the solvent. Thus while all the DL-glutamates studied here are in the coil form in $Me_2SO$ and two samples are partially coil in dimethylformamide, only the very lowest molecular weight has a coil component in chloroform. Poly($\gamma$-benzyl DL-aspartate) of fairly low molecular weight (DP $\simeq$ 60) has been shown to be largely coil in chloroform while poly($\beta$-methyl DL-aspartate) of somewhat greater molecular weight is entirely coil in the same solvent. The ability of the nmr spectrum to estimate the fraction of coil conformation in DL copolymers means that in combination with ORD measurements on the same solution, a precise conformational analysis is possible in terms of coil, RH helix and LH helix. This has been possible previously only by estimating the coil fraction in the solid state and the relative proportions of RH and LH helix from solutions. Such an approach is clearly subject to uncertainties as a result both of the dependence of coil content on the solvent and of the necessity to compare solid state with solution data. The nmr–ORD method is to be preferred.

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# Further Conformational Studies of Immunoglobulin Hinge Peptides†

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ABSTRACT: In a previous paper, low-energy structures of human immunoglobulin G1 hinge peptide (structure I of text) were described. It was found that because of the existence of a twofold symmetry axis, a single backbone conformation could give rise to different double-strand structures when the cysteine side chains had more than one conformation compatible with the existence of such an axis. It was tentatively suggested that this fact might account for the hinge mechanism. The comparison is now made with human immunoglobulin G2 hinge peptide (see structure II of text). It is assumed that this molecule also possesses a twofold axis. Because the presence of the four disulfide bridges must lead to drastic geometrical requirements within the peptide, to begin with, a geometrical approach has been used. Two low-energy conformations of the IgG1 hinge peptide ($d^{280\ 130}$ d d $d^{290\ 270}$ and $d^{290\ 250}$ d d $d^{290\ 270}$) are taken as starting points and only structures having the assumed twofold axis are taken into account. The first results suggest that, once again, a single backbone conformation (of nine amino acids, this time) may give rise to two different double-strand structures notwithstanding the presence of the two additional disulfide bridges. Energy calculations are compatible with this model but they must be further developed, so as to confirm the hypothesis.

In a previous paper,[1] the conformational energy of the central double pentapeptide of the human immunoglobulin G1 hinge region

(Cys-Pro-Pro-Cys-(Pro))
| |
Cys-Pro-Pro-Cys-(Pro)

I

has been calculated with the aid of semiempirical potential functions. In Figure 1, the general shape of an IgG1 molecule is given so as to show the location of the peptide in the molecule. It was found that a small number of families of backbone conformations are energetically allowed and that, due to the existence of a twofold symmetry axis passing through the middle of the two S–S bridges,[2] different dimer structures can result from an identical single-chain backbone conformation when more than one cysteine side-chain conformation is compatible with the closure of the disulfide bridges. It was tentatively suggested that this fact might be related to the hinge mechanism.

Consequently, it is interesting to make the comparison with hinge peptides of other immunoglobulins. The double nonapeptide of the human IgG2 hinge region,[3]

(Cys-Cys-Val-Glx-Cys-Pro-Pro-Cys-(Pro))
| | | |
Cys-Cys-Val-Glx-Cys-Pro-Pro-Cys-(Pro)

II

which has the same C-terminal pentapeptide as the IgG1, was chosen because of the hindrances imposed by the presence of two additional disulfide bridges located only two residues away in the direction of the amino end of the heavy chain. Assuming the existence of a similar twofold symmetry axis, it can easily be tested (and without attempting a calculation of the tertiary structure of the IgG2 hinge) whether or not the model proposed for the IgG1 hinge remains valid for the IgG2, despite the additional geometrical requirements.

The following procedure is used. Two low-energy structures of the IgG1 hinge peptide are chosen. Their single chains only differ by the cysteine side-chain conformations, the backbone having an identical secondary structure. For the sake of convenience, these single chains are called nuclei. The test should establish whether or not, for both nuclei, an identical backbone conformation for the

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(1) C. Renneboog-Squilbin, *J. Mol. Biol.*, 64, 221 (1972).
(2) W. D. Terry, B. W. Matthews, and D. R. Davies, *Nature (London)* **220**, 239 (1968).
(3) B. Frangione, C. Milstein, and J. R. L. Pink, *Nature (London)* **221**, 145 (1969).

