

Improving the efficiency of protein structure determination from NMR

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Abstract

Comprehensive computational experiments were performed to evaluate efficiency of the newly proposed COMBINE procedure on protein structure calculations from NMR data. This procedure is intended to combine merits of the previously developed FISINOE method with the DIANA program, widely used for NMR structure calculations. The new version of the FISINOE program, FISINOE-3, was developed to determine local conformations of proteins consistent with short-range NMR data (intraresidue and sequential distance constraints and coupling constants). For each residue, the program determines the allowed ranges of ϕ , ψ and χ_1 torsion angles consistent with the NMR data. The benchmark calculations were carried out on three proteins: bovine pancreatic trypsin inhibitor, crambin and avian pancreatic polypeptide. The results of the calculations obtained by the COMBINE protocol were compared with the results obtained by the STANDARD run of the DIANA program. The COMBINE procedure allowed one to significantly narrow ranges of the dihedral angle constraints before the structure calculations that, in turn, resulted in more stereospecific assignments. The numbers of βCH_2 groups unambiguously assigned using the COMBINE procedure were significantly greater in comparison with those assigned by the STANDARD protocol. For all three proteins, the use of the COMBINE procedure almost doubled the numbers of unambiguously assigned βCH_2 groups in comparison with STANDARD. The computational experiments clearly showed that the use of allowed ranges for torsion angles obtained by the COMBINE procedure as input data for the DIANA program provides a higher precision and accuracy of 3D protein structures reproduced from NMR constraints. The COMBINE procedure may be incorporated into any protocol using as input data the allowed ranges of torsion angles consistent with a given set of NMR constraints. Since the COMBINE procedure proved to be effective, reliable and robust, it may be recommended for general use in 3D structure determination of proteins and peptides from NMR data. © 1997 Elsevier Science B.V.

Keywords: Protein structure determination; NMR; Refinement

1. Introduction

In past years, methods of nuclear magnetic resonance (NMR) have been intensively implemented as powerful tools for studies of 3D protein structures in solution [1–5]. Multidimensional NMR spectroscopy

provides a lot of structural information that can include estimates of distances between relatively closed protons and of intervals for some torsion angles consistent with NMR data. To reconstruct protein structures that agree with the NMR-derived constraints, a number of computational methods and procedures have been proposed [6–13].

Goodness of any computational method may be characterized by its precision and accuracy of protein

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structures reconstructed by the method. Precision is a measure of the variation within the reproduced structures, while accuracy is the measure of the closeness of the reproduced structures with the “true” structure (“gold standard”). By the benchmark calculations, each of the computational methods was shown to introduce its own systematic bias [14]. Taking into account the accuracy of experimental data provided by NMR, the attainable accuracy of the protein structure calculations was shown to be varying by 1 to 2 Å, while the precision within a family of the calculated structures can reach 0.4–0.7 Å [14]. Both precision and accuracy depend on the method used, on precision and accuracy of the input data and on the size of the molecule [14,15].

It was shown previously that the quality of the calculated structures can be improved by increasing the number of stereospecific assignments for prochiral groups of protons, in particular, for β -methylene protons [16–18]. The stereospecific assignments can be obtained by matching the experimental spin–spin coupling constants and the intraresidual and sequential NOEs with the calculated ones for allowed conformations provided by either a systematic conformational search [16,18] or analysis of crystallographic databases on known protein structures [18,19].

The crystallographic information on high-resolution protein structures derived from the structural databases was shown to be beneficial for protein structure calculations from NMR [18–20]. Indeed, the problem of NMR structure determination falls into the category of *ill-posed* mathematical problems [19,20], since the number of variables (coordinates of atoms) to be determined is usually larger than the number of experimental constraints derived from NMR data. The way to decisively improve this situation is to use additional information that is not contained directly in NMR data. The Brookhaven Protein Data Bank (PDB) [21] is an excellent source of such information accumulated on physical realities of many proteins that may be used for the NMR structure calculations.

The previously developed FISINOE approach [20,22,23] attempts to combine the data from PDB with the NMR measurements. The PDB information was used to provide an a priori probability distribution for protein conformations. The probable values of torsion angles for any amino acid residue in the

protein sequence can be determined based on intensities of NOE cross-peaks and coupling constants [20,22,23]. It was shown that the interproton distances measured from NOE data and coupling constants in conjunction with structural information derived from PDB provided refinement of the ϕ , ψ angles, determination of χ_1 conformations, and stereospecific ^1H assignments of β -protons [20].

Using methods of cluster analysis for (ϕ , ψ , χ_1) angle distributions observed in high-resolution proteins from PDB, it was shown that these distributions are well clustered in 16 clusters [24,25]. For every cluster, the first statistical moments, i.e. the mathematical expectations of the ϕ , ψ and χ_1 angles, and their standard deviations were estimated. The statistical data of the 16 clusters were used as a priori data in a new version of the FISINOE approach, the FISINOE-3 program [26].

