chains interspersed with polar groups. This ring is composed of Tyr-34, Tyr-51, Glu-52, and Tyr-93 of both monomers, and Asp-97 of monomer 2. Only the residues from monomer 2 are associated with site A. In the crystal lattice, the site (A′) adjacent to monomer 1 and potentially equivalent to site A is blocked by constituents of a symmetry-related dimer. The side chain of Leu-110 from monomer 2 of this second molecule protrudes into site A′, and occupies a position between Tyr-34 and Glu-52. Site B is situated at a deeper level and extends from the Phe-99 side chains near the middle of the main cavity to four aromatic residues (Tyr-38 and Phe-101) at the base. Four seryl residues (36 and 91) are available for hydrogen bonding in site B. Sites A and B can be covalently labeled with 1-fluoro-5-iodo-2,4-dinitrobenzene, which reacts to the same extent with Tyr-34 and Tyr-38 of monomer 2. The total quantities of bound dinitrophenylleucine or triacetin are also evenly divided between sites A and B. Methadone and ε-dansyllysine bind only in site A, while caffeine and theophylline prefer site B. Bis(dinitrophenyl)lysine is not removed from sites A and B by soaking the crystals in buffered ammonium sulfate for 2 weeks, while ε-dinitrophenyllysine is displaced by the same treatment. The pocket containing site C is lined primarily by hydrophobic groups, including those of Tyr-38, Pro-46, Tyr-89, and Phe-101 of both monomers. 5-Acetyluracil binds isomorphously and with high occupancy in site C. Menadione, meperidine, 1,10-phenanthroline, *p*-nitrophenylphosphorylcholine, phenylmercuric compounds, and ω-bromoheptanoate also bind in significant quantities in site C. However, the crystals are physically damaged by these compounds, and the difference Fourier maps show additional troughs and peaks in the vicinity of site C. In the case of menadione, the changes were interpreted in terms of a displacement of a section of polypeptide chain between Lys-44 and Ala-45 in monomer 2 of the native protein. This chain is involved in one of the few interactions between the amino domains. It appears that a large entering group with the appropriate affinity can induce a fit in the deeper sites by disrupting such weak interactions along the solvent channel.

Myeloma proteins have been extensively used in reactions simulating the binding of antigens by functional antibodies, which are usually heterogeneous and difficult to isolate in large quantities (Eisen *et al.*, 1967, 1968, 1970; Schubert *et al.*, 1968, 1970; Terry *et al.*, 1970a,b; Ashman and Metzger, 1970; Goetzl and Metzger, 1970; Hadler and Metzger, 1971, 1973; Givol *et al.*, 1971; Jaffe *et al.*, 1971; Haimovich *et al.*, 1972; Riesen and Morell, 1972; Porter, 1972; Potter, 1972). Myeloma proteins and antibodies of the class IgG consist of two light (mol wt 22,000–23,000) and two heavy chains (mol wt 50,000–55,000). In some myeloma patients, free light chain dimers appear in the urine as Bence-Jones proteins (Edelman and Gally, 1962). We have isolated and characterized an IgG1 myeloma protein and the λ-type light chain dimer (Bence-Jones protein) from the patient Mcg (Deutsch, 1971; Deutsch and Suzuki, 1971; Fett *et al.*, 1973; Edmundson *et al.*, 1970, 1971, 1972; Ely *et al.*, 1973; Schiffer *et al.*, 1973). The three-dimensional structure of the Bence-Jones dimer at 3.5-Å resolution showed a cavity which we proposed would have properties similar to an antigen-binding site (Schiffer *et al.*, 1973). At approximately the same time Padlan *et al.* (1973) reported that the Fab fragment from a murine IgA(K) protein contained a binding "cleft" approximately 15 Å wide at the mouth, 12 Å deep, and 20 Å long. These dimensions are similar to those of the "cavity" in the Bence-Jones dimer. Poljak *et al.* (1973) found that the corresponding region in a human Fab′ fragment was a more shallow groove, 15 Å × 6 Å × 6 Å. The cavity, the cleft, and the groove are all partially lined by constituents of the "hypervariable" loops.

The present work was undertaken to determine the types of compounds which can be bound in the light chain cavity, and their steric relationships to the cavity lining.

The specificity of an antibody is associated with the variable

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‡ On leave of absence at the Max Planck Institute for Biochemistry, Munich, West Germany.

Present address: Department of Chemistry, University of South Carolina, Columbia, S. C. 29205.

§ Present address: Department of Physiological Chemistry, University of Wisconsin, Madison, Wis. 53706.

(V) regions (or "domains") of the light and heavy chains (Edelman and Gall, 1969; Milstein and Pink, 1970). These V domains contain about 110 amino acid residues, derived from the N-terminal regions of the light and heavy chains. Within the V domains are "hypervariable" sequences, some of which appear to be essential to the antigen-binding sites (Wu and Kabat, 1970; Kabat and Wu, 1971; Capra *et al.*, 1971; Kehoe and Capra, 1971; Franěk, 1971; Hilschmann *et al.*, 1972). "Affinity labeling" has been used to identify specific tyrosyl, lysyl, or histidyl residues which may be at or close to a hapten binding site (Wofsy *et al.*, 1962; Singer, 1967; Thorpe and Singer, 1969; Fleet *et al.*, 1969; Haimovich *et al.*, 1970, 1972; Goetzl and Metzger, 1970; Hadler and Metzger, 1971, 1973; Press *et al.*, 1971; Cebra *et al.*, 1971). The main cavity between the V domains of the Bence-Jones dimer contains Tyr-34 and -93 from the hypervariable regions, but lysyl and histidyl side chains are not present.

The patient from whom the dimer was obtained had amyloidosis, and Bence-Jones proteins from such individuals tend to have more affinity for tissue components than those from patients without this dyscrasia (Osserman *et al.*, 1964). It is therefore possible that the Mcg dimer has antibody activity toward some tissue component (see Deutsch, 1971). Rather than search for such an antigen, however, we chose to consider the molecule as a constitutive and primitive immunoglobulin of unknown biological specificity. Compounds were selected to define the chemical specificity of the protein for hapten-like molecules by criteria based mainly on the large number of aromatic residues in the main cavity and pocket. The selection was also strongly influenced by the experiences of other workers. For example, compounds containing dinitrophenyl (Dnp)[1] groups are bound by a surprising number of human myeloma proteins (Eisen *et al.*, 1967; Ashman and Metzger, 1970; Terry *et al.*, 1970a,b; Riesen and Morell, 1972). Murine myeloma proteins with specificities for Dnp groups or for phosphorylcholine have also been identified (Potter, 1972; Barstad *et al.*, 1974). The latter compound was added to the Mcg Bence-Jones crystals for comparison with results of the crystallographic study of the murine (McPC 603) Fab fragment with activity against phosphorylcholine (Padlan *et al.*, 1973, 1974).

Poljak *et al.* (1972, 1973) are determining the structure of a human (New) Fab′ fragment from an IgG myeloma protein binding vitamin K_1. Other myeloma proteins binding Dnp compounds have shown cross-reactivity to members of the vitamin K series (*e.g.*, menadione) and/or to purine and pyrimidine derivatives such as caffeine and 5-acetyluracil (Schubert *et al.*, 1968, 1970; Smith and Potter, 1969; Underdown and Eisen, 1971; Riesen and Morell, 1972).

In the present investigations Dnp and other aromatic compounds (*e.g.*, dansyllysine), as well as molecules known to cross-react with anti-Dnp proteins, were diffused into crystals of the Bence-Jones dimer. Dipeptides, monosaccharides, and lipid components were also used for screening. Among the lipids, triacetin was chosen because of its small size and relatively high solubility. Tributyrin was used to test the effects of increasing the size and hydrophobicity of the triglyceride, and ω-bromoheptanoate was selected because of its solubility, hydrophobic straight chain, and bromine "heavy atom."

