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The Structure Determination of the Variable Portion of the Bence-Jones Protein Au

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Abstract. The structure of a $\varkappa$-type Bence-Jones protein variable fragment Au has been determined by molecular replacement methods using the known structure of an other Bence-Jones variable fragment Rei (Epp *et al.*, Eur. J. Biochem. **45**, 513 (1974)). The crystallographic R factor is 0.31 for about 4000 significantly measured reflections between 6.8 to 2.5 Å. The Au protein forms a dimer across a crystallographic two fold axis. The spatial relationship of the two monomers, the conformation of the backbones and of the internal residues is extremely similar to that found in Rei.

Key words: Immunoglobulin — Bence-Jones — X-Ray — Structure — Rotation-Function — Patterson Search — Translation Function — Molecular Replacement.

The structure of Fab fragments (Padlan *et al.*, 1974; Poljak *et al.*, 1973), a Bence-Jones dimer (Schiffer *et al.*, 1973) and a dimer made up of Bence-Jones protein (Rei) variable fragments (Epp *et al.*, 1974), have been determined by X-ray diffraction. All three light chain domains show a very close structural resemblance. These domains are related by pseudo-two fold axes in the crystal, the structure of the dimer so formed seems to be identical in all three compounds. Assuming this similarity also holds for other light chain variable domains molecular replacement methods (for a review see Rossmann, 1972) may be applied. We have thus determined the structure of the variable portion of the Bence-Jones protein Au, using the known structure of the Rei protein.

Proteins Au and Rei both belong to the $\varkappa_1$ subgroup and differ in only 16 amino acids, two of which are interchanges of glutamine for glutamic acid; see Table 1 (Palm and Hilschmann, 1973; Schiechl and Hilschmann, 1971).

The preparation, characterization and crystallization of the variable fragment of the Bence-Jones protein Au has been described (Schramm *et al.*, 1970; Schramm,

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Table 1. Amino acid differences between protein Rei and Au (Palm and Hilschmann, 1973; Schiechl and Hilschmann, 1971)

Residue Number	Residue Name Rei	Au
30[a]	Ile	Ser
31[a]	Lys	Asp
39	Thr	Lys
50[a]	Glu	Asp
55	Gln	Glu
56	Ala	Ser
65	Ser	Gly
69	Thr	Ala
70	Asp	His
71	Tyr	Phe
92[a]	Gln	Asp
93[a]	Ser	Tyr
96[a]	Tyr	Trp
104	Leu	Val
105	Gln	Glu
107[b]	Thr	Lys

[a] Residues in the hypervariable regions.
[b] No electron density found for this residue in either crystal.

1971). Intensity data of the native protein were measured by diffractometer, ω-2θ integrations were performed and background was counted at both sides. All reflections were measured at least twice. Absorption corrections were applied (Huber and Kopfmann, 1969). Comparison of equivalent reflexions showed that many of these measurement were unsatisfactory probably due to crystal slippage. These reflexions were discarded. The final data set contained 3500 reflections (83%) of that between 10 to 2.7 and 500 (43%) between 2.7 to 2.5 Å resolution.

Protein Au crystallizes in space-group $P6_122$ with unit cell dimensions $a = 82.3$ Å, $c = 77.0$ Å with one variable domain in the asymmetric unit. The Rei protein on the other hand crystallizes with two variable domains in the asymmetric unit of the related space-group $P6_1$ ($a = 75.8$ Å, $c = 98.2$ Å). The two V domains forming the chemically significant dimer are related by a local diad perpendicular to and intersecting the Z axis at $Z = 0.68$ Å and making an angle of 42.5° with the positive X axis (see Epp *et al.*, (1974) for details). If the dimer structure is to be maintained in Au, it's diad has to lie on one of the crystallographic 2 fold axes in $P6_122$, i.e. along the X-axis of Au or at an angle of 30° to it (Fig. 1). In the latter case the three-fold screw axis would pass through the dimer and the length of the unit cell ($c = 77$ Å) and the minimum thickness of the dimer (36 Å) do not permit this.