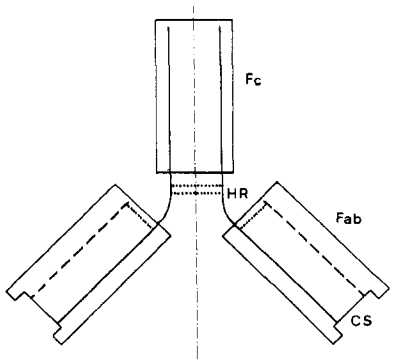



**Figure 1.** Schematic diagram of an IgG1 molecule as an example of the IgG class: (—) heavy polypeptide chains, (---) light polypeptide chains, (. . .) interchain disulfide bridges, (-·-·) twofold symmetry axis, (CS) antibody combining site, (HR) hinge region. The molecule consists of three domains (one FC and two Fab fragments) connected by a hinge region.

four additional N-terminal amino acid residues is compatible with the formation of a dimer. This implies that for a similar backbone conformation, two different arrangements of the cysteine side chains must be found so that the four disulfide bridges are located perpendicular to a straight line which passes through their centers.

The first part of the study is essentially geometrical but it is followed by energetic considerations.

## I. Method

(a) The peptide geometry and the conventions[4] described previously[1] are used. The two low-energy structures of the IgG1 hinge peptide taken as starting points are $d^{280\,130}$ d d $d^{290\,270}$ and $d^{290\,250}$ d d $d^{290\,270}$.[5] They correspond respectively to nucleus A and nucleus B. A search is made for conformations of the N-terminal tetrapeptide with the $S_2M_2$ and $S_3M_3$ bonds (see Figure 2) nearly perpendicular to the $M_6M_9$ symmetry axis (the maximum deviation allowed from perpendicularity is 10°) and with the requirement that the $M_2M_9$ and $M_3M_9$ axes make an angle with $M_6M_9$ not exceeding 10°.

The distances of the N,$C^\alpha$ and C′ backbone atoms to the symmetry axis are also calculated so that structures where the axis passes "through" the polypeptide chain are eliminated. The criterion used is: the minimum accepted distance between symmetrical atoms corresponds to a van der Waals repulsion of 50 kcal/mol.

As a first approximation, Liquori's stereochemical code[6] is used and backbone conformations are expressed as "words," these two terms being synonymous in this paper. The code is merely a convenient way of choosing low local van der Waals energy conformations for the $C_{i-1}{}^\alpha$–$C_{i+1}{}^\alpha$ segments of the polypeptide chain in order to reduce computer time. States a, b, c, d, and a* (Table I) are taken into account for the four amino-terminal residues but it is obvious that from a geometrical point of view, state d is equivalent to state b for cysteine-2. The side-chain $\chi$ angles of cysteines-2 and -3 are varied by steps of 60°, the unfavorable 0° value being excluded. The rest of the molecule stays fixed. That means that $4 \times 5^3 = 500$ different backbone conformations are tested for each nucleus and that for each backbone conformation, $5^4 = 625$ different cysteine SM bond arrangements are possible.

(b) In a second step, the van der Waals and electrostatic energies of the nonapeptides which comply with the symmetry conditions given above are calculated. The valine and glutamic acid residues are replaced by alanine residues in order to simplify the calculation and the contributions of their side chains will consequently be neglected. Sulfur atoms are bound to the cysteine side chains in order to represent the disulfide bridges. The potential functions previously described[1] are used.

(c) Finally, dimers are generated from chosen low-energy nonapeptides by a 180° rotation around the twofold symmetry axis and their energies were calculated as the sum of three contributing factors: (1) the so-called cycle energy ($E_C$) which corresponds to the pairwise interactions between all the atoms of the carboxy-terminal pentapeptide starting from the $C_5{}^\alpha$ and which remains constant in the present treatment; (2) the so-called monomer energy ($E_M$) which corresponds to the pairwise interactions between the atoms of one single chain; and (3) the so-called variable energy ($E_V$) which corresponds to two times the pairwise interactions between the atoms of the amino-terminal tetrapeptide of one chain and all the atoms of the other chain. The total dimer energy is equal to $E_C + 2E_M + E_V$.

**Table I**
**Liquori's Stereochemical Code[6]**

| State | $\phi$ (deg) | $\psi$ (deg) |
|---|---|---|
| a | −63 | −49 |
| b | −155 | 155 |
| c | −150 | 70 |
| d | −75 | 155 |
| a* | 52 | 60 |

## II. Results

(a) One hundred and sixty conformations which may be classified in 31 words fulfill the geometrical requirements for nucleus A and 55 conformations (18 words) for nucleus B. In Table II, a classification of these words is given so that the common features of the structures become apparent. The following rule has been adopted. First, the Cys-Val-Glx tripeptide has been considered; the five backbone parameters which have an influence on the position of the cysteine-3 side chain are $\psi_3$, $\phi_4$, $\psi_4$, $\phi_5$, and $\psi_5$. The words with cysteine-3 in b or d (and in c or a*) are consequently placed on the same line since, when starting at the $C_3{}^\alpha$ atom, they have an identical (or nearly identical) set of $\phi$ and $\psi$ angles.