[1] Abbreviations used are: Dnp, 2,4-dinitrophenyl; IDnp, 5-iodo-2,4-dinitrophenyl; Dnp-F, 1-fluoro-2,4-dinitrobenzene; IDnp-F, 1-fluoro-5-iodo-2,4-dinitrobenzene; dansyl, 5-dimethylaminonaphthalene-1-sulfonyl; Tos-PheCH$_2$Cl, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone.

Materials and Methods

Preparation of 1-Fluoro-5-iodo-2,4-dinitrobenzene (IDnp-F). The reagent was synthesized by nitration of 1-fluoro-3-iodobenzene by the method of Schramm (1972). *Anal.* Calcd: C, 23.10; H, 0.65; N, 8.98; O, 20.51; F, 6.09. Found: C, 23.35; H, 0.84; N, 9.15; O, 20.59; F, 6.36. Iodine analyses gave high values (*e.g.*, 47.1% *vs.* a theoretical value of 40.7%), which are presently unexplained. In reactions with the Bence-Jones dimer in the crystals and in solution, the synthesized IDnp-F was indistinguishable from a sample of the reagent kindly provided by Dr. Schramm.

Reaction of Bence-Jones Dimer with Dnp-F and IDnp-F in Solution. To conserve IDnp-F reagent, all preliminary experiments were performed with Dnp-F (see Hirs, 1967). The reactions were carried out in aqueous solutions for 90 min in the dark and under a blanket of nitrogen. A 30–100-fold excess of reagent was added initially and the solution was kept saturated by vigorous stirring. A constant pH was maintained by addition of 0.1 N NaOH delivered from a Radiometer SBR 2/TTT1/ABU 1 pH-Stat.

Samples of 10 mg of the Bence-Jones protein were allowed to react at 40° with Dnp-F at pH 7.4 and 6.2. After 90 min the excess reagent was removed by extraction with diethyl ether and the solution was lyophilized. Samples of ~1.5 mg of protein were hydrolyzed in 6 N HCl or in 3 M *p*-toluenesulfonic acid containing 0.2% 3-(2-aminoethyl)indole (Liu and Chang, 1971) at 110° for 24 hr in sealed, evacuated tubes. The hydrolysates were subjected to quantitative amino acid analysis by the method of Spackman *et al.* (1958).

A sample of 200 mg of protein was treated with IDnp-F at 27° and at pH 6.2. The derivatized protein was separated from the reagent and its hydrolysis product (iododinitrophenol) on a 2 × 20 cm column of Sephadex G-25, equilibrated with deaerated 0.1 M phosphate (pH 6.2), and protected from light with aluminum foil. The eluent was concentrated to 20 mg of protein/ml by vacuum dialysis in the dark, and attempts were made to crystallize the derivative in ammonium sulfate by the method used for the native protein (Edmundson *et al.*, 1971). These attempts have been unsuccessful.

Another sample of 200 mg of protein was allowed to react with IDnp-F by the same procedure. After removal of aliquots for amino acid analysis, the derivatized protein was reduced with 2-mercaptoethanol and alkylated with ethylenimine at pH 8.6 by the method of Raftery and Cole (1966). The solution was dialyzed exhaustively against deionized water and lyophilized.

The aminoethylated protein was treated with Tos-PheCH$_2$Cl-treated trypsin at 40° and at pH 8.0 for 24 hr in the dark. Initially, the ratio of trypsin to substrate was 4% by weight; two additional aliquots of trypsin, each equivalent to 1% by weight, were added after 1.5 and 3 hr. The pH was lowered to 3.5 with 50% (v/v) acetic acid to terminate the hydrolysis. The digest was centrifuged to give soluble and insoluble ("core") fractions.

The soluble peptide fraction was placed on a 3.8 × 141 cm column of Bio-Gel P-6, equilibrated with 0.1 M acetic acid at 22°. The column was shielded from light with aluminum foil. The eluent was monitored at 230 and 280 nm with a Gilford Model 2000 spectrophotometer. The absorbance of each fraction of 5 ml was next determined at 365 nm to detect the IDnp-labeled peptides. Fractions absorbing at 365 nm were lyophilized and subjected to paper electrophoresis at pH 3.5. Peptides were further purified by electrophoresis at pH 1.9 and eluted from the paper with 1 M acetic acid. After lyophilization, the peptides were hydrolyzed for 24 hr in 3 M *p*-toluenesulfonic

TABLE I: Summary of Binding Studies.

Derivative	Concn (mM)	Soak Period (Days)	Site	Mol. Bound/ Mol. Dimer
IDnp			A	0.82
			B	0.82
		Backwash[a]	A	0.80
			B	0.71
Bis(Dnp)Lys	0.8	2	A + B	0.30
			C	0.01
		Backwash[a]	A + B	0.31
			C	0.01
Bis(Dnp)Lys	0.8	24	A + B	0.50
			C	0.09
		Backwash[a]	A + B	0.37
			C	0.12
ϵ-Dnp-Lys	1.0	22	A + B	0.36
			C	0.05
		Backwash[a]		
ϵ-Dinitrophenylamino-caproate	1.0	14	A + B	0.16
			C	0.03
ϵ-Dinitrophenylamino-caproate	1.0	42	A + B	0.22
			C	0.05
Dnp-Leu	0.8	5	A	0.42
			B	0.43
Dnp-Leu	0.8	20	A	0.51
			B	0.47
		Backwash[a]		
Dnp-Trp	0.8	7	A + B	0.52
Dnp-Trp	0.8	35	A + B	0.58
			C	0.12
Dnp-Phe	0.8	4	A + B	0.32
Dnp-Phe	0.8	23	A + B	0.40
			C	0.07
Bis(Dnp)Tyr	1.0	6	A + B	0.08
			C	0.01
Bis(Dnp)Tyr	1.0	51	A + B	0.47
			C	0.15
Dinitrophenol	1.0	29		
Dinitroaniline	1.0	36		
p-Nitroaniline	1.0	26	C	0.10
p-Nitrophenyl-phosphorylcholine	5.0	52	A	0.29
			C	0.49
Phosphorylcholine	10.0	28		
ϵ-Dansyl-Lys	1.0	31	A	0.36
Methadone	0.5	1	A	0.30
1,10-Phenanthroline	1.0	13	A	0.98
			C	0.18
Morphine	1.0	39	A	0.08
			C	0.02
Colchicine	0.5	3	A + B	0.62
Caffeine	0.8	10	A	0.06
			B	0.44
Theophylline	1.0	10	B	0.30
5-Acetyluracil	1.0	25	C	0.71
Menadione (vitamin K_3)	0.5	6	C	0.67
Meperidine (Demerol)	1.0	36	A	0.09
			C	0.23
Triacetin	Saturated	94	A	0.20
			B	0.17
			C	0.04
Tributyrin	Saturated	51	A + B	0.07
			C	0.03

TABLE I (*Continued*)

Derivative	Concn (mM)	Soak Period (Days)	Site	Mol. Bound/ Mol. Dimer
ω-Bromo-heptanoate	10.0	7	A	0.15
			B	0.03
			C	0.30
Leu-Trp	1.0	10		
Gly-Leu	1.0	8		
Gly-Ile	1.0	58		
His-Phe	1.0	35		
L-Rhamnose	2.0	16		
L-Fucose	2.0	38		
D-Galactose	2.0	27		
N-Acetyl-D-galactosamine	2.0	36		
D-Glucosamine	1.0	15		

[a] In the backwash experiments crystals containing Dnp compounds were washed with 1.9 M ammonium sulfate, buffered at pH 6.2, and then stored in a fresh aliquot of this solution in the dark for 14 days (except for the IDnp-labeled crystals, which were stored for 1.5 years).

acid at 110° in sealed, evacuated tubes and subjected to amino acid analysis.

