Review Letter

How good are predictions of protein secondary structure?

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Received 17 February 1983

The three most widely used methods for the prediction of protein secondary structure from the amino acid sequence are tested on 62 proteins of known structure using a program package and data collection not previously available. None of these methods predicts better than 56% of the residues correctly, for a three state model (helix, sheet and loop). The algorithms of Robson et al. [J. Mol. Biol. (1978) 120, 97–120] and Lim [J. Mol. Biol. (1974) 88, 873–894] are the best of those tested. New methods, now under development, can be tested against this benchmark.

1. INTRODUCTION

The explosive increase in our knowledge of DNA sequences (currently at about 1 Megabase) has led to increased use of protein secondary structure predictions from the amino acid sequence. Typically, one wants to know what structural type of protein the DNA codes for and whether the protein is related to one of known function or structure. The most interesting practical use has been the prediction of antigenic oligopeptides as potential vaccines [3,4]. For any of these uses it is important to know how well secondary structure prediction methods work.

Assessment of available prediction methods is best made by comparing predictions with the crystallographically determined structure. Such comparisons have been made [5], but have been hindered by two facts:

(i) There are ambiguities in two of the best known methods, those of Chou [6] and Lim [2], in that they often give different results in the hands of different people and are therefore not programmable without extension or modification;

(ii) There is considerable variation in the definitions of secondary structure given by crystallographers.

We have now solved both of these difficulties and report the results of a completely objective, up-to-date assessment of the most widely used prediction methods on 62 proteins with more than 10000 residues. For a three-state definition of secondary structure (helix, sheet, loop/turn) the overall prediction accuracy for new protein structures does not exceed 56% for the best of these methods and is only 50% for the most widely used (Chou) method. We caution against the over-interpretation of predictions made by presently available methods and provide a benchmark against which new methods, now under development, can be tested.

2. METHODS

Ambiguities in the method of Chou [6] were overcome by selecting possible secondary structure segments such that the sum of preference parameters over all chosen segments is maximal; technically, this is a difficult optimization problem.
but was achieved by a recursive algorithm which was added to a program written by C. Oefner [7].

Turn prediction, done separately by Chou [8], was not included. Conceivably the overall success of Chou’s method can be improved by rules for eliminating overlaps of predicted turns with predicted helix/sheet residues. Ambiguities in the method of Lim [2] were overcome by a simplified iterative procedure for segment selection which was added to a program written by J.A. Lenstra [9]. The (unambiguous) method of Robson was used as programmed by the authors [1].

Known methods not compared here include: Nagano [10] (bad beta prediction in our hands); Maxfield and Scheraga [11] (similar to Robson’s, reportedly 57% accurate for five states); Pitsyn and Finkelstein [12] (new version just published [13]); Palau and Argos [14] (reportedly 56% accurate for four states).

Objective and accurate assignment of secondary structure was achieved by a pattern recognition algorithm [15] which extracts hydrogen-bonded features from the full atomic coordinates as deposited with the Protein Data Bank [16].

### 3. RESULTS AND DISCUSSION

Predictive success is given in table 1 for each protein, averaged over the three structure states.

<table>
<thead>
<tr>
<th>Protein Name</th>
<th>Fraction Correct [%]</th>
<th>Protein Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-Lactate Dehydrogenase</td>
<td>54</td>
<td>A-P-S</td>
</tr>
<tr>
<td>Myoglobin (Fe)</td>
<td>45</td>
<td>A-P-S</td>
</tr>
<tr>
<td>Hemoglobin (Deoxy)</td>
<td>65</td>
<td>A-P-S</td>
</tr>
<tr>
<td>Methionine</td>
<td>78</td>
<td>A-P-S</td>
</tr>
<tr>
<td>Lactate Dehydrogenase, apo</td>
<td>55</td>
<td>A-P-S</td>
</tr>
<tr>
<td>Myoglobin (Fe)</td>
<td>45</td>
<td>A-P-S</td>
</tr>
<tr>
<td>Hemoglobin (Deoxy)</td>
<td>65</td>
<td>A-P-S</td>
</tr>
</tbody>
</table>

All methods are compared according to how well they predict the three states α-helix, β-sheet and loop (everything else) or older (a) and newer (b) protein structures. Fraction correct is the number of residues predicted correctly in any state divided by the total number of residues. The protein name is preceded by the protein data bank [16] identifier IDEN and the number of residues RES. The * indicates the percentage of correctly predicted residues one can expect in applying the methods of Robson and Lim to newly determined sequences.

protein and each method as the percentage of residues predicted correctly in a three state description of secondary structure. The result of the comparison is similar to that of Busetta and Hospital [17] who have 47% success for Chou and 57% success for Robson on 34 proteins. The method of Lim has a surprising 65% success rate for protein structures known in 1974 when his method was published, but this drops to 56% for proteins elucidated after 1974. The difference can be understood to be due to special rules tailored to particular proteins in Lim’s method.