(b) As can be seen in Table II, a great number of words (139 of 215) with a van der Waals energy greater than 50 kcal/mol may be eliminated. Only three sets of words (II, VII, and X) are found to be energetically favorable in some cases. Sets II and VII are common to both nuclei whereas set X is at first glance only applicable to nucleus B. One may wonder if words belonging to set X could not also be found for nucleus A when the rotation angle values are allowed to deviate slightly from their "standard" value. The answer is yes. To give an example, conformations ($a^{60\ 180}$b (with $\psi_3 = 120°$)$^{90\ 210}$ a b (with $\phi_5 = -170°$) $d^{280\ 130}$ d d $d^{290\ 270}$) and ($b^{300\ 240}$ $b^{30\ 60}$ a b $d^{280\ 130}$ d d $d^{290\ 270}$) are found after this treatment.

(c) The molecular models of all the low-energy single-chain structures (total energy lower than 10 kcal/mol) were built into dimers with the aid of Dreiding stereomodels. A reasonable cysteine side-chain arrangement was chosen from the available structures for the abcc nucleus A and abcc nucleus B monomers (set VII) as well as for the abab nucleus A and abab nucleus B (set X) monomers. These monomers were taken as starting points for

(4) IUPAC-IUB Commission of Biochemical Nomenclature, *Biochemistry*, 9, 3471 (1970). It should be noted that in Figure 3 of ref 1, "$\Phi = \psi = 0°$ should read $\Phi = \psi = 180°$."

(5) For the meaning of the letters, see Table I. The exponents indicate the cysteine $\chi$ angle values.

(6) A. M. Liquori, *Quart. Rev. Biophys.*, 2, 65 (1969).

**Table II**
**Classification of the Conformations of the N-Terminal Tetrapeptide of the IgG2 Hinge Nonapeptide[a]**

| | Nucleus A: $d^{280\ 130}$ d d $d^{290\ 270}$ | | | | | | | | Nucleus B: $d^{290\ 250}$ d d $d^{290\ 270}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Words | 3 | 4 | 5 | Words | 3 | 4 | 5 | Words | 3 | 4 | 5 | Words | 3 | 4 | 5 |
| I | | | | | | | | | $a_2\ a_1\ b\ a^*$ | 2 | | | | | | |
| | | | | | | | | | $b_1\ a_1\ b\ a^*$ | 1 | | | | | | |
| | $a_1^*\ a_4\ b\ a^*$ | 4 | | | | | | | $a_2^*\ a_1\ b\ a^*$ | 2 | | | | | | |
| II | $b_2\ b_3\ b\ a^*$ | 6 | 2 | | | | | | | | | | | | | |
| | $c_4\ b_3\ b\ a^*$ | 12 | 3 | | $c_3\ d_3\ b\ a^*$ | 9 | 2 | | $c_1\ b_4\ b\ a^*$ | 4 | | | | | | |
| | $a_2^*\ b_3\ b\ a^*$ | 6 | 2 | | $a_3^*\ d_3\ b\ a^*$ | 9 | | | $a_2^*\ b_4\ b\ a^*$ | 8 | 3 | | | | | |
| III | | | | | $a_1\ a_2^*\ d\ a^*$ | 2 | | X | | | | | | | | |
| | $b_1\ c_2\ d\ a^*$ | 2 | | X | | | | | | | | | | | | |
| | $c_3\ c_2\ d\ a^*$ | 6 | | X | | | | | $c_1\ c_3\ d\ a^*$ | 3 | | | | | | |
| | $a_4^*\ c_2\ d\ a^*$ | 8 | | X | | | | | $a_1^*\ c_3\ d\ a^*$ | 3 | | | | | | |
| IV | $c_2\ b_1\ a^*\ a^*$ | 2 | | | | | | | | | | | | | | |
| | $a_2^*\ b_1\ a^*\ a^*$ | 2 | | | | | | | | | | | | | | |
| V | $a_1\ a_2\ a\ c$ | 2 | | X | | | | | | | | | | | | |
| | $b_2\ a_2\ a\ c$ | 4 | | X | | | | | | | | | | | | |
| VI | | | | | $a_2\ a_2^*\ a\ c$ | 4 | | | | | | | | | | |
| | $b_1\ c_1\ a\ c$ | 1 | | | | | | | $b_2\ c_1\ a\ c$ | 2 | | | | | | |
| | $c_1\ c_1\ a\ c$ | 1 | | | | | | | | | | | | | | |
| | $a_1^*\ c_1\ a\ c$ | 1 | | | | | | | | | | | | | | |
| VII | $a_9\ b_3\ c\ c$ | 27 | 20 | | $a_4\ d_3\ c\ c$ | 12 | 9 | | $a_9\ b_1\ c\ c$ | 3 | 2 | | $a_1\ d_1\ c\ c$ | 1 | | |
| | $b_1\ b_3\ c\ c$ | 3 | 1 | | $b_1\ d_3\ c\ c$ | 3 | 1 | | | | | | | | | |
| | $c_3\ b_3\ c\ c$ | 9 | 4 | | | | | | | | | | | | | |
| | $a_4^*\ b_3\ c\ c$ | 12 | 4 | | | | | | | | | | | | | |
| VIII | $a_1\ a_1\ b\ d$ | 1 | | | | | | | | | | | | | | |
| IX | $a_3\ c_1\ b\ d$ | 3 | | | $a_2\ a_1^*\ b\ d$ | 2 | | | | | | | | | | |
| | $b_2\ c_1\ b\ d$ | 2 | | | $b_1\ a_1^*\ b\ d$ | 1 | | | | | | | | | | |
| | $c_2\ c_1\ b\ d$ | 2 | | | | | | | | | | | | | | |
| | $a_2^*\ c_1\ b\ d$ | 2 | | | | | | | | | | | | | | |
| X | | | | | | | | | $a_4\ b_1\ a\ b$ | 4 | 3 | | $a_2\ d_1\ a\ b$ | 2 | 2 | |
| | | | | | | | | | $b_2\ b_1\ a\ b$ | 2 | 2 | | $b_2\ d_1\ a\ b$ | 2 | 2 | |
| | | | | | | | | | $c_5\ b_1\ a\ b$ | 5 | 5 | X | $c_4\ d_1 q\ a\ b$ | 4 | 3 | |
| | | | | | | | | | $a_3^*\ b_1\ a\ b$ | 3 | 3 | X | $a_4^*\ d_1\ a\ b$ | 4 | 3 | |
| | | 118 | 36 | | | 42 | 12 | | | 42 | 18 | | | 13 | 10 | |