Reaction of Crystalline Protein with IDnp-F. Approximately 5 molar quantities of IDnp-F in ~3 μl of methanol were added to crystals in 1.9 M ammonium sulfate, which was 0.1 M in pH 6.2 phosphate (Edmundson *et al.*, 1971). The amount of protein in each crystal was crudely estimated from the weight of a comparable crystal (range of 100–200 μg) and the fractional volume (~0.4) of protein (Edmundson *et al.*, 1971). The reactions were carried out at 22° in the dark. Within 6 hr the crystals had turned yellow, and surface cracks transverse to the long axis appeared after 12 hr. Supernatant solutions were removed after 24 hr, and the crystals were washed with 1.9 M ammonium sulfate. The cracks deepened during the wash step, but posed no serious problem because crystals are routinely cut in the transverse direction before mounting in quartz capillaries for X-ray diffraction. Diffraction data were collected within 3 days and also after 1.5 years of soaking in the ammonium sulfate solution.

Noncovalent Binding of Various Compounds in the Crystals. Crystals of the Bence-Jones dimer were transferred from mother liquor to vials containing 1.9 M ammonium sulfate buffered at pH 6.2. Solutions of the compounds listed in Table I were added to the vials, which were stored at 22° in the dark. Crystals were mounted in quartz capillaries immediately before use with the diffractometer. The soaking periods generally were longer than 5 days (see Table I), but were as short as 1 day when surface cracks appeared in the crystals. Among the compounds causing physical damage to the crystals were ε- and bis(dinitrophenyl)lysine, bis(dinitrophenyl)tyrosine, *p*-nitrophenylphosphorylcholine, methadone, 1,10-phenanthroline, colchicine, menadione, ω-bromoheptanoate, and phenylmercuric salts. Dnp-Leu, Dnp-Phe, Dnp-Trp, dinitrophenol, dinitroaniline, ε-dansyllysine, caffeine, theophylline, 5-acetyluracil, meperidine, triacetin, tributyrin, and various peptides and monosaccharides did not crack the crystals. Crystals containing bis(dinitrophenyl)lysine, ε-Dnp-Lys, or Dnp-Leu were removed from the reagent and soaked in 1.9 M ammonium sulfate (pH 6.2) for 2 weeks.

Crystallography. Data for all crystals were collected to 6.5 Å with an automated Picker four-circle diffractometer by the procedure previously described (Edmundson *et al.*, 1972). The sites occupied by the IDnp or other groups were identified by difference Fourier analysis, using the protein phases determined earlier. The data for the IDnp derivatives were extended to 3.5 Å.

"Residual maps" (Matthews, 1970) were calculated after the refinement of phase angles and heavy-atom positions for all data to 2.5-Å resolution. Inspection of the maps for each heavy-atom derivative used to solve the structure of the Bence-Jones dimer revealed no additional binding sites. The residual electron density in the regions of the A, B, and C sites discussed in this article averaged only about half the lowest contour level considered significant. Peaks appearing in these sites on the difference Fourier maps for the IDnp and other derivatives are therefore not attributable to heavy-atom "ghosts."

To calculate the approximate number of molecules bound per dimer, the electron density values were summed for each derivative peak and compared with the corresponding sum for the mercury atom in the S–Hg–S derivative (see Ely *et al.*, 1973). To avoid overestimating the results, the summation was limited to values exceeding the noise level in each map. The noise level was defined in terms of peaks of electron density which could not be explained in a stereochemical manner (see Henderson and Moffat, 1971). In the case of the S–Hg–S derivative, the noise level was about 16% of the maximum value in the peak corresponding to the mercury atom inserted into the interchain disulfide bond of each dimer.

Model Building. A model of the segments forming the main cavity and the pocket between the V domains of the Bence-Jones dimer was constructed with Watson-Kendrew skeletal models fitted to a 2.5-Å electron density map with the aid of an optical comparator (see Schiffer *et al.*, 1973, for details of the 3.5-Å map). The amino acid sequence data of the Mcg light chain were obtained by Fett and Deutsch (1974).

Results and Discussion

Reaction of Bence-Jones Dimer with Dnp-F or IDnp-F in Solution. At pH 7.4 and 40°, approximately 2.0 lysyl, 0.7 histidyl, and 1.5 tyrosyl residues in each light chain were found by amino acid analyses to have been derivatized with Dnp-F in solution. The reaction with lysine decreased to ~0.4 residue per

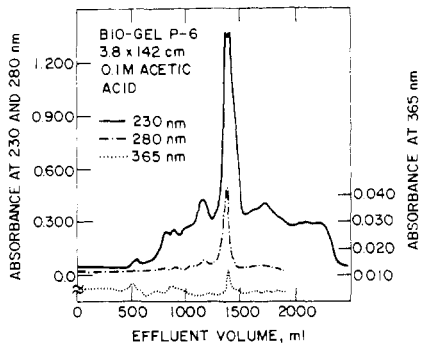

FIGURE 1: Elution profile for the preliminary fractionation of soluble tryptic peptides of the reduced and alkylated IDnp derivative of the Bence-Jones protein. A sample of 100 mg of protein was used; the eluent was monitored at three wavelengths as listed on the drawing. Fractions of 10 ml were collected. The band absorbing at 365 nm and appearing after the emergence of about 1400 ml of effluent was subjected to paper electrophoresis at pH 3.5 and 1.9 to obtain a peptide containing IDnp-His in the sequence Ser-IDnp-His-Arg.

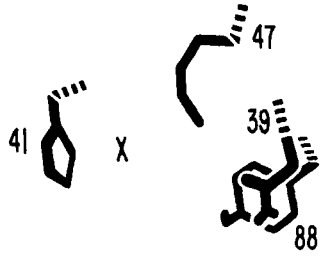

FIGURE 2: Schematic drawing of the side chains around one of the major sites for binding organic mercurials outside the solvent channel. The side chains belong to His-41, Lys-47, Gln-39, and Tyr-88; the bond between the α and β carbon atoms is represented in each case by a striated line. The general location of bound mercurials such as *o*-chloromercuriphenol is denoted by X. While accessible to mercurials in the crystal, His-41 did not react with IDnp-F under the conditions used in the present investigation.

monomer at pH 6.2, while the derivatization of histidine (~0.8 residue) and tyrosine (1.5 residues) did not change appreciably. However, it should be noted that hydrolytic losses of underivatized tyrosine make accurate estimates difficult.

The elution profile for the soluble tryptic peptides from protein reacted with IDnp-F at pH 6.2 and 27° is shown in Figure 1. Only one peak included a component absorbing at 365 nm. After purification by electrophoresis at pH 3.5 and 1.9, this component was found to have the following amino acid composition: Ser, 0.93; Arg, 1.07. The degradation product of IDnp-His formed during hydrolysis was not identified by amino acid analysis. In the same mixture was an underivatized peptide in a yield four times as great as the IDnp peptide and with the composition: Ser, 0.93; His, 1.00; Arg, 1.07. There are only three arginyl and three histidyl residues in the Mcg protein, and the two peptides represent the Ser-His-Arg sequence at positions 191-193.

Arg-193, which is associated with the Oz antigenic locus (Appella and Ein, 1967), and the adjacent histidyl residue are on the surface of the molecule in the crystal structure (Schiffer *et al.*, 1973). His-201 is not very accessible, while His-41 is available for the binding of organic mercurials (see Figure 2). However, a peptide containing His-41 as an underivatized residue and having the following composition (Lys, 1.04; His, 1.05; Glx, 1.93; Gly, 1.02; Ala, 0.98; Tyr, 0.89) was released from the IDnp-labeled protein by the combination of tryptic hydrolysis at Lys-44 and a chymotryptic-like split at Trp-37. Without the latter cleavage, this region of the polypeptide chain is part of an insoluble tryptic "core," which includes residues 1-44. The major fraction of the IDnp label was probably present in this insoluble peptide, since the yield of the only derivative (Ser-His-Arg) among the soluble peptides was too low to account for the results of the crystallographic analysis (see next section).

The heterogeneity produced in side reactions such as those with His-192 probably accounts for the failure of the IDnp-labeled protein to crystallize.