**Structure predictions can be evaluated in more...**
detail by calculation of assorted quality indices [5] which indicate how well a particular state is predicted, whether there is over- or underprediction etc. All of these indices can be calculated from the predicted/observed matrix in table 2 which indicates, say, how many of the 2295 observed helical residues are correctly predicted as helical (H) and how many are wrongly predicted as loop/turn (L) or sheet (E, for extended); or, how many of the 2684 residues predicted as helical by Lim are sheet, loop or helical in the crystallographic structure. One such quality index for each state is the ‘fraction correct of observed’ in table 2. For example, we see that 74% of the observed loop residues are correctly predicted by Lim, while only 36% of the observed sheet residues are correct; this imbalance is related to an overall underprediction (1690/2295) of sheet and an over-

Table 2

Predictive success of the three most widely used secondary structure prediction methods: details of sheet (E), loop/turn (L) and helix (H) prediction averaged over all proteins

<table>
<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>observed</td>
<td>predicted</td>
<td>predicted</td>
<td>predicted</td>
</tr>
<tr>
<td>helix</td>
<td>H</td>
<td>H</td>
<td>H</td>
</tr>
<tr>
<td>loop</td>
<td>L</td>
<td>L</td>
<td>L</td>
</tr>
<tr>
<td>sheet</td>
<td>E</td>
<td>E</td>
<td>E</td>
</tr>
<tr>
<td>observed</td>
<td>1195</td>
<td>1444</td>
<td>820</td>
</tr>
<tr>
<td>predicted</td>
<td>1244</td>
<td>1444</td>
<td>820</td>
</tr>
<tr>
<td>fraction correct (%)</td>
<td>93.7</td>
<td>92.6</td>
<td>99.1</td>
</tr>
</tbody>
</table>

Suppose you have predicted a residue as helical and want to know the chances of being right. For a particular method, the average ‘fraction correct of predicted’ (table 2) defined as:

\[
PC(S) = \frac{N \text{ (correctly predicted in state } S)}{N \text{ (predicted in state } S)} \times 100
\]

is a direct measure of the probability of correct prediction having predicted a residue to be in state S. Curiously, \(PC(S)\) does not appear among the quality indices commonly used [5], but is perhaps the most useful in prediction practice (after all, in a truly unknown protein structure no reference can be made to observed states). For example, when Lim’s method predicts a sheet strand, we can estimate from table 2 that there is a 49% chance of correct prediction. Note the high probability of correct loop prediction of 63–68% which is related to the high fraction (50%) of observed loop residues.

Suppose you do not care about the details of secondary structure assignments but merely want to use a secondary structure prediction method to predict the helix/sheet content of a protein; for example, for comparison with spectroscopic determinations (such as circular dichroism). The root-mean-square average difference between predicted and observed secondary structure content for the 62 proteins is 12–17 residues/100 residues (table 2). For example, a prediction by Robson of sheet content has a typical uncertainty of \(\pm 12\%\). An uncertainty of this size renders present comparisons of predicted secondary structure content with circular dichroism experiments useless in all but extreme cases.

We conclude that one may expect a success rate, for three states, of about 50% with Chou’s method and of 55–56% with either Robson’s or Lim’s method. In any event, an error rate of 44% is unacceptable for many purposes and newly developing methods must do better. We estimate that empirical–statistical prediction of secondary structure alone may eventually reach 70% accuracy for three states; higher accuracy will, in our opinion, only come with a protein-folding theory aiming at prediction of the complete three-dimensional structure.
ACKNOWLEDGEMENTS

A detailed comparison for 9 proteins is reported in C. Oefner's thesis [7]. We thank G.E. Schulz for his active role in organizing the implementation of published prediction methods in our laboratory and the Deutsche Forschungsgemeinschaft for financial support to the project 'Protein Structure Theory'. The automated program package or predictions based on it are available on a collaborative basis.

REFERENCES