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Polypeptide chains with similar amino acid sequences but a distinctly different conformation

Bovine and porcine phospholipase A_2

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The primary structures of bovine and porcine pancreatic phospholipase A_2 differ only by about 15%. Nevertheless, a 12 residue loop, with only one substitution (Val→Phe) has a quite different conformation, whereas the rest of the molecules have a very similar folding indeed. From this observation it is concluded that prediction of a 3-dimensional structure on the basis of sequence similarity of short segments alone might give erroneous results.

Amino acid sequence *Prediction of conformation* *Phospholipase A_2* *Sequence similarity*

1. INTRODUCTION

X-ray crystallographic analysis has shown that proteins with homologous amino acid sequences have similar 3-dimensional structures. Classical examples are sperm whale myoglobin and horse α- and β-haemoglobin (~18% identical residues) [1,2], the serine proteases trypsin, chymotrypsin and elastase [3,4] with 25% identical residues, and the sulfhydryl proteases actinidin and papain [5] with 48% identical residues.

From the above it can be inferred that the 3 dimensional fold of a protein is preserved over a longer evolutionary period than the amino acid sequence. This deduction has been the foundation of the use of molecular replacement methods in solving the crystal structure of a protein related to a protein with a known 3-dimensional structure [6].

Here, however, we present evidence that the above generalization is not necessarily valid when comparing polypeptide chains of limited length, which have a similar sequence. This will be illustrated with an example derived from the structures of bovine and porcine phospholipase A_2.

2. MATERIALS AND METHODS

For the comparisons, we used the crystal structures of bovine pancreatic phospholipase A_2, refined at 1.7 Å resolution [7] and porcine pancreatic phospholipase A_2, refined at 2.6 Å resolution [8]. To obtain optimal superposition of the 2 models a method was used similar to the one described in [9, 10]. The superimposed models were examined on an Evans and Sutherland Picture System II, using the versatile program Guide [11].

3. RESULTS

The amino acid sequences of bovine and porcine phospholipase A_2 are highly homologous: 85% of the 124 residues are identical [12]. Also, their 3 dimensional structures are very similar: the rms difference between 112 equivalent Cα-atoms is 0.47 Å [8]. However, one loop of 12 residues (59–70), which has one substitution only at position 63 (bovine Val → porcine Phe) has a completely different conformation. In the bovine enzyme the first part of this loop is an α-helix

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(residues 59–66) and the second part forms a surface loop. The side chain of Val 63 points towards the solvent (fig. 1). A phenylalanine at this position would not cause any steric hindrance with other residues. Nevertheless, in the porcine enzyme the side chain of Phe 63 is in the molecule's interior, and this has caused a complete rearrangement of the loop 59–70. The short α-helix in the bovine enzyme has disappeared and, instead, residues 67–71 form a short 3_{10} helix. Fig. 1 shows a comparison of the folding of the two molecules and table 1 gives quantitative information on the extent of the conformational change. It is clear that the remaining parts of the molecules have a very similar conformation.

4. DISCUSSION

The two phospholipases are homologous proteins with similar structures. The substitution at position 63 has caused a rearrangement of part of the main chain in one of the descendants. No other substitutions occur in the very vicinity of residue 63. Neither is residue 63 involved in contacts with neighbouring molecules in the crystal structure. As indicated in fig. 1 there are some substitutions at the surface of the phospholipase molecule further away from position 63 (Lys 53 → Arg 53; Asn 72 → Ser 72), but it is hard to see how they could induce a different conformation around residue 63.

Thus the cause of the rearrangement of the peptide chain most probably lies in the substitution itself. The volume of the Val side chain is about 60 $Å^3$ less than the volume of a Phe side chain [13]. Consequently, a Phe → Val substitution would provide space for 2–3 water molecules buried in the hydrophobic interior of porcine phospholipase.