[a] The subscripts indicate the number of cysteine side-chain conformations compatible with the formation of a dimer possessing a twofold symmetry axis. 1, set of words; 3, total number of single-chain tertiary structures geometrically compatible with the formation of the dimer; 4, number of single-chain tertiary structures with a van der Waals energy lower than 50 kcal/mol; 5, cross when short distances (van der Waals energy greater than 50 kcal/mol) arise between symmetrical N, $C^\alpha$ of C′ atoms in the dimer.

the generation of the dimers. A narrowing down of the range of some rotation angles was performed in order to verify the influence of small variations of the rotation parameters on the molecule geometry and on its energy (Table III). The results are summarized in Table IV and the details of one of the best conformations obtained from the four conformations started with are given in Table V.

It can be concluded as a result of the examination of the different contributions to the dimer energy, that if the monomer energy remains favorable in all the geometrically allowed structures (as in line 1 and 4[7] of Table IV), the number of low-energy dimers depends on whether or not, short distances arise between the symmetrical chains. In the two other cases (line 2 and 3 of Table IV) the dimer energy does not parallel the $E_C$ energy because of the unfavorable monomer energy. Bearing in mind that the energy resulting from the dimer formation ($E_C + E_V$) never falls below −7 kcal/mol, it is interesting to scrutinize the monomer energy contributions which appear twice in the total energy. When two conformations corresponding to the same word are considered as in line 1 and 2 or 3 and 4 (Table IV), the comparison must be made at the level of the side chains since the backbone conformation remains unchanged. In line 1, the cysteine-2 side-chain conformation is about 10 kcal/mol lower than in line 2 but the opposite happens in the cysteine-3 side chain. Therefore, one could expect an $E_M$ of the same order of magnitude for both lines (see the example in Table V). However, less favorable interactions which arise between cysteine-2 and -3 side chains in line 2 explain why the energy of the conformations derived from line 2 is always higher than that of the conformations derived from line 1. On the other hand, in line 3, the energy of both cysteine side chains is about 10 kcal/mol greater than in line 4. This fact accounts for the 40-kcal/mol difference between the dimers obtained from the conformations of line 3 and 4 (see the examples in Table V).

(7) In line 4, the 22 nonnegative $E_M$ are small. Hence, the energy of the dimers derived from them is negative.

## III. Discussion

From the study of the human IgG1 hinge double pentapeptide (see structure I) it was possible to draw a certain number of conclusions which can be briefly summarized as follows. (1) The geometrical criterion (the disulfide bridges perpendicular to the symmetry axis passing through their centers) is not restrictive enough to make a choice between the different single-chain backbone conformations. Indeed, for all of the 72 tested words, at least

