Communications to the Editor

Structural Role of Valine and Isoleucine Residues in Proteins. A Proposal

In previous papers of this series[1-3] it has been shown that the β structure of homo-heptamers from the amino acid residues with β-branched side chain L-valine and L-isoleucine is more stable than that of homo-heptamers from L-alanine, L-norvaline, L-norleucine, L-leucine, β-cyclohexyl-L-alanine, *S*-methyl-L-cysteine, and L-methionine. This experimental observation is of particular interest for the prediction of protein structure since it corroborates the results of a statistical analysis[4,5] which showed that valine and isoleucine occur with the greatest frequencies in the β regions of proteins.

From our conformational investigation on the various homo-heptapeptides carried out by a number of spectroscopic techniques[6-9] indication was also obtained that the β structure of hepta-L-valine and hepta-L-isoleucine is probably more stable in the parallel-strand arrangement. This is reasonable on steric grounds since in a regular antiparallel β-pleated sheet every C^β atom has one short distance between the corresponding atoms of the two residues in the adjacent peptide strands, so that destabilizing interactions can arise between their substituents. In addition, in the regular parallel β-pleated structure all these C^β atoms are equidistant; this property could give rise to more favorable interstrand interactions of the β-branched side chains of the narrowly fluctuating (i.e., with a restricted distribution of the ϕ and ψ dihedral angles)[10,11] valine and isoleucine residues. This proposal is supported by: (i) the results of Komoto et al.[12] who described the conformations and conformational variations of poly-L-valine and poly-L-isoleucine in the course of polymerization; (ii) the observed stereoselectivity in the polymerization of valine *N*-carboxy anhydride;[13] (iii) the study of Cung et al.[14,15] on the stereoselective dimerization of the model "dipeptides" *N*-acyl-L-valine *N*-alkylamides; and (iv) the x-ray diffraction analysis of *N*-acetyl-L-valine-*N*-methylamide,[16] which showed that the conformation about the valine C^α atom is $\phi = -118°, \psi = 113°$ very near to the parallel-strand β-pleated sheet structure model proposed by Pauling and Corey ($\phi = -110°, \psi = 113°$).[17]

If this proposal is correct one would expect that, when amino acid residues at the same level in adjacent strands are considered, doublets of the type Val···Val, Val···Ile, and Ile···Ile, or triplets (or quadruplets, etc.) containing exclusively these two residues, are commonly found in β regions of proteins with parallel orientation of strands, while they should occur rarely in β regions with antiparallel orientation of strands.

To test this hypothesis, an analysis of the globular proteins of known sequence, the structure of which has been solved by x-ray diffraction, is currently in progress in our laboratory. In the present communication the preliminary results obtained on the dehydrogenases are reported. These proteins have been examined first since they contain both a region of parallel β-pleated sheet (in the coenzyme-binding domain) and a region of antiparallel β-pleated sheet (in the catalytic domain).[18-23] Only the residues have been considered which are internal in a sheet, part of a strand with identical orientation to both sides, and forming the hydrogen bonds typical of regular parallel or antiparallel β-pleated sheets with both their NH and CO groups.

According to the above limitations, Figures 1 and 2 show the amino acid residues in the parallel-strand β sheet of lobster D-glyceraldehyde 3-phosphate dehydrogenase (GPDHase)[18-20] and the M_4 isoenzyme from dogfish lactate

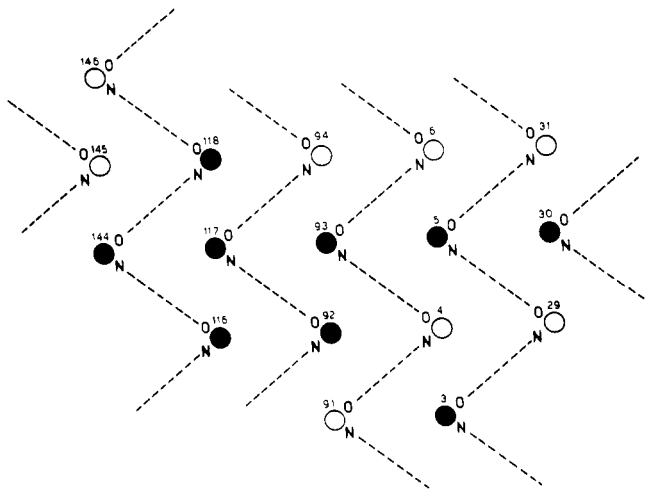

Figure 1. Amino acid residues in the parallel-strand β sheet of lobster D-glyceraldehyde 3-phosphate dehydrogenase[18-20] (see text for explanation). Black circles represent valine and isoleucine residues and white circles other residues. Dashed lines indicate interstrand hydrogen bonds.