Recently, we have shown that combined use of the FISINOE approach with the systematic scanning of sterically allowed conformational space, implemented in the COMBINE procedure, results in a more precise determination of angular constraints and in an increased number of stereospecific assignments of β -protons [27]. The COMBINE procedure integrates merits of the FISINOE method with the systematic scanning of sterically allowed conformational space, implemented in the HABAS program [16], which is supplemental to the DIANA program [17] widely used for NMR structure calculations. Using the same input data (short-range NMR data) FISINOE and HABAS are based on fundamentally different principles. FISINOE is a knowledge-based approach implemented to estimate mathematical expectations and standard deviations for ϕ , ψ and χ_1 angles by combining a previously known distribution function for these dihedral angles with a measured set of experimental data, while HABAS aims to obtain stereospecific ^1H NMR assignments for a pair of β -methylene protons by the systematic scanning of the (ϕ , ψ , χ_1) space. As a by-product of the scanning, HABAS also determines sterically allowed intervals for the angles consistent with a given set of NMR data. Our COMBINE procedure strives to find the angular intervals that match both probabilistic and steric conditions.

In this paper, the efficiency of the COMBINE protocol for 3D protein structure determination from

the simulated NMR data was evaluated by the benchmark calculations on three proteins: bovine pancreatic trypsin inhibitor (BPTI), crambin and avian pancreatic polypeptide (APPT). The results obtained with the COMBINE protocol were compared with those of the standard protocol of the DIANA program [17,28].

2. Methods

2.1. Data and software used

The computational experiments with simulated NMR data were carried out to evaluate the quality (accuracy and precision) of the protein structure determination for BPTI, crambin and APPT molecules. The DIANA program with REDAC strategy [28], implemented in the SYBYL 6.2 software package [29], was used in the calculations utilizing either the STANDARD protocol, with angle constraints and stereospecific assignments conventionally determined by HABAS [16], or the COMBINE protocol with narrowed angle ranges and newer assignments. Artificial NMR-type data were simulated from X-ray coordinates taken from the PDB files: 1ppt.pdb [30], 1crn.pdb [31] and 4pti.pdb [32], corresponding to APPT, crambin and BPTI. The SYBYL 6.2 was used to add hydrogens to heavy atoms, to calculate interproton distances and to estimate the values of the ϕ , ψ and χ_1 angles based on X-ray atomic coordinates. The Karplus-type relations [16,33] were used to generate the coupling constants $^3J_{\alpha N}$ and $^3J_{\alpha\beta}$. To mimic typical NOE data used in a protein structure determination, the values of all interproton distances shorter than 4.0 Å were substituted by corresponding upper limits on these distances in the following way. As the intraresidue and sequential constraints, the upper limit equal to 2.5 Å was applied to all distances shorter than 2.5 Å, the limit 3.0 Å to all distances within the interval from 2.5 to 3.0 Å and the limit 4.0 Å to all distances within the interval from 3.5 to 4.0 Å. As the long-range constraints, the upper limit of 4.0 Å was used for all interproton distances shorter than 4.0 Å.

2.2. The FISINOE-3 program

FISINOE-3 uses, as a priori data, a set of clusters for ϕ , ψ and χ_1 angles [25] and, as input data, intraresidue

and sequential distance constraints and coupling constants (short-range NMR data). For each residue, the program determines a subset of clusters for ϕ , ψ and χ_1 angles consistent with the NMR restraints. Posterior probabilities, mathematical expectations and standard deviations of the angles are estimated for each (ϕ , ψ , χ_1) cluster, as are overall ranges of these angles. The allowed range for a torsional angle is considered as a confidence interval, determined as a product of the half-width (input parameter of FISINOE-3) and the standard deviation, σ , of the angle from its mathematical expectation. The lowest limit within the confidence intervals for the angle within subset of the (ϕ , ψ , χ_1) clusters, consistent with the NMR restraints, is taken as the lower limit of the overall range of the angle. Analogously, the uppermost limit is taken as the upper limit of the overall range. The FISINOE-3 program is written on C++ and compiled under IRIX 5.3 and Solaris 2.5 operating systems. The program is available through the World Wide Web (<http://www.unmc.edu/Eppley/ECCC3/fisinoe3.htm>).

2.3. The STANDARD protocol

The DIANA program with REDAC strategy, implemented in SYBYL 6.2 [29], was used in the calculations utilizing the STANDARD protocol. The angle constraints and stereospecific assignments for β -methylene protons were determined by the HABAS program. The DIANA calculations were performed with the standard selection of minimization levels and parameters [17,29]. The computational experiments, with 50 and 250 randomly generated structures, were performed by the STANDARD protocol.

