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COMPUTER-AIDED DESIGN OF THERMOSTABLE PROTEINS(U) JOHNS
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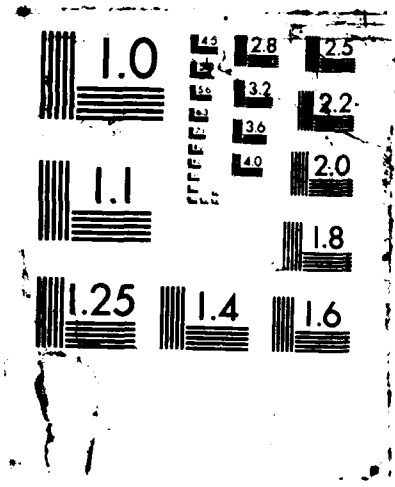
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We are developing strategies for computer-aided protein design. Our strategies emphasize simple geometric aspects of protein structure, and our computer program (PDB_PROTEUS) allows us to systematically test a very large number of alternative sequences and conformations.

We have written programs to systematically search a protein structure for places where we can add disulfide bonds, new salt bridges, or favorable aromatic interactions. We also search for places where the backbone can accommodate glycine ->alanine mutations and for places where prolines can be accommodated. (These changes -like the disulfide bond- should stabilize the folded structure by reducing the entropy of the unfolded form.)

We have tested each of these approaches by trying to design thermostable variants of the lambda repressor. The most stable variant - ala46ala4cys88lys93 - contains 4 mutations and is 17 degrees more stable than the wild-type protein. Since mutations that reduce the entropy of the unfolded form seem to have the largest stabilizing effect on repressor, we are searching for ways to introduce new metal-binding sites and chemical crosslinks between side chains.

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Computer-Aided Design of Thermostable Proteins

We are developing methods for computer-aided protein design and are testing these strategies by constructing thermostable variants of the lambda repressor. Repressor's DNA-binding domain normally denatures at 54° C; our goal is to construct a variant that will bind normally to DNA and yet be stable to 100° C. To date, we have constructed a quadruple mutant that is stable to 71° C and binds DNA as well as the wild type protein.

→ Our fundamental goal is to develop methods for de novo protein design, and we are proceeding by treating the problem of protein design as an "inverted" version of the protein folding problem (Pabo, 1983). In the protein folding problem, one is given an amino acid sequence and must predict how this folds in three dimensions. Protein design can be approached in quite a different way - one can begin by choosing a folded arrangement of the polypeptide backbone and then try to pick an amino acid sequence that will stabilize this structure. "Inversion" eliminates the problem of predicting long-range interactions, since residues which will interact in the final tertiary or quaternary structure already are close in space when they are added to the prefolded backbone. One should be able to pick residues which will have favorable interactions with their neighbors.

We are developing a program, called PDB_PROTEUS, for computer-aided protein design. Our program uses simple geometric aspects of protein structure and frequently uses local coordinate systems so that the geometric relationships are easier to visualize (Pabo and Suchanek, 1986). There are many advantages to using a computer program: A program can easily check millions of possible sequences and conformations. Using a program also makes it easy to try several variations of a particular search strategy or to apply the same strategy to many different proteins. *Keywords: Computer aided design, molecular biology, computer programs, synthesis chemistry, computer simulation* ←

Much of our work during the past year has focussed on developing and refining the PDB_PROTEUS system. Although the program is written in FORTRAN, we have tried to develop a programming strategy that will be very flexible. The core of the system is a library of subroutines. Each performs a discrete

operation - like adding a residue or changing the coordinate system - and our library now contains about 120 subroutines. The main programs use these subroutines (almost like a higher level programming language). Since we believe these subroutines and programs could be useful for many other projects, we have paid careful attention to our programming style and are making this system available to other laboratories.

In our attempts to stabilize the repressor, we have written programs to search the repressor structure for the best residues to change and then have experimentally tested each of these predictions. We have tried five different types of changes, and our results are summarized below:

1) Disulfide Bonds: The search program used all the disulfide bond conformations found in the Protein Data Bank and also used a library of conformations that were closely related to the left handed spiral configuration (Richardson, 1981). Modelling suggested that an intermolecular disulfide bond could be introduced by changing Tyr 88 to Cys (Pabo and Suchanek, 1986). Experimental studies show that this disulfide bond forms spontaneously, stabilizes the repressor dimer against thermal denaturation, and increases the affinity for DNA (Sauer et. al., 1986).

2) Salt Bridges: The program searches for any position where a new salt bridge could be introduced by changing a single residue. The best position appeared to be at the C-terminal end of helix 5, where changing Ser 92 to Lys should allow a salt bridge with Glu 89. This has no effect on the thermal stability of repressor, but introducing Lys 93 (effectively adding a residue to the C-terminal end of the helix!) does stabilize the protein by about 0.5° C. Although this approach does not seem to be very promising, we need to test additional positions.

3) Aromatic Interactions: Studies of aromatic-aromatic interactions in proteins suggest that these can stabilize a protein if the aromatic rings are about 5.5 Å apart and are approximately perpendicular to each other (Burley and Petsko, 1985). We have searched for places where aromatic residues could be added to make favorable contacts with an existing aromatic residue.