**Table III**
**Angle Values (in Degrees) Used in the Dimer**

| | Backbone | | | | | | | | Side Chain | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | For Nucleus A | | | | For Nucleus B | | | |
| | $\phi_2$ | $\psi_2$ | $\phi_3$ | $\psi_3$ | $\phi_4$ | $\psi_4$ | $\phi_5$ | $\psi_5$ | $\chi_2^1$ | $\chi_2^2$ | $\chi_3^1$ | $\chi_3^2$ | $\chi_2^1$ | $\chi_2^2$ | $\chi_3^1$ | $\chi_3^2$ |
| abcc | −63 | −49 | −155 | 155 | −150 | 70 | −150 | 70 | 180 | 180 | 120 | 240 | 60 | 240 | 60 | 180 |
| | | | −140 | 120 | −180 | | | | 210 | 210 | 150 | 270 | 90 | 270 | 90 | 210 |
| | | | | | | | | | 150 | 150 | 90 | 210 | 30 | 210 | 30 | 150 |
| abab | −63 | −49 | −155 | 155 | −63 | −49 | −155 | 155 | 60 | 180 | 120 | 240 | 300 | 240 | 60 | 240 |
| | | | −170 | 120 | −70 | | −170 | | 90 | 210 | 150 | 270 | 330 | 270 | 90 | 270 |
| | | | | | | | | | 30 | 150 | 90 | 210 | 270 | 210 | 30 | 210 |

**Table IV**
**Results of the Energetical Calculations for the Dimers**

| | Structures | No. of Tested Structures | No. of Geom Allowed Structures | $E_c$ (kcal/mol) | No. of Neg $E_M$ | No. of Neg $E_V$ | No. of Dimers with a Neg Total Energy |
|---|---|---|---|---|---|---|---|
| 1 | $a^{180\ 180}\ b^{120\ 240}\ c\ c\ d^{280\ 130}\ d\ d\ d^{290\ 270}$ | 648 | 39 | −4.2 | 39 | 31 | 31 |
| 2 | $a^{60\ 240}\ b^{60\ 180}\ c\ c\ d^{290\ 250}\ d\ d\ d^{290\ 270}$ | 648 | 10 | −4.6 | 2 | 10 | 2 |
| 3 | $a^{60\ 180}\ b^{120\ 240}\ a\ b\ d^{280\ 130}\ d\ d\ d^{290\ 270}$ | 1296 | 93 | −4.2 | 16[a] | 82 | 8[b] |
| 4 | $a^{300\ 240}\ b^{60\ 240}\ a\ b\ d^{290\ 250}\ d\ d\ d^{290\ 270}$ | 1296 | 114 | −4.6 | 92 | 84 | 86 |

[a] There is no negative $E_M$. Number of $E_M$ smaller than 9 kcal/mol. [b] Number of dimers formed from the 16 $E_M$ smaller than 9 kcal/mol which have no short interchain distances (their energy is smaller than 13 kcal/mol).

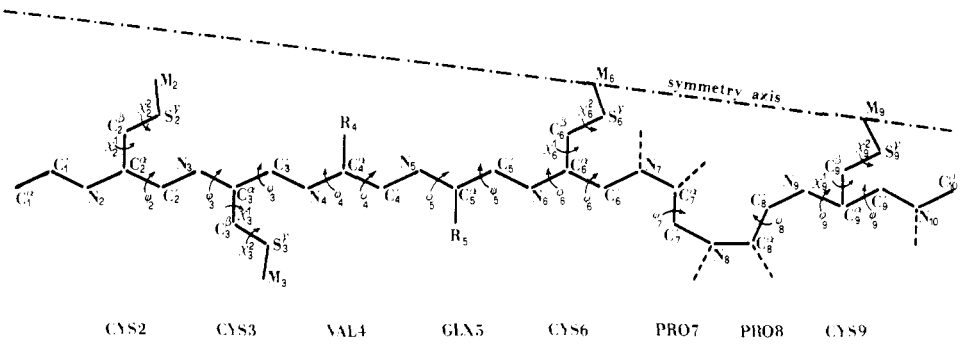



**Figure 2.** Schematic diagram of the human IgG2 hinge peptide.

1 of the 1296 possible cysteine side-chain arrangements is compatible with the formation of a structure possessing a twofold symmetry axis. Hence the calculation of the energy of the dimers is necessary when making a choice between the different words. (2) For a certain number of backbone conformations, more than one cysteine side-chain arrangement compatible with the formation of a low-energy dimer is found. In other words, different double-strand structures are generated from a single chain with an identical backbone conformation owing to the different orientations of the cysteine side chains of that single chain.

It was suggested in ref 1 that the hinge mechanism (*i.e.*, the change in the angle between both Fab fragments[8] (see Figure 1)) might simply be the result of a conformational change of the cysteine side chains, the backbone conformation remaining unchanged (or nearly unchanged).

Such a hypothesis is to be tested in the case of other hinge peptides and particularly in the case where additional constraints could be incompatible to the proposed hinge mechanism. The most rigid hinge peptide seems to be the human IgG2 peptide which possesses two additional and adjacent cysteine residues located two residues away in the direction of the amino end of the heavy chain. The problem was formulated in the following manner. Can in this case too, a single chain with the same backbone conformation give rise to two different dimer structures possessing a twofold symmetry axis?

It should be emphasized here that the applied geometrical test is a rough estimation and consequently the subsequent dimer energy calculation is only indicative.