Locations of the Binding Sites in Crystals of the Bence-Jones Dimer. The structural formulae for representative compounds used in the binding studies are given in Figure 3. Tracings of the appropriate sections of the three-dimensional 6.5-Å difference Fourier maps for the IDnp, Dnp-Leu, bis(dinitrophenyl)lysine, Dnp-Trp, methadone, 1,10-phenanthroline, colchicine, caffeine, and theophylline derivatives are shown in

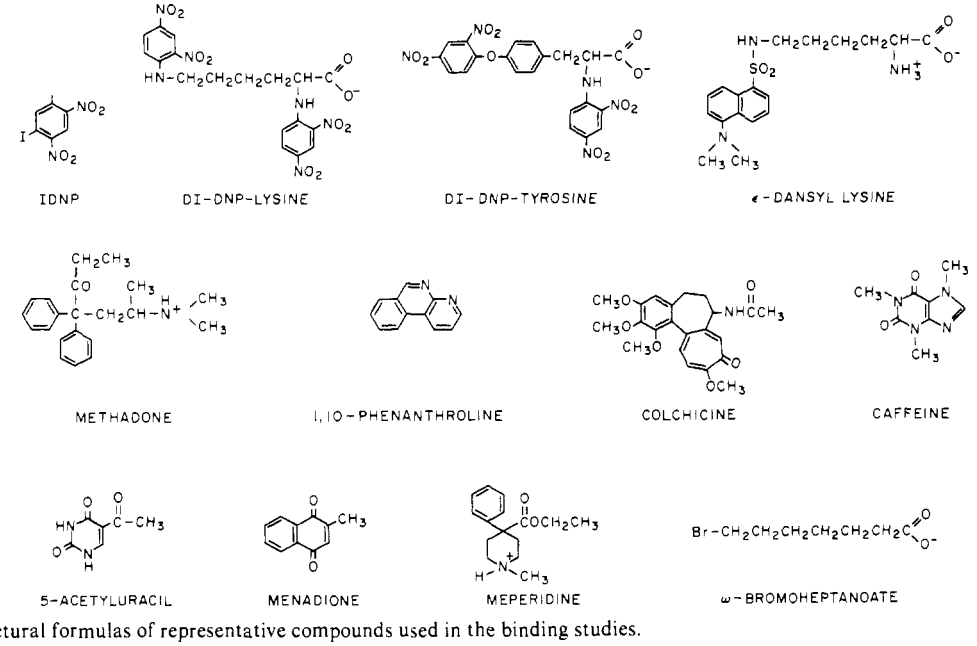

FIGURE 3: Structural formulas of representative compounds used in the binding studies.

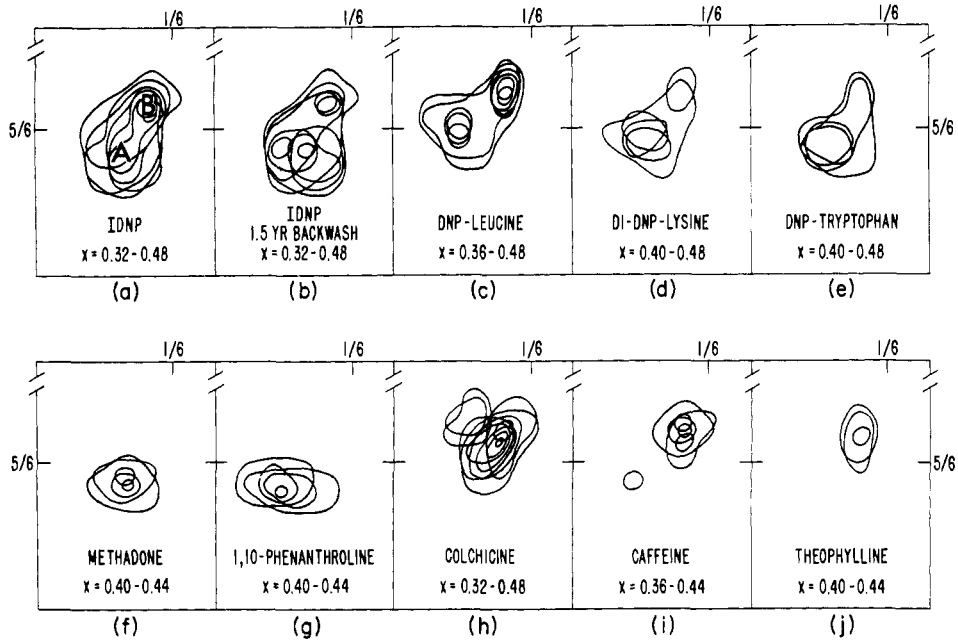

FIGURE 4: Superposed sections of 6.5-Å difference Fourier maps for compounds binding in sites A and B. Sections are normal to the a^* axis, with y the ordinate and z the abscissa. The range of x values in the superposed sections is listed in each panel. The contours on all maps are on the same arbitrary scale, with five contours set at the maximum electron density. The zero contour is omitted. Sites A and B are defined in terms of the centers of density for the two peaks representing the IDnp groups (see panel a and Table II). The pattern in panel b was obtained after crystals of the IDnp derivative were washed and placed in buffered ammonium sulfate for 1.5 years. The maps for 1,10-phenanthroline, bis(dinitrophenyl)lysine, and Dnp-Trp contain an additional peak in site C (see text), although models of these compounds do not fit into this site without distortion of the protein structure.

Figure 4. The difference maps for the 5-acetyluracil and menadione derivatives are presented in Figure 5. The coordinates for the peak maxima in the IDnp and 5-acetyluracil maps are listed in Table II. During the search for isomorphous derivatives to determine the structure of the protein, several organic mercurials were found to bind in the main cavity or adjacent pocket (see Schiffer *et al.*, 1973). The coordinates of the mercury atoms in two of these compounds are also presented in Table II. The positions of IDnp, 5-acetyluracil, and the mercury atoms were first located on the 2.5-Å electron density map for the native protein and then related to the skeletal model fitted to this map. The sites occupied by other compounds are given in Table I with the conditions for reactions with the protein crystals.

IDnp groups, ϵ- and bis(dinitrophenyl)lysine, ϵ-dinitrophenylaminocaproate, Dnp-Leu, Dnp-Trp, Dnp-Phe, bis(dinitrophenyl)tyrosine, *p*-nitrophenylphosphorylcholine, ϵ-dansyllysine, methadone, 1,10-phenanthroline, morphine, colchicine, caffeine, theophylline, meperidine, triacetin, tributyrin, and ω-bromoheptanoate, as well as the mercurials sodium mersalyl, sodium merthiolate, mercuhydrin, and methylmercuric chloride, were all found in the main cavity.

The IDnp groups, Dnp-Leu, triacetin, sodium mersalyl, and sodium merthiolate occupied two distinct positions, A and B (see Figure 4 and Tables I and II). ϵ-Dansyllysine, methadone, and 1,10-phenanthroline were found in site A, while caffeine and theophylline were bound mainly in site B. The mercury atom in mercuhydrin, a complex of theophylline and the sodium salt of methoxyhydroxymercuriopropylsuccinylurea, was centered between sites A and B (Schiffer *et al.*, 1973). This finding implies that the theophylline moiety in mercuhydrin was also located in site B, since the mercury atom interacts

TABLE II: Coordinates of Centers of Peaks in Binding Sites A, B, and C.

Derivative	Site	x	y	z
IDnp	A	0.44	0.88	0.13
	B	0.40	0.78	0.14
5-Acetyluracil	C	0.60	0.27	0.16
Sodium merthiolate[a]	A	0.428	0.864	0.129
	B	0.405	0.780	0.146
o-Chloromercuriphenol[a]	C	0.609	0.256	0.158

[a] Coordinates for organic mercurials are taken from Schiffer *et al.*, 1973.

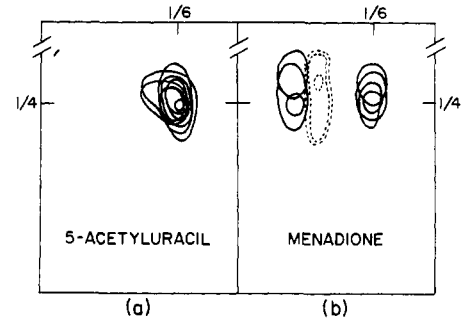

FIGURE 5: Superposed sections of the 6.5-Å difference Fourier maps for 5-acetyluracil and menadione (vitamin K_3), which bind in site C. Range of x values of superposed sections: 5-acetyluracil, x = 0.56–0.64; menadione, site C, x = 0.56–0.68; negative trough, x = 0.60–0.64; second positive peak, x = 0.52–0.60. The 5-acetyluracil forms an isomorphous derivative and does not damage the crystals. Menadione cracks the crystals and gives rise to the additional peaks to the left of site C.