2.4. The COMBINE protocol

For each amino acid residue in the protein sequence, a set of sterically allowed conformations and overall ranges for the ϕ , ψ and χ_1 angles (angular constraints), consistent with a set of its short-range NMR data, was determined by the FISINOE-3 program. The angular constraints, along with short-range NMR data, were used as input data into HABAS. The renewal angular constraints and the stereospecific assignments of β -methylene protons determined by HABAS were used for protein structure calculations by the DIANA program. The calculations were carried out with 50

randomly generated structures. The half-width of the allowed angular intervals determined by the FISNOE-3 program was taken as equal to 3σ .

2.5. Estimation of quality of the structure determination

In each of the computational experiments, a family of 20 structures was selected by the lowest values of the target function and taken for further consideration. The selected structures were superimposed with the crystal structure, from which NMR data were simulated, to determine the root mean square deviation (RMSD) between coordinates of corresponding atoms in the structures. Mean squared error (MSE) of each structure was estimated as the square of the corresponding RMSD. The overall precision within each structural family was determined as the RMSD of variances between the 20 structures and their average structure. To calculate the average structure, the 20 structures belonging to the same structural family were superimposed, by minimizing atomic RMSDs and then averaged. Accuracies were estimated as atomic RMSDs between the average structure of each family and the ‘‘gold standard’’. Precision, accuracy and MSE were estimated for both backbone atoms ($C\alpha$, C' and N) and all heavy atoms.

All calculations were performed on the Silicon Graphics Indigo² workstation with the R4400 processor.

3. Results and discussion

The COMBINE procedure allowed one to significantly narrow ranges of the dihedral angle constraints before the structure calculations that, in turn, resulted in more stereospecific assignments yielded from HABAS. For example, the total lengths of the angular

intervals for ϕ , ψ and χ_1 angle for the BPTI molecule were decreased to 1.9, 2.4 and 1.8 times, correspondingly [27]. The numbers of the stereospecific assignments for β -methylene protons obtained by using HABAS with STANDARD and COMBINE sets of the dihedral angle constraints are compared in Table 1.

As can be seen in Table 1, numbers of βCH_2 groups unambiguously assigned by the use of the COMBINE procedure were significantly greater in comparison with those assigned by STANDARD. For all three proteins, the use of the COMBINE procedure almost doubled the numbers of unambiguously assigned βCH_2 groups in comparison with STANDARD.

The precision and accuracy of structure calculations obtained by the use of the COMBINE procedure in comparison with the STANDARD run of DIANA were assessed for the 20 selected structures with the lowest values of target function in each computational experiment. The calculations were carried out for 50 and 250 starting structures produced by STANDARD (STD50 and STD250, correspondingly) and for 50 structures produced by COMBINE (COMB50). The atomic RMSDs between the average of those structures and the original crystal structure were used as a measure of the accuracy of structure determination, while RMSDs between the average and the members of the structural family served as a measure of precision. These data are presented in Tables 2 and 3.

In Tables 2 and 3, the accuracy and precision of the 20 best structures selected by the lowest values of the target function determined by the COMBINE procedure are compared with those determined by STANDARD.

Comparing the results for the same 50 starting structures, the overall quality of the structures produced by the COMBINE procedure was always better than when it was produced by the STANDARD protocol. The precision of the STANDARD calculations starting with 250 structures (about 5 times longer computations)

Table 1
Stereospecific assignments of β -methylene protons obtained by the STANDARD and COMBINE procedures

Protein	Number of residues	Number of βCH_2 groups (without Pro)	Stereospecific assignments	
			STANDARD	COMBINE
APPT	36	23	12 (52%)	22 (96%)
Crambin	46	19	7 (37%)	16 (84%)
BPTI	58	36	19 (53%)	31 (86%)

Table 2
Accuracy of the structures reproduced by STANDARD and COMBINE protocols

Protein	RMSD for all heavy atoms			RMSD for backbone atoms		
	STD50	STD250	COMB50	STD50	STD250	COMB50
APPT	1.00	1.02	0.91	0.69	0.70	0.65
Crambin	0.70	0.63	0.63	0.50	0.51	0.46
BPTI	0.93	0.92	0.89	0.51	0.55	0.45

was close to the results obtained by COMBINE with 50 starting structures, while for all three proteins the accuracy of the COMBINE calculations was always better. Interestingly, the fivefold increase in the number of starting structures did not guarantee the better accuracy (see Table 2, all heavy atoms for APPT and backbone atoms for all three proteins). In all calculations, the accuracy provided by the COMBINE procedure was better than that provided by STANDARD starting with 50 or 250 structures.

For the STANDARD protocol, the precision and accuracy were dependent upon the number of randomly chosen trial structures. An increase in the number of trial structures improved the precision to some extent but sometimes decreased the accuracy. For the same number of randomly chosen trial structures, the precision of the 20 accepted structures determined by COMBINE was always