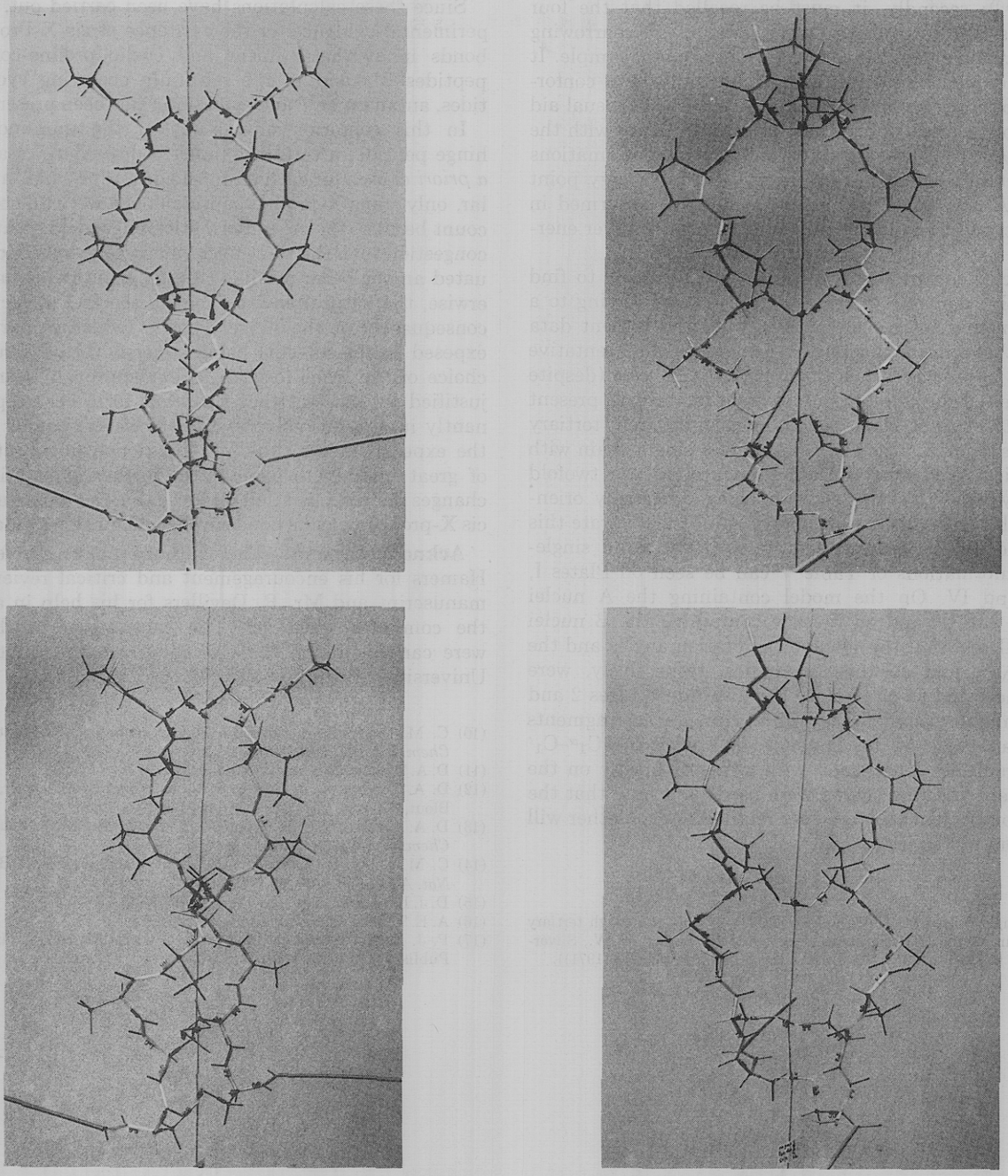
As expected and contrary to the IgG1 hinge peptide, the geometrical hindrances are drastic and justify *a posteriori* the approach which has been used. Of the 500 available words, only 31 (6%) are realized for nucleus A and 18 (3.5%) for nucleus B; 8 of these 18 words are common to both nuclei. The geometrical study, even in its most rudimentary form, thus gives quite a clear answer. A single chain with an identical backbone conformation may give rise to different double-strand structures owing to different arrangements of the four cysteine side chains, in the case of the IgG2 hinge peptide, also. In a final step, structures where the geometrical requirements are perfectly

(8) N. M. Green, *Advan. Immunol.*, 11, 1 (1969).