Concerning a Val → Phe substitution, it is worthwhile to consider the hydrophobic interactions of the peptide chain 59–70 with the rest of the molecule. An estimate of the extent of the hydrophobic interactions can be obtained from a

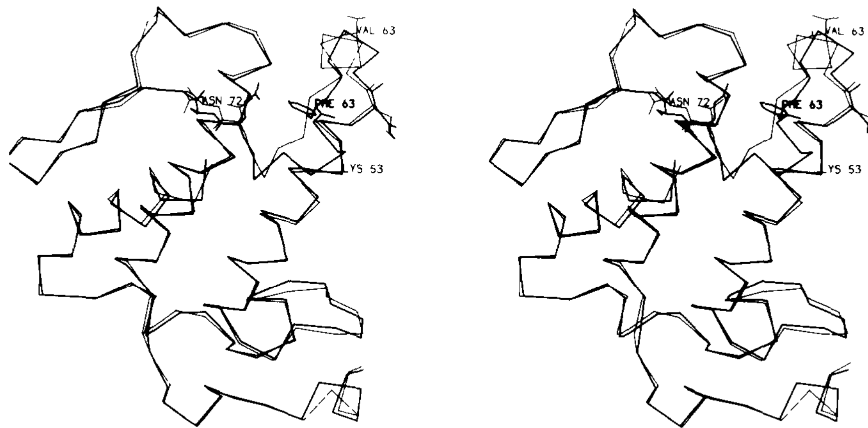

Fig. 1. Stereodrawing of the superposition of $C\alpha$ atoms of bovine phospholipase (thin lines) and porcine phospholipase (heavy lines). Indicated are amino acid substitutions at position 63 and its vicinity.

Table 1

Structural differences between bovine and porcine phospholipase A_2 after optimal superposition of the complete molecules

	58		60			↓		65					70
Sequence in bovine phospholipase	Leu	Asp	Ser	Cys	Lys	Val	Leu	Val	Asp	Asn	Pro	Tyr	Thr
Sequence in porcine phospholipase	Leu	Asp	Ser	Cys	Lys	Phe	Leu	Val	Asp	Asn	Pro	Tyr	Thr
Distance between corresponding $C\alpha$-atoms (Å)	0.6	1.8	1.9	1.0	2.8	8.3	8.2	7.9	6.1	3.7	0.8	1.1	0.5

calculation of the solvent accessible surface area, which becomes buried upon recombining the peptide chain 59–70 with the rest of the molecule [14]. In the bovine phospholipase structure with a Val or a (hypothetical) Phe at position 63, the buried surface area is 923 $Å^2$. In the porcine conformation with a Phe at position 63 this is 1066 $Å^2$. The difference of about 140 $Å^2$ corresponds with about 3–3.5 kcal/mol [14] by which the porcine conformation is stabilized over the bovine one by hydrophobic interactions.

As to hydrophilic interactions, we considered the number of hydrogen bonds in which N and O atoms present in the 59–70 loop are involved. In the bovine conformation there is one extra hydrogen bond between a main chain N atom and a carbonyl oxygen atom. This extra hydrogen bond (partly) compensates the hydrophobic stabilization in the porcine structure. In this respect it is of interest to compare the structure of the 59–70 loop with its conformation in bovine prophospholipase, the structure of which enzyme is known at a resolution of 3.0 Å [15]. In this case the peptide chain 63–72 was found to be disordered or very flexible. This latter finding also indicates that the peptide chain 59–70 might easily adopt different conformations.

It is obvious from the above that one should be very careful in predicting a similar spatial conformation for two peptides on the basis of sequence similarity alone, especially when comparing relatively short sequences. A somewhat similar situation has been observed in [16] when comparing the folds of a stretch of 19 residues in *p*-hydroxybenzoate hydroxylase [17] with glutathione reductase [18], the structures of which are known in atomic detail. In spite of the high similarity (9 identical amino acid residues out of 19; 47%), the folds are very different. Therefore, only when residues known to be at characteristic positions in the 3-dimensional fold (half-cystines, glycines, prolines) or key-residues essential for catalysis or the binding of substrates or effectors are present at equivalent positions in the primary structure, a much greater reliability in a prediction based on sequence similarity can be presumed to be present. In fact a paradox is presented by nature: very dissimilar amino acid sequences appear to fold in extremely similar ways (e.g., the nucleotide binding domains in the dehydrogenases [19]), where as at the same time much more similar sequences can have, surprisingly, a completely different folding.

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