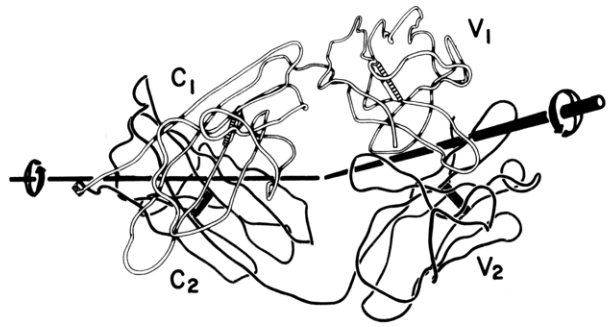

FIGURE 6: Tracing of a photograph of a model of the Bence-Jones dimer, with the local pseudo-twofold axes of rotation included (see Schiffer *et al.*, 1973). Monomer 1 is shown in white and monomer 2 in black. The amino or "variable" domains are labeled V, and the carboxyl or "constant" domains are labeled C with the appropriate subscript for monomer 1 or 2. The pseudodiad on the right runs through the center of the main cavity between the amino domains. The views presented in Figures 7, 8, and 9 are taken down this axis.

with N-7 of theophylline. The peak for dansyllysine was in an intermediate position, but closer to the center of site A than B. The broad peaks for ϵ- and bis(dinitrophenyl)lysine, ϵ-dinitrophenylaminocaproate, Dnp-Trp, Dnp-Phe, and bis(dinitrophenyl)tyrosine extended across sites A and B, although the electron density was higher in the region of site A (see Figure 4).

The solvent channel continuing from the main cavity contains an additional binding site (C), in which such disparate compounds as ϵ- and bis(dinitrophenyl)lysine, ϵ-dinitrophenylaminocaproate, Dnp-Trp, Dnp-Phe, bis(dinitrophenyl)tyrosine, *p*-nitroaniline, *p*-nitrophenylphosphorylcholine, 1,10-phenanthroline, 5-acetyluracil, menadione, meperidine, triacetin, tributyrin, ω-bromoheptanoate, *o*- and *p*-chloromercuriphenol, phenylmercuric compounds, and methylmercuric chloride have been found (see Figure 5 and Tables I and II). The Dnp compounds generally appear in sites A and B before significant quantities are detected in site C (see Table I). Dinitrophenol, dinitroaniline, phosphorylcholine, and various dipeptides and monosaccharides were not bound to any significant degree.

Three-Dimensional Structures of the Binding Sites. A tracing of a photograph of the model of the polypeptide chains of the Bence-Jones dimer is shown in Figure 6, with the local pseudo-twofold axes of rotation included in the drawing. A stereophotograph and schematic drawings of the skeletal models which illustrate the orientations of residues 34–52 and 88–112 are presented in Figures 7–9. In each case the view is down the pseudo-twofold axis between the amino domains.

The circular entrance to the main cavity between the V domains is located at the tip of the dimer (see Figures 7 and 8 and Schiffer *et al.*, 1973). This cavity is shaped like a truncated cone 15 Å in diameter at the entrance and 10 Å across at the base ("floor"). The distance from the entrance to the floor along the pseudo-twofold axis between the V domains is about 17 Å. The solvent separating the monomers continues through a 5–6-Å opening between the two pairs of aromatic residues (Tyr-38 and Phe-101) forming the floor (see Figure 8). Then the solvent channel enlarges into an ellipsoidal pocket with dimensions of about 8 × 8 × 10 Å (length × width × height).

Most side chains projecting into the main cavity are constituents of the three hypervariable regions (residues 23–36, 50–56, and 91–100). The cavity lining consists of eight tyrosyl (34, 38, 51, and 93 from both monomers), four phenylalanyl (99 and 101), two valyl (48), two glutamyl (52), one aspartyl (97 from monomer 2), and four seryl (36 and 91) side chains. The appearance of the entrance to the cavity is dominated by a ring of phenolic groups contributed by Tyr-34, -51, and -93 from both monomers (see Figures 7 and 8). Interspersed among the aromatic side chains are acidic groups of Glu-52 from both monomers and Asp-97 from monomer 2. Asp-97 from monomer 1 lies at the edge of the cavity but does not protrude into it.

The hydrophobicity of the cavity lining increases with distance from the entrance (see Figure 8). The Phe-99 side chains of the two monomers are found at a distance of 10–11 Å, and the two valyl residues (48) are located between Phe-99 and the four aromatic residues on the floor. However, there are also two pairs (Ser-36 and -91) of polar hydroxyl groups beyond Phe-99, in addition to the two phenolic groups of Tyr-38.

Tyr-38 and Phe-101 from the two monomers provide the roof of the pocket as well as the floor of the main cavity (see Figures 8 and 9). The walls of the pocket consist of two tyrosyl (89), two prolyl (46), and two glutaminyl (40) residues.

In comparison with the carboxyl halves, the two amino domains have few points of contact, particularly along the solvent channel. Among the few are contacts between Tyr-51 and Asp-97 side chains at the entrance of the main cavity and between the Gln-40 side chains flanking one end of the pocket (see Figure 9). Another constituent of the pocket, Pro-46 from mono-

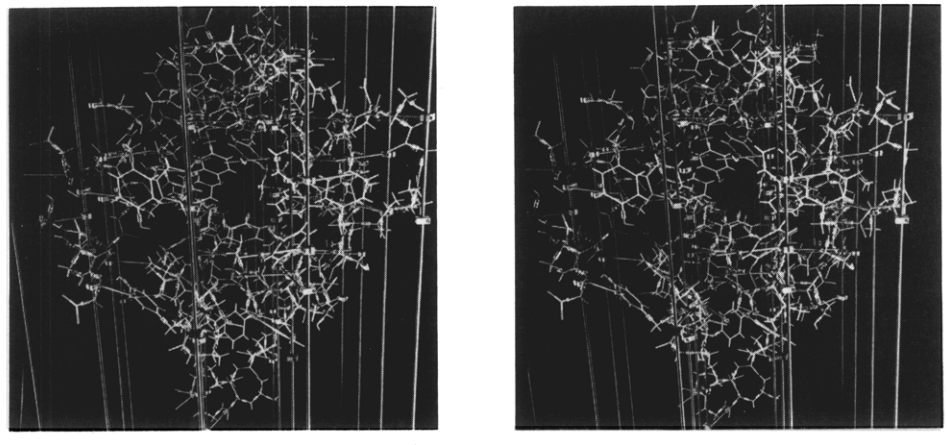

FIGURE 7: Stereophotograph of the Watson-Kendrew skeletal models of residues 34–52 and 88–112, as viewed down the pseudo-twofold axis between the amino domains. Note the size of the solvent channel and the large number of aromatic rings lining it. Monomer 1 is above and to the left of the pseudo-twofold axis (see Figure 8). The amino acid sequence used in building the model was obtained by Fett and Deutsch (1974).