This was confirmed and the rotational parameter of the dimer around the two-fold axis determined by Patterson search techniques. Details of this search were as follows: First structure factors were calculated from a Rei dimer with its diad placed parallel to the Y* axis in the Rei unit cell. The Y* axis is one of the axes of rotation in the methods used, and therefore this simplifies the interpretation of the

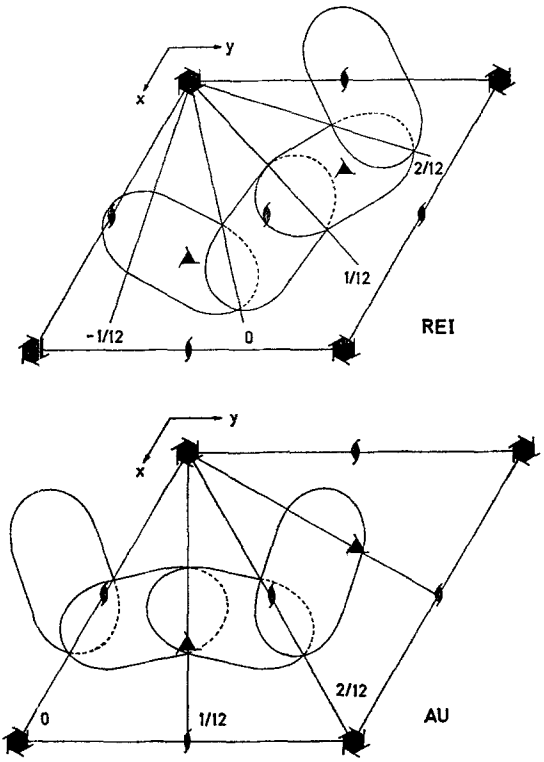


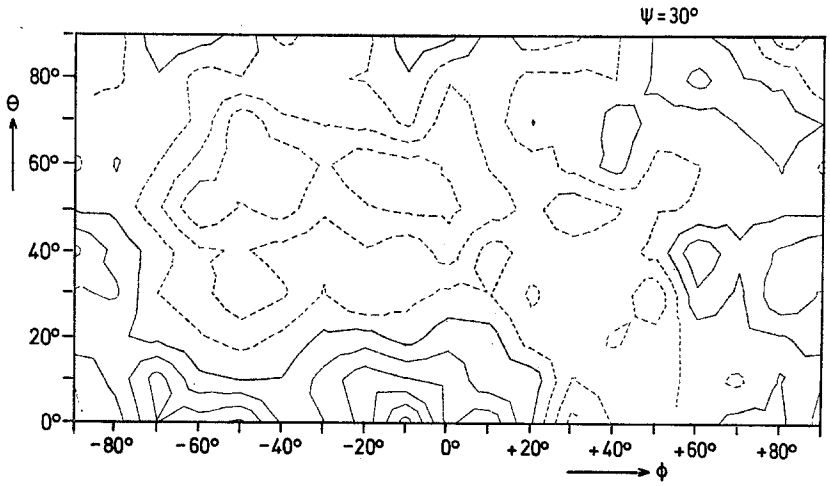
Fig. 1. Packing of the Rei and Au molecules in the unit cell projected along the Z axis. The crystallographic and local symmetry elements are indicated; — 1/12; 0; 1/12; 2/12 mark the height of the diads above the XY plane. The chemically significant dimer lies across the axis at 42.5° from + X in Rei, and across the X axis in the Au unit cell

results[1]. Rotational search with the "dimer" vector set was done in the Au Patterson function, using a method and programmes described previously (Epp *et al.*, 1972; Hoppe, 1957; Huber, 1965, 1969). Data to 2.8 Å resolution was used. 2526 grid points representing the highest values of the Patterson function observed at the 15800 grid points within a sphere of radius 21 Å around the origin were selected in this search.

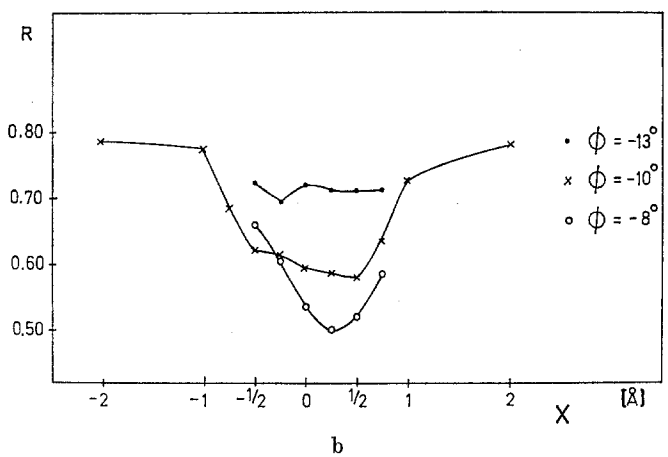
The maximum of the product function was at $\chi = 30°$, $\theta = 0$, $\varphi = -10°$, and had the value 100. The next highest peak was 74. The average of the function was 24. The first search was done in step-widths of 10° for all angles, and was repeated in 1° intervals in the area around the peak. The refined value so obtained was $\chi = 30°$, $\theta = 0°$, $\varphi = -8°$ with a height of 103 (Fig. 2a).