**Table V**
**Details of the Four Structures Given in the Example**

| | Nucleus A | | | | | | | | | | | | Nucleus B | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | abccdddd | | | | | | | | | | | | |
| | $\phi_2$ | $\psi_2$ | $\chi_2^1$ | $\chi_2^2$ | $\phi_3$ | $\psi_3$ | $\chi_3^1$ | $\chi_3^2$ | $\phi_4$ | $\psi_4$ | $\phi_5$ | $\psi_5$ | $\phi_2$ | $\psi_2$ | $\chi_2^1$ | $\chi_2^2$ | $\phi_3$ | $\psi_3$ | $\chi_3^1$ | $\chi_3^2$ | $\phi_4$ | $\psi_4$ | $\phi_5$ | $\psi_5$ |
| | −63 | −49 | 150 | 210 | −155 | 120 | 150 | 270 | −150 | 70 | −150 | 70 | −63 | −49 | 60 | 240 | −155 | 155 | 60 | 180 | −150 | 70 | −150 | 70 |
| | | | | | Cysteine-2 | | | | | Cysteine-3 | | | | | | | Cysteine-2 | | | | | Cysteine-3 | | |
| 1[a] | | | | | 0.060 | | | | | 0.118 | | | | | | | 0.176 | | | | | 0.176 | | |
| 2[b] | | | | | −0.099 | | | | | −0.186 | | | | | | | 0.145 | | | | | 0.075 | | |
| 3[c] | | | | | 4.32 | | | | | 5.29 | | | | | | | 6.18 | | | | | 5.64 | | |
| 4[d] | | | | | 6.26 | | | | | 5.58 | | | | | | | 7.81 | | | | | 9.36 | | |
| | | | | | Electrostatic Energy | | | | | van der Waals Energy | | | | | | | Electrostatic Energy | | | | | van der Waals Energy | | |
| Monomer | | | | | −9.1 | | | | | 5.3 | | | | | | | −10.1 | | | | | 9.2 | | |
| Cycle | | | | | 0.1 | | | | | −4.3 | | | | | | | 0.1 | | | | | −4.7 | | |
| Variable | | | | | 0.1 | | | | | −2.2 | | | | | | | 0.1 | | | | | −0.5 | | |
| Dimer total energy | | | | | | | | −14.0 | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | −6.9 | | | | | | |
| | | | | | | | | | | | | ababdddd | | | | | | | | | | | | |
| | $\phi_2$ | $\psi_2$ | $\chi_2^1$ | $\chi_2^2$ | $\phi_3$ | $\psi_3$ | $\chi_3^1$ | $\chi_3^2$ | $\phi_4$ | $\psi_4$ | $\phi_5$ | $\psi_5$ | $\phi_2$ | $\psi_2$ | $\chi_2^1$ | $\chi_2^2$ | $\phi_3$ | $\psi_3$ | $\chi_3^1$ | $\chi_3^2$ | $\phi_4$ | $\psi_4$ | $\phi_5$ | $\psi_5$ |
| | −63 | −49 | 60 | 180 | −155 | 120 | 150 | 240 | −70 | −49 | −170 | 155 | −63 | −49 | 270 | 210 | −170 | 120 | 30 | 210 | −70 | −49 | −155 | 155 |
| | | | | | Cysteine-2 | | | | | Cysteine-3 | | | | | | | Cysteine-2 | | | | | Cysteine-3 | | |
| 1[a] | | | | | 0.073 | | | | | 0.184 | | | | | | | 0.049 | | | | | 0.087 | | |
| 2[b] | | | | | 0.140 | | | | | −0.006 | | | | | | | −0.108 | | | | | −0.148 | | |
| 3[c] | | | | | 4.20 | | | | | 6.14 | | | | | | | 3.94 | | | | | 5.15 | | |
| 4[d] | | | | | 4.98 | | | | | 6.20 | | | | | | | 4.11 | | | | | 6.84 | | |
| | | | | | Electrostatic Energy | | | | | van der Waals Energy | | | | | | | Electrostatic Energy | | | | | van der Waals energy | | |
| Monomer | | | | | −10.8 | | | | | 18.0 | | | | | | | −10.1 | | | | | −2.2 | | |
| Cycle | | | | | 0.1 | | | | | −4.3 | | | | | | | 0.1 | | | | | −4.7 | | |
| Variable | | | | | 0.1 | | | | | −1.2 | | | | | | | 0.1 | | | | | −0.9 | | |
| Dimer total energy | | | | | | | | 9.2 | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | −30.1 | | | | | | |

[a] 1 = distance of M to the symmetry axis divided by the length M $M_9$. [b] 2 = cosine of the angle formed by the symmetry axis and the SM segment. [c] 3 = distance of $S^\gamma$ to the symmetrical $S^\gamma$ (given in Å). [d] 4 = distance of $C^\beta$ to the symmetrical $C^\beta$ (given in Å). The energies are expressed in kcal/mol, angles in degrees.