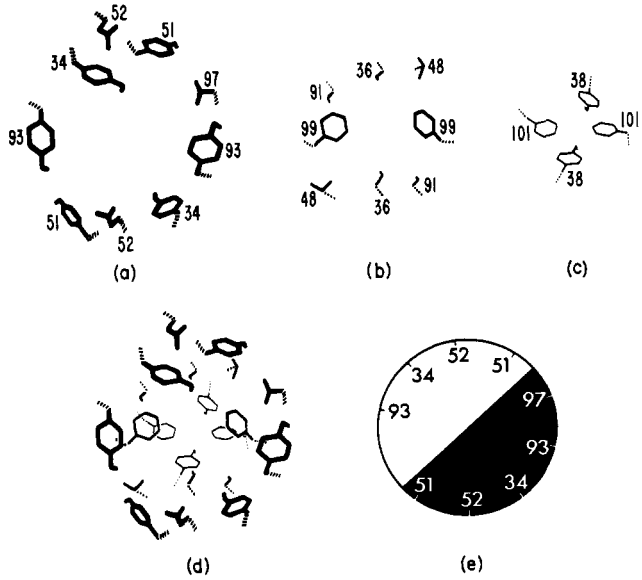

FIGURE 8: Schematic and composite drawings of the side chains lining the main cavity between the amino domains. The side chains are in the same orientations as in Figure 7; the bond between the α and β carbon atoms is represented by a striated line. (a) The entrance to the cavity, lined by Asp-97, Tyr-93, Tyr-34, Glu-52, and Tyr-51 from monomer 2 (reading clockwise), and by Tyr-93, Tyr-34, Glu-52, and Tyr-51 from monomer 1. Binding site A is close to Tyr-34 of monomer 2 (see Figure 10). Note that the twofold pseudosymmetry does not hold for the Tyr-34 side chains. (b) Intermediate level in the cavity. The Phe-99 side chains are about 10–11 Å from the entrance. The side chains of Ser-36, Ser-91, and Val-48 lie between Phe-99 and the base. Binding site B is located in this region (see Figure 10). (c) The base ("floor") of the cavity, about 16–17 Å from the entrance, consisting of Tyr-38 and Phe-101 from the two monomers. (d) Composite drawing of the cavity lining. (e) Key to the distribution of the side chains between monomer 1 (white half of circle) and monomer 2 (black half).

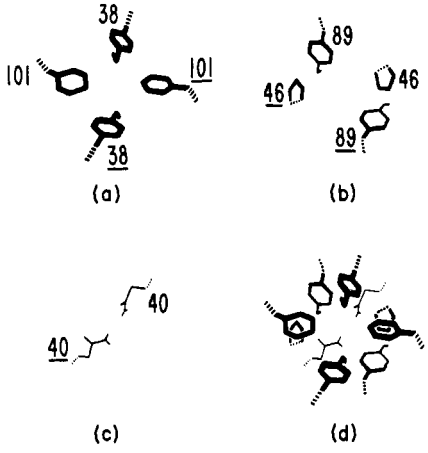

FIGURE 9: Schematic and composite drawings of the side chains lining the pocket in the solvent channel. The sequence numbers of the side chains from monomer 2 are underlined. (a) The entry proximal to the main cavity: Tyr-38 and Phe-101 serve as both the floor of the main cavity and the roof of the pocket. The Tyr-38 side chains in particular have sufficient space to rotate about their β carbon atoms to enlarge the opening into the pocket. (b) Binding site C, which is centered between the two Pro-46 residues. Tyr-89 from monomer 1 is closer to the center of site C than its counterpart in monomer 2. (c) The Gln-40 side chains at one end of the pocket. (d) Composite drawing of the pocket lining.

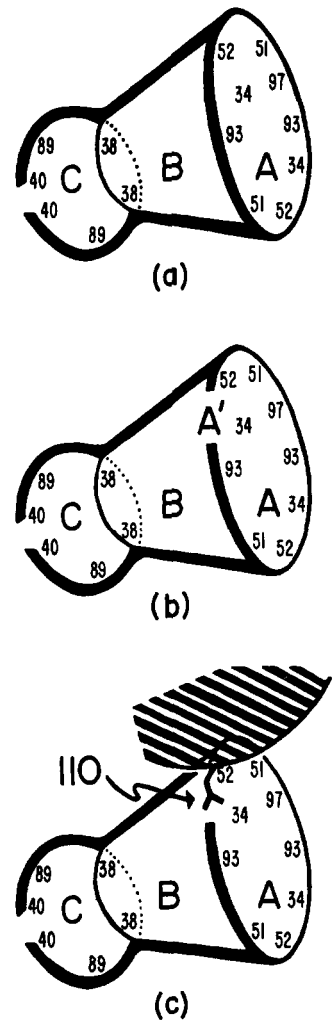

FIGURE 10: Schematic drawings of the solvent channel in profile. The main cavity is represented by a truncated cone and the pocket by an ellipsoid. Binding sites are indicated by letters, and the positions of side chains are indicated by their sequence numbers. (a) Binding sites A, B, and C: the centers of sites A and B were initially defined from the difference Fourier map for the IDnp derivative (see Figure 4 and Table II). IDnp groups are covalently linked to Tyr-34 and Tyr-38 of monomer 2. The location of site C was obtained from the difference map for 5-acetyluracil. Site A is more spacious than the others, but is largely restricted to the constituents of monomer 2 for possible contact residues. The side chains of both monomers contribute to sites B and C. (b) Site A′: small molecules are not bound in the region related to site A by the pseudo-twofold axis. A′ marks the position expected for the Tyr-34 side chain of monomer 1 on the basis of local twofold pseudosymmetry. (c) Schematic drawing of the relation between site A′ and the Leu-110 side chain from monomer 2 of a symmetry-related dimer in the crystal lattice.

mer 1, is situated near the side chain of Tyr-89 from monomer 2. Tyr-89 from monomer 1 is close to the polypeptide backbone of monomer 2 at the junction of Lys-44 and Ala-45.

Binding site A is significantly closer to monomer 2 than monomer 1, while site B is located near the pseudo-twofold axis down the cavity (see Figure 10). The most probable contact residues for site A are Tyr-34, Glu-52, and Tyr-93 of monomer 2, with Tyr-51 in close proximity. The Phe-99 side chains form the junction between sites A and B. With a bias toward side chains of monomer 2, all residues between Phe-99 and the base are potential contributors to site B (*i.e.*, pairs of Phe-99, Ser-36, Ser-91, Tyr-38, and Phe-101 in order of descent). The two Val-48 side chains are somewhat removed from site B.

The center of site C is located between the Pro-46 residues of adjacent chains (see Figures 9 and 10). The four aromatic rings of Tyr-38 and Phe-101 face the binding site on the proximal side of the prolines, with the monomer 1 residues closer to the site than those of monomer 2. On the distal side of the pro-

lyl residues, both the polar (OH) and apolar (phenyl) groups of the two Tyr-89 side chains are accessible with the residue from monomer 1 again being nearer the center of site C.

Space Available for Binding. A skeletal model (2 cm = 1 Å) of bis(dinitrophenyl)lysine in its most extended form occupies sites A and B from the entrance to the floor of the main cavity, and can be used as a rough measure of the amount of space available for binding. With smaller compounds such as triacetin, sodium merthiolate, or IDnp groups, it is possible to place two models in the cavity at the same time. The detection of 1.6 IDnp groups per dimer confirms that both sites A and B are occupied simultaneously (see Table I). Like the three compounds just mentioned, Dnp-Leu is attracted to both sites A and B. However, the quantities of bound Dnp-Leu (0.4–0.5 molecule per site; see Table I) do not indicate conclusively whether both sites are occupied in a single dimer. Site C is just large enough for the binding of one molecule of an acetylated pyrimidine ring like 5-acetyluracil (see Figure 3) or of an ortho-substituted phenyl ring like *o*-chloromercuriphenol.

Covalent Binding of IDnp Groups. Of the residues capable of reacting covalently with IDnp-F (see Hirs, 1967), only tyrosine is present in either the main cavity or the pocket. The two tyrosyl side chains closest to the positions of the Dnp peaks are those of Tyr-34 and -38 of monomer 2 (see Figure 8). The corresponding residues in monomer 1 are not directly involved.

This finding may explain the major difficulties in the interpretation of chemical degradation studies. If only monomer 2 is derivatized, Tyr-34 and -38 labeled with IDnp cannot be recovered from the dimer in yields greater than 50%. The isolation of the unlabeled soluble peptide containing Tyr-38 (and His-41; see preceding section) can therefore be attributed to enzymatic cleavage of monomer 1. The derivatized peptide(s) from monomer 2 apparently remained in the insoluble fraction of the tryptic hydrolysate.