A nearly equivalent value was obtained with Crowther's Fast Rotation Function (Crowther, 1972). This calculation was done both with data from 10 to 6 Å and from 10 to 5 Å. The sphere around the origin of the Patterson function

[1] Eulerian angle convention: ψ rotation about Z' axis, θ rotation about new X' axis, φ rotation about new Y' axis. $X'Y'Z'$ describes a Cartesian system related to the hexagonal crystal system: $X' \equiv X$, Y' in the X-Y plane perpendicular to X (Y^*), $Z' \equiv Z$.



a



b

Fig. 2. a) Section θ, ψ through the product rotation function at $\varphi = 30°$. Intervals are one half of the average value. The highest dashed level (next to the first solid) corresponds to the average value 24. b) The R factor for $hk0$ reflections between 7 to 4 Å resolution of the position of the dimer along the X axis. The origin is chosen on the X-axis at a distance from the 6_1 axis identical to the distance between the center of the dimer and the 6_1 axis in Rei

used had a radius of 35 Å and 29 Å respectively. The highest peak (100) in both maps was at $\psi = 30°$, $\theta = 0°$, $\varphi = -13°$ (in the same angle convention as above); the next highest peaks had values of 73 and 72. The maps were calculated in 5° intervals, the exact values of the rotation angles were found by extrapolation. For these calculations the highest 30% of the structure factors within the specified ranges were used for both proteins. When this search was later repeated with the strongest 60% of the model (Rei) data, within the 10 to 5 Å resolution range, the result was altered to $\psi = 30°$, $\theta = 0°$, $\varphi = -7°$. The maximum was 100 and the next highest peak was 74.

The peak heights referred to in the four orientational search calculations are unrelated and are adjusted in the individual calculations to yield a highest value of 100.

Because the twofold axis of the dimer is the crystallographic X axis, the translational search is reduced to a one-dimensional problem along this axis. This search can be further narrowed by packing considerations. These require the center of the dimer to be placed at about $X = 1/2$. In the Rei crystal, the center of the dimer is also about the same distance from the 6 fold axis measured along its diad.

To find the exact position, the coordinate set of Rei was rotated, to the orientation found above. The distance from the 6 fold axis to the center of the dimer was now the same as that found in Rei. This position is the origin of the translational search (Fig. 2b). The effect of the translation on the crystallographic R factor:

$$\left(= \frac{\Sigma \, ||F_0| - |F_c||}{\Sigma \, |F_0|} \right)$$

for the $hk0$ reflections was monitored. This was first done for $\varphi = -10°$, then to check the correctness of the rotation parameter φ the above procedure was also done at $\varphi = -8$, and $-13°$. The minimum was found at $+3/8$ Å (from the zero position as defined above) in the positive X direction. A deeper minimum (by 0.015) was found with $\varphi = -7°$ when refined coordinates (see below) of the Rei dimer were used.

The Translation Function (Crowther and Blow, 1967) for the 6 fold screw axis (6_1) gave an almost identical result with the ratio of the highest to next highest peak being 157:70. The resulting X coordinates agreed with those determined from the R factor search to within 0.5 Å. This calculation also establishes the correct space group $P6_122$. A translation calculation for a 6_5 axis had as highest value 101 only.

The constrained crystallographic refinement (Deisenhofer and Steigemann, 1974) of the Rei dimer has been proceding in our laboratory, the R factor for reflections between 6.8 to 2.2 Å has been reduced to 0.29. When these new coordinates were transformed and the non-identical residues (after $c\beta$) removed (except Gln 55, Tyr 71, Gln 105) from the structure factor calculation, the R factor was 0.36 for 3968 reflections of the Au protein between 6.8 to 2.5 Å resolution. Three cycles of constrained crystallographic refinement (Deisenhofer and Steigemann, 1974; Huber, Kukla, Bode, Schwager, Bartels, Deisenhofer and Steigemann, 1974; Diamond, 1971), and the adjustment of the overall temperature factor from 25 to 16 lowered the R factor to 0.31.

The difference Fourier map calculated with this model showed about 50 maxima and minima between 0.2 and 0.4 e/Å^3. Several of these are present also in the difference Fourier map of the Rei protein, indicating imperfections of the model. Several maxima are at positions where bound water molecules have been found in Rei.