Unfortunately, the only position that appears plausible (residue 33) changes a key residue involved in nonspecific contacts with the DNA. Studies in Robert Sauer's laboratory at M.I.T. have shown that this mutant is stable (Hecht et. al., 1984) but it is not useful to us because it disrupts DNA binding.

4) Glycine to Alanine Changes: Hecht and Sauer (1986) have shown that repressor can be stabilized by changing two glycines to alanines. We have set up a program that automatically searches for places that Gly to Ala changes might be made. This does not find any other plausible positions in repressor, but the program should be useful with other proteins.

5) Prolines: Proline residues may stabilize proteins by reducing the conformational entropy of the unfolded protein. Obviously, they can introduce unfavorable strain if they are put at the wrong positions, but we have search repressor for positions where the backbone conformation should allow a proline residue to be introduced. Two positions appeared plausible and have been tested. We found that changing Tyr 60 to Pro has a mild destabilizing effect, but changing Gln 9 to Pro stabilizes the protein by 0.6° C.

Since we expect that a set of changes will be necessary to stabilize repressor to 100° C, it was important to determine whether the effects of multiple mutations are additive. Our initial results are quite encouraging. To test the effects of multiple mutations, we combined our disulfide mutant with the two glycine to alanine changes in helix 3. We found that the wild type protein denatured at 54°, the Cys 88 mutant denatured at 62°, the Ala46Ala48 double mutant denatured at 62°, and the Ala46Ala48Cys88 mutant was stable to 70° (Stearman et. al., 1988). More recently, we have shown that that the Ala46Ala48Cys88Lys93 quadrupule mutant is stable to 71°. We are now introducing the Pro 9 mutation to see whether this gives a further incremental increase in the stability.

During the last year we also have continued with the crystallographic refinement of repressor, and it is clear that a highly refined structure is very important for modelling and design. Our initial predictions had used the repressor structure obtained by fitting to an isomorphous electron density map at 3.2 Å

resolution (Pabo and Lewis, 1982). We have much better data from our repressor-operator cocrystals (Jordan et. al., 1985) and this structure has been refined to an R factor of 24.5% using data from 8.0 to 2.5 Å resolution. Comparisons have shown that our model-building predictions are very sensitive to differences between these coordinate sets. The initial, less accurate, coordinates gave several predictions (not discussed above because they were not obtained with the better coordinates) that were thermally unstable.

Although it is too early for a firm conclusion, our data suggest that it may be easiest to stabilize a protein by introducing mutations that reduce the entropy of the unfolded form, and we will focus on this strategy during the final year of our contract. Our disulfide search program can readily be rewritten to search for any types of crosslinks between side chains. (The program only requires that we can predict plausible conformations for the crosslinks, because we do need to know the relative spatial arrangement of the proximal and distal residues that are connected by the crosslink.) Specifically, we plan to introduce cysteines that can be connected by reagents like dimercaptoethanol, dithiothreitol, or can be bridged by a mercury ion. (Many other crosslinking reagents can be used, but we think it will be advantageous to begin with ones that react reversibly, since it should be easier to analyze the products.) These approaches may allow us to reach our goal of designing a repressor variant that is stable to 100° C.

References

Burley, S.K. and Petsko, G.A. (1985) Aromatic-Aromatic Interaction: A Mechanism of Protein Structure Stabilization **Science** **229**, 23-28.

Hecht, M.H., Sturtevant, J.M., and Sauer, R.T. (1984) Effects of Single Amino Acid Replacements on the Thermal Stability of the NH2-terminal Domain of Phage Lambda Repressor **Proc. Natl. Acad. Sci. USA** **81**, 5685-5689.

Hecht, M.H., Sturtevant, J.M., and Sauer, R.T. (1986) Stabilization of Lambda Repressor Against Thermal Denaturation by Site-Directed Gly to Ala Changes in Helix 3 **Proteins** **1**, 43-46.

Jordan, S.R., Whitcombe, T.V., Berg, J.M., and Pabo, C.O. (1985) Systematic Variation in DNA Length Yields Highly Ordered Repressor-Operator Cocystals **Science** **230**, 1383-1385.

Pabo, C.O. (1983) Molecular Technology: Designing Peptides and Proteins **Nature** **301**, 200.

Pabo, C.O. and Lewis, M. (1982) The Operator-binding Domain of Lambda Repressor: Structure and DNA Recognition **Nature** **298**, 443-447.

Pabo, C.O. and Suchanek, E.G. (1986) Computer-Aided Model-Building Strategies for Protein Design **Biochemistry** **25**, 5987-5991.

Richardson, J. (1981) Protein Anatomy and Taxonomy **Advances Protein Chemistry** **34**, 167-339.

Sauer, R.T., Hehir, K., Stearman, R.S., Weiss, M.A., Jeitler-Nilsson, A., Suchanek, E.G., and Pabo, C.O. (1986) An Engineered Intersubunit Disulfide Enhances the Stability and DNA-Binding of the N-terminal Domain of Lambda Repressor **Biochemistry** **25**, 5992-5998.

Stearman, R.S., Frankel, A.D., Freire, E., Liu, B., and Pabo, C.O. (1988) Combining Thermostable Mutations Increases the Stability of Lambda Repressor, **Biochemistry**, submitted

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