In the crystals there are steric reasons for Tyr-34 and -38 of monomer 2 to be more reactive than their counterparts in monomer 1. Tyr-34 and Glu-52 in the latter are partially shielded by at least one side chain (Leu-110) and a section of polypeptide backbone from monomer 2 in a symmetry-related dimer in the crystal lattice (see Figure 10c). The phenolic group of Tyr-38 in monomer 1 is located at a slightly greater depth in the cavity and is therefore less accessible to IDnp-F than the corresponding side chain in monomer 2.

Tyr-34 and Tyr-93 have been identified by affinity labeling as constituents in or near the binding sites of anti-Dnp antibodies (*e.g.*, see Franěk, 1971). Both residues are accessible in monomer 2 of the Bence-Jones dimer. In the crystal lattice, however, the side chain of Tyr-93 is in close proximity with the main chain at positions 95 and 96, as well as with the side chain of Glu-202 from another molecule. Tyr-93 is therefore expected to be less reactive than Tyr-34.

The 3.5-Å difference Fourier map for the IDnp derivative showed binding details not clearly defined in the 6.5-Å map. A series of troughs and peaks indicated a shift of 2.5 Å in the main chain extending from Tyr-34 to Ser-36 of monomer 2. The phenolic ring of Tyr-34 was also displaced about 3 Å as a result of the reaction. There were no detectable displacements of side chains in site B. The IDnp ring, covalently linked to Tyr-38 of monomer 2, was in van der Waal's contact with the side chain of Ser-36 of monomer 1 and Phe-99 of both monomers.

Noncovalent Binding of Dnp Compounds. The peaks representing the noncovalently bound Dnp compounds in the difference maps closely correspond to those for the IDnp groups (see Figure 4). The asymmetry in the binding relative to monomers 1 and 2 is maintained. Tyr-34 and -38 from monomer 2 and Phe-99 from both monomers are still likely to be primary contact residues for the Dnp rings.

With two Dnp rings, bis(dinitrophenyl)lysine binds in 2 days to approximately the same extent as ϵ-Dnp-Lys in 3 weeks (see Table I). Moreover, the bis(dinitrophenyl)lysine was not removed from the cavity by soaking the crystals in ammonium sulfate for 2 weeks, while ϵ-Dnp-Lys and Dnp-Leu were displaced by the same treatment. As expected, the covalently bound IDnp groups were not removed when the crystals were bathed in ammonium sulfate for 1.5 years (see Figure 4). These findings should be considered in light of observations that dinitrophenylhaptens are not readily dissociated from high affinity anti-Dnp antibodies, even in solutions of guanidine hydrochloride (Cathou *et al.*, 1968).

A charge-transfer complex of a Dnp group with a tryptophyl residue on the heavy chain has been proposed in some anti-Dnp antibodies to explain the red spectral shift on binding of the hapten (Little and Eisen, 1967; Eisen *et al.*, 1968). The only tryptophyl residue (37) in the amino domain of the Bence-Jones protein is not present in the cavity (Schiffer *et al.*, 1973), but it is sterically possible for a Dnp ring to form a charge-transfer complex with Tyr-34 of monomer 2.

Side chains other than Tyr-34 and -38 undoubtedly participate in the binding of Dnp compounds and influence the nature of the groups that can be bound. For example, Dnp-Leu and Dnp-Trp bind to about the same extent as bis(dinitrophenyl)-lysine in the same period of time (see Table I) and do not cause the physical damage seen in crystals of the lysine derivative. The amount of bound ϵ-dinitrophenylaminocaproate is only about half as large as that of ϵ-Dnp-Lys. The close proximity of the side chain of Glu-52 to that of Tyr-34 may result in repulsion of the carboxyl group of the caproate, an effect partially offset by the positively charged α-amino group on ϵ-Dnp-Lys. Moreover, dinitrophenol, which is present as the negatively charged phenoxide ion at the pH (6.2) of the crystals, does not bind in detectable quantities. The failure to bind dinitroaniline is less easily explained, since this compound is a weak base with no charge at pH 6.2.

Outside the main cavity and pocket, electrostatic effects are expected to be damped by the presence of large quantities of ammonium sulfate (1.9 M) in the crystals. There are no positively charged side chains inside the solvent channel, and sulfate ions presumably exert no marked effect on the binding reactions with Dnp compounds. Hydrated sulfate ions are probably excluded from the pocket, which is sufficiently wide to accommodate only about three water molecules. Sulfate ions are believed to be present in the binding site of a crystalline Fab fragment with activity against phosphorylcholine. Padlan *et al.* (1973) concluded from their Fourier maps that a sulfate ion was displaced from the phosphate binding region by addition of the "hapten," 2-(5′-acetoxymercury-2′-thienyl)ethyl-phosphorylcholine.

Noncovalent Binding of Other Small Compounds. The propensity of the main cavity to bind Dnp groups extends to multiring systems ranging from naphthalene and purine derivatives to alkaloids (see Table I). On the difference Fourier maps ϵ-dansyllysine, 1,10-phenanthroline, and methadone are represented by sharp peaks in site A, as are the methyl derivatives of xanthine (caffeine and theophylline) in site B (see Figure 4). In contrast, the alkaloid colchicine, bound to the extent of 0.62 mol/dimer, gives a large, diffuse pattern of electron density extending beyond sites A and B into other parts of the cavity. Since physical damage to the crystals accompanies the binding of colchicine, the difference Fourier map probably reflects con-

formational changes in the cavity lining. Phenanthroline, which is bound in site A in even larger quantities than colchicine (see Table I), also causes the crystals to crack. In this case, however, the damage is probably associated with binding in site C, into which a phenanthroline model cannot be placed without some distortion of the protein structure.

Sites A and B have residues suitable for both hydrogen bonding and hydrophobic interactions, but it was surprising that triacetin and the ring compounds were bound in the same locations. In the balance of the polar and nonpolar forces in triglycerides, the polar properties become more important as the fatty acid chain length decreases. For example, the carbonyl groups in triacetin are accessible for hydrogen bonding, and the methyl groups are not very hydrophobic. As the chains lengthen, the polymethylene groups begin to predominate. The crystal structure of trilaurin, for instance, resembles that of a long-chain hydrocarbon, and the effect of the glyceryl residue is small by comparison (Vand and Bell, 1951). Tributyrin has properties intermediate between those of triacetin and trilaurin. Although present in small amounts because of its low solubility, tributyrin is bound in the main cavity like triacetin, in site C like ω-bromoheptanoate (see Table I), and in three other sites in spaces between dimers. Although it is difficult to fit more than one polymethylene chain of a triglyceride model into the pocket, the difference map for tributyrin showed a small but significant peak in site C, as well as in site B. It is sterically possible for two butyric acid chains and most of the glyceryl moiety to be wedged into the base of the main cavity, with the third chain extending toward site C.

Both tributyrin and menadione, a "fat-soluble" vitamin, are practically insoluble in the ammonium sulfate solutions used for crystallization. While the tributyrin is slowly drawn into site C from the suspension over the crystals, menadione enters rapidly and causes the crystals to begin cracking within 2 hr. In the difference Fourier map for menadione (see Figure 5b), the peak in site C is adjacent to a large negative trough, which in turn is next to a positive peak. In the native protein the position of the trough minimum coincides with that of a segment of polypeptide chain between Lys-44 and Ala-45 in monomer 2. The positive peak is located in an open space outside the pocket but immediately adjacent to this section of chain. The additional peaks indicate that menadione induces changes in the protein structure. As mentioned in the description of the solvent channel, the displaced chain is one of the few participants in interactions between the variable domains.

Other compounds like ω-bromoheptanoate, *p*-nitrophenylphosphorylcholine, and phenylmercuric compounds also bind in site C with high occupancies (see Table I) and also cause crystal cracking and conformational changes. In the case of phenylmercuric compounds investigated as possible heavy-atom derivatives, the difference Fourier maps have a high background level, and the known contributions of the heavy atoms do not fully account for the intensity changes in the diffraction patterns (Schiffer *et al.*, 1973).