Most of the peaks occur at or near external amino acid side chains indicating different conformations of these residues. A prominent change is a rotation around C_α to C_β of Tyr 49, which is a constituent of the hapten binding site (see

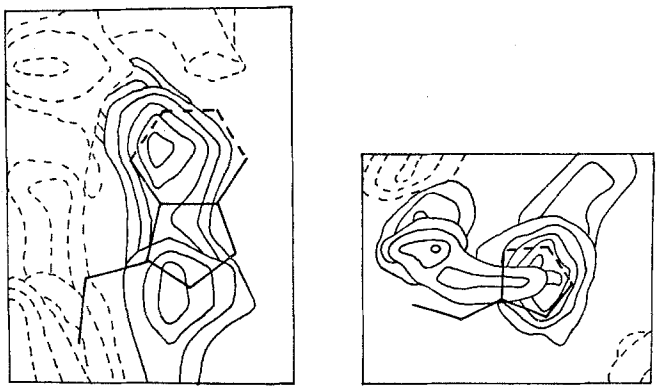


Fig. 3. The electron-density for residues His 70 and Trp 96 in the difference Fourier map. Atoms after $c\beta$ were not included in the phase calculation. The atom positions are superimposed. The first solid contour line is at 0.1 $e/Å^3$, the next are steps of 0.05 $e/Å^3$, 3 layers are superimposed

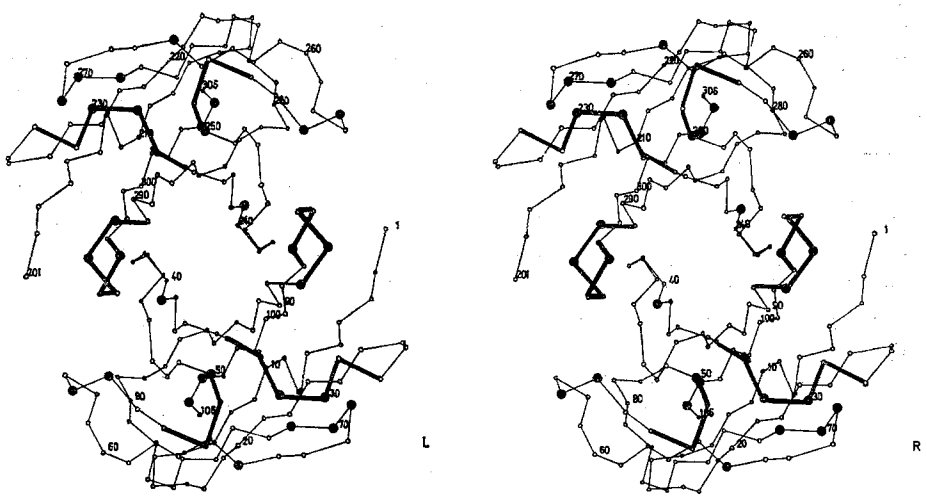


Fig. 4. Stereo drawing of the α-carbon atoms of the dimer viewed along its diad, taken from Epp *et al.* (1974). The second subunit has 200 added to the amino acid numbers. The positions of residues which differ between Rei and Au are marked by full circles and the hypervariable segments are indicated by heavy lines

Fig. 7 in Epp *et al.*, 1974). This conformational change might be a consequence of the replacement of Tyr 96 by a Try in Au.

Several maxima could be attributed to the non-identical residues. The most prominent peaks represented His 70 and Trp 96 (whose electrondensity is shown in Fig. 3). Tyr 93 was not found; this residue is on the surface of the molecule in an ill defined outside loop.

The positions of residues at which Au protein differs from Rei is as marked in Fig. 4. 6 of the 16 changes occur in the hypervariable region, but only one of these,

the exchange of Tyr to Trp at position 96, occurs in the binding cavity. The orientation of the Trp residue is very similar to that of the Tyr it replaces, with the 6 membered ring of Trp extending towards the dimer axis, (decreasing the dimensions of the cavity; see Fig. 7 in Epp *et al.* (1974)). The other exchanged residues of the hypervariable region are on the surface of the molecule, at the periphery of the binding cavity. Residues Tyr 71 and Leu 104 on the inside of the molecule are replaced in Au by the hydrophobic residues Phe and Val respectively. The remaining residues are on the surface of the molecule, they probably have an effect on the packing within the crystal.

The contact between neighbouring molecules in Au across the second 2 fold axis, is very similar to that observed in the Rei *V* dimer. The $-7°$ difference in φ rotation causes a flattening of the 6_1 helix and therefore a shortening of the *c* axis.

The conformation of the backbone and that of the internal residues of the Au and Rei variable fragments seems identical. Further data collection of the Au protein and refinement of both protein structures, now in progress, is required before detailed comparison of the external side chains can be made.

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