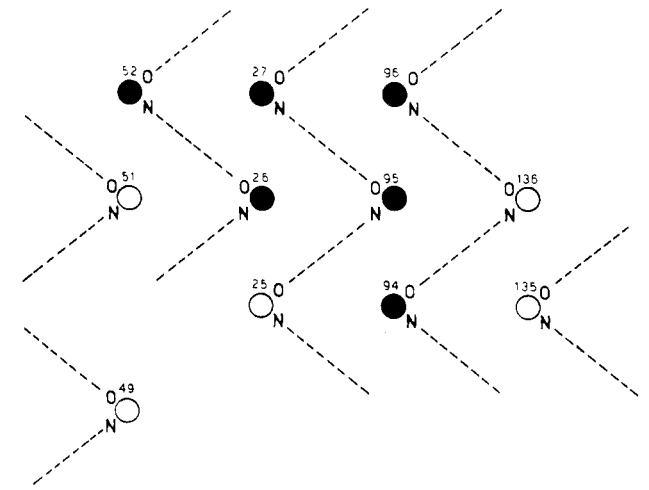

Figure 2. Amino acid residues in the parallel-strand β sheet of the M_4 isoenzyme from dogfish lactate dehydrogenase[19,21] (see text for explanation). Black circles represent valine and isoleucine residues and white circles other residues. Dashed lines indicate interstrand hydrogen bonds.

dehydrogenase (LDHase),[19,21] respectively. It is evident that almost 50% of the doublets are formed by valine and isoleucine residues; the Val^{144}···Val^{117}···Val^{93}···Ile^{5}·····Val^{30} interstrand sequence of the lobster GPDHase and the Val^{52}···Val^{27}···Ile^{96} interstrand sequence of the dogfish LDHase are particularly intriguing. If the primary sequences in the parallel-strand β-sheet region of lobster,[19,20] pig,[19,20] yeast,[19,20] and *Bacillus stearothermophilus*[22] GPDHases, aligned according to the principle of topological equivalence of Rossmann et al.,[23] are compared, it will be seen that the mutations involving the β-branched amino acid residues shown in Figure 1 are structurally conservative in about 90% of times (Table I). Significantly, yeast[19,20] and *Bacillus stearothermophilus*[22]

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Table I
Structurally Conservative Mutations of the Valine and Isoleucine Residues Shown in Figure 1[b]

GPDHase (lobster)	Lobster[19,20]	Pig[19,20]	Yeast[19,20]	*B. stearothermophilus*[22]
116	Val	Val	Val	Val
92	Ile	Val	*a*	Ile
144	Val	Val	Val	Ile
117	Val	Ile	Val	Ile
93	Val	Val	Ile	Val
5	Ile	Val	Ile	Ile
30	Val	Ile	*a*	Val

[a] Mutations structurally nonconservative. [b] The sequences are aligned according to the method of Rossmann et al.[23]

GPDHases exhibit an additional doublet of the type Ile···Val corresponding to the amino acid residues in positions 91 and 3, respectively, of Figure 1.

On the other hand, again in agreement with our working hypothesis, only one of such doublets (Val^{305}···Val^{244}) occurs in the antiparallel-strand β sheet of lobster GPDHase;[18] interestingly, this doublet is absent in pig[20] and *Bacillus stearothermophilus*[22] GPDHases due to structurally nonconservative mutations of the valine residue in position 244.

References and Notes

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CORRECTION

"Helical Structures of Poly(D-L-peptides). A conformational Energy Analysis", by F. Colonna-Cesari, S. Premilat, F. Heitz, G. Spach, and B. Lotz, Volume 10, Number 6, November–December 1977, page 1284.

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