In the Watson-Kendrew model fitted to the 2.5-Å map, there is no apparent way for the small molecules to enter site C except through the opening in the base of the main cavity. This opening will accommodate models up to 11–12 cm (*i.e.*, 5.5–6.0 Å) in width without alteration of the protein model. Larger models can be passed through the opening if the two phenolic rings of the Tyr-38 residues are rotated about their β carbon atoms. However, the models of some compounds do not completely clear the opening when entering the pocket. If the *p*-nitrophenyl group of *p*-nitrophenylphosphorylcholine is placed in site C, for example, the choline moiety lies outside the pocket. The *p*-nitrophenyl group is required for binding, since phosphorylcholine is not bound in significant amounts to the protein (see Table I).

While the binding is primarily dependent on hydrophobic interactions, most of the tested compounds showing specificity for site C also have accessible polar groups. The best example is 5-acetyluracil, which binds with high specificity (see Table I) without cracking the crystals, and which has both polar groups and aromatic properties. The difference Fourier map indicates that 5-acetyluracil does not alter the protein structure (see Figure 5a). The size difference between 5-acetyluracil and a purine derivative like caffeine explains why the latter is not bound in site C. It is not clear why 5-acetyluracil fails to bind in site B.

Blocking of Site A′ by Another Protein Molecule in the Crystal Lattice. It was emphasized earlier that a potential binding site (A′) in the main cavity, close to monomer 1 and structurally similar to site A, is effectively blocked by constituents of a symmetry-related dimer in the crystal lattice.

The main chain in positions ~108–111 of monomer 2 of the symmetry-related dimer lies directly over site A′. The isobutyl group of Leu-110 from the second molecule penetrates into site A′ and occupies a position between Glu-52 and Tyr-34 (dimer I). On the basis of the local twofold pseudosymmetry, the relative position of the Tyr-34 side chain is different from that of its counterpart in site A (see Figure 7 and 8a); Leu-110 is located near the position expected for Tyr-34.

The surfaces of the symmetry-related dimers are highly complementary in this region of the crystal lattice. Among the interactions along these surfaces are those involving Tyr-93 of monomer 2, dimer I, and Glu-202 of monomer 2, dimer II. There is also a close contact between Asp-97 of monomer 2, dimer I, and Lys-114 of monomer 2, dimer II.

Under such highly favorable conditions of complementarity, it is thus possible for a single side chain on a protein to enter one of the two most accessible binding sites in the Bence-Jones dimer without seriously altering the geometry of the cavity lining (*i.e.*, other than the possible displacement of the side chain of Tyr-34). Fortunately for the binding studies, the interactions between the proteins in the crystal lattice are asymmetrical and do not substantially reduce the capacity of the remainder of the solvent channel to bind smaller molecules.

Multiple Site Binding. If the Leu-110 side chain from dimer II is considered as being "bound" in site A′, the addition of any of the compounds previously discussed results in multiple site binding. A and B can act as discrete sites for the binding of two small molecules like triacetin, merthiolate, or IDnp groups, or as a combined site for the binding of larger molecules like bis-(dinitrophenyl)lysine, with more than one suitable chemical grouping. Sites A and C can also be occupied in tandem, usually by relatively large compounds like 1,10-phenanthroline or bis(dinitrophenyl)lysine which damage the crystals. Finally, elongated compounds like tributyrin (and possibly *p*-nitrophenylphosphorylcholine) can be wedged between sites B and C through the opening in the floor of the main cavity.

Protein Conformational Changes Associated with Binding. The local conformational changes accompanying the covalent binding of an IDnp group to Tyr-34 in site A were discussed previously. Large compounds like colchicine cause more extensive disruption of the protein structure in this region. The ease with which compounds like menadione can disrupt the crystal structure emphasizes the small number of stabilizing interactions that have to be interrupted to gain access to the deeper sites and/or to enlarge them. This flexibility in the dimer permits an oversized entering group with the proper affinity to

"induce a fit" (see Koshland, 1973) at the appropriate level in the binding channel.

Differences in Structure between the Mcg Bence-Jones Dimer and the Light Chains in the Parent IgG Molecule; Binding of Compounds by Light Chains Alone. The electron density map at 3.5-Å resolution revealed striking differences in the conformations of the two light chains of the Mcg Bence-Jones dimer (Schiffer *et al.*, 1973). These conclusions have been confirmed with the current 2.5-Å map. In contrast the presence of a crystallographic twofold axis between the halves of the parent IgG protein (with a combination of one light and one heavy chain in each half) requires the two light chains in this molecule to be identical in conformation (Edmundson *et al.*, 1970). The Bence-Jones and IgG proteins were obtained from one patient (Mcg) and the amino acid sequences of the light chains are presumed to be the same in both proteins (see Deutsch, 1971; Fett *et al.*, 1973). These proteins therefore illustrate the fact that different conformations can be derived from the same amino acid sequence when the second member of the associating pair of molecules is another light chain (instead of a heavy chain as in the IgG protein). There are precedents for this observation in crystal structures of dimers of insulin (Blundell *et al.*, 1971), chymotrypsin (Tulinsky *et al.*, 1973), and malate dehydrogenase (Hill *et al.*, 1972). The monomers in these proteins are not identical, although the differences are far less pronounced than in the Mcg Bence-Jones dimer.

A comparison of the structures of the Bence-Jones dimer with human and murine Fab fragments (Poljak *et al.*, 1973; Padlan *et al.*, 1973, 1974) indicates that monomer 2 closely resembles the light chains associating with the amino halves of the heavy chains in the Fab fragments; monomer 1 does not. The spatial relations between V and C domains of monomer 1 are similar to those in the heavy chains. For example, when cylindrical envelopes are drawn around the V and C domains of the Bence-Jones monomers, the angle between the long axes of the envelopes is ~70° in monomer 1 and ~110° in monomer 2 (Schiffer *et al.*, 1973). Poljak *et al.* (1973) reported that the corresponding angles in the Fab fragment were 80–85° in the heavy chain and 100–110° in the light chain. Moreover, an additional disulfide bond between the V and C domains in rabbit light chains (Strosberg *et al.*, 1972) is sterically possible between the homologous positions (residues 82 and 174) in monomer 2 and in the light chain of the human Fab fragment. Formation of the bond is not possible in monomer 1, since positions 82 and 174 are ~25 Å apart.

If the Bence-Jones monomers were related by a crystallographic twofold axis (*i.e.*, identical in conformation), the binding sites would not exist as we see them in the electron density maps.

The combined crystallographic results in this article bear on the problem of the binding of haptens by light chains alone (Goodman and Donch, 1965; Mangalo *et al.*, 1966; Yoo *et al.*, 1967; Painter *et al.*, 1972; Nakamura *et al.*, 1973; Stevenson, 1973). The level of specific binding is generally low when compared with that of the parent antibody. Light chains can be removed from specific antibodies under dissociating conditions after reduction and alkylation of the light–heavy interchain disulfide bonds. In binding studies the light chains are allowed to "renature" and to dimerize before testing for activity. If the formation of binding sites comparable to those in the Mcg Bence-Jones dimer is a prerequisite for activity, one of the light chains would have to adopt a conformation similar to that of monomer 1 during the "renaturation" process. The low activity of light chain dimers may therefore be partially influenced by folding problems as well as by the loss of their complementary heavy chains.

We have presented evidence that the Mcg Bence-Jones dimer binds hapten-like molecules in sites comparable in size and location to the binding site for phosphorylcholine in a murine Fab fragment (Padlan *et al.*, 1973). With the light chains displaying different conformations (one resembling the amino half of a heavy chain), we suggest that the Bence-Jones dimer can serve as a model for a primitive antibody. Multiple binding sites are available in the solvent channel between the V domains, and the main cavity and pocket have chemical reactivities like those of "cryptic" sites in antibodies which show specificity for compounds unrelated to the immunogen (Eisen *et al.*, 1967). The combined properties of the Bence-Jones dimer provide strong evidence for economy of the immune response to the challenge of the vast number of possible antigenic determinants.

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