**Plates I–IV.** Photographs taken at two different angles (along the $S_2$–$S_2$ bridge for Plates I and III and perpendicular to it for Plates II and IV) of the two dimers which can be generated from a single chain having the abccdddd conformations of Table V. Plates I and II correspond to the single-chain conformation "$a^{150\ 210}$ b (with $\psi_3$ = 120)$^{120\ 150}$ c c ($d^{180\ 130}$d d $d^{290\ 270}$ = nucleus A)" and plates III and IV to "$a^{60\ 240}b^{60\ 180}$ c c ($d^{290\ 250}$ d d $d^{290\ 270}$ = *nucleus B*)." *The vertical string represents the twofold symmetry axis.* The main-chain bonds have been emphasized with pieces of plastic (pieces of drinking straws were used) and the $C_1^{\alpha}$–$C_i$ bonds have been lengthened.

fulfilled should be obtained by a refinement of the rotation angle values.

Even though the selection is very severe from a geometrical point of view, an energy estimation is necessary to get an idea of the validity of our hypothesis. Indeed, a certain number of the backbone conformations that were found are to be excluded because of the existence of interactions between residues located far from each other in the sequence. Fortunately, however, low-energy single-chain structures are available for both nuclei. They are found namely in sets VII and X (Table II) which are also particularly favored from an entropic point of view for nucleus A and B, respectively. That is why our attention has been concentrated on these sets and why conformations of the amino-terminal tetrapeptide derived from the abab word were scrutinized for nucleus A.

It was then possible to tentatively make a comparison between the abccdddd words (from set VII) for both nuclei and also between the ababdddd words (from set X) when certain chosen backbone angles and the cysteine-2 and -3 side-chain angles were allowed to vary slightly.

Even though the energy values are only an indication, it is obvious that the abcc nucleus A conformation is entropically favored relative to the abcc nucleus B conformation (Table IV) and that the energy difference between the conformations derived from abab nucleus A and abab nucleus B is too large not to be taken into account. However, the following features of the study must be kept in mind. First of all, let us mention the quite arbitrary choice of the two nuclei. The same kind of study should be made starting from other nuclei detected in our previous study (for instance, $c^{190\ 40}$ d d $d^{300\ 100}$ and $c^{200\ 210}$ d

d $d^{290\ 140}$); secondly, it must be recalled that the four monomers used for generating the dimers, with narrowing down of the angle values, are after all only an example. It must be emphasized that the cysteine side-chain conformations of abab nucleus A were chosen with the visual aid of the molecular model so that they would relate with the symmetry axis. These cysteine sidė-chain conformations are unfortunately not too favorable from an energy point of view. A new angle refinement should be performed in order to try to find side-chain arrangements of lower energy.

In conclusion, although we have not been able to find two equally probable dimer structures corresponding to a same monomer backbone conformation, the present data suggest that on the basis of a geometric and tentative energetic approach, the IgG2 hinge peptide can (despite the severe tension due to the four disulfide bridges present in a length of eight amino acids) exist in different tertiary structures as a result of the rotation of a single chain with an identical backbone conformation around the twofold symmetry axis, this single chain being differently orientated with respect to the symmetry axis. To illustrate this point, the dimers generated from both abccdddd single-chain conformations of Table V can be seen on Plates I, II, III, and IV. On the model containing the A nuclei (Plates I and II) and on the one containing the B nuclei (Plates III and IV), the $\chi_2^2$ and $\phi_4$ rotation angles and the $\psi_2$, $\phi_3$, $\chi_3^2$, and $\psi_4$ rotation angles, respectively, were slightly adjusted so as to close the disulfide bridges 2 and 3. One might visualize the Fab fragments as fragments making an angle of, for instance, 90°[9] with the $C_1^{\alpha}$–$C_1'$ bonds which are lengthened with strips of plastic on the model (here drinking straws were used) and note that the orientation of the two fragments relative to each other will be different in Plates I and III.

(9) The angle chosen is arbitrary since until now only the rough tertiary structure of the IgG1 molecule is known V. R. Sarma, E. W., Silverton, D. R. Davies, and W. D. Terry, *J. Biol. Chem.*, **246**, 3753 (1971)).

Since these calculations have been carried out, new experimental evidence for the existence of cis X-Pro peptide bonds in synthetic linear and cyclic proline-containing peptides[10-14] and in the naturally occurring cyclic peptides, antanamide[15] and evolidine[16] has been presented.

In this conformational study of the immunoglobulin hinge peptide, a certain number of low-energy states were *a priori* chosen for each amino acid residue[6] and in particular, only trans X-proline peptide bonds were taken into account because the cis conformation engenders greater steric congestion than the trans form when the prolyl residue is situated among other residues in a polypeptide chain.[17] Otherwise, the natural environment of the IgG molecules and consequently of the hinge peptide (which is particularly exposed to the solvent) being aqueous (blood serum), the choice of the trans form in a first approach, seems to be justified by the fact that the trans form occurs predominantly in aqueous solvents.[10-16] However, having in mind the experimental results[10-16] mentioned above, it will be of great interest to examine in further calculations, the changes in conformation induced by the introduction of cis X-proline peptide bonds in the IgG hinge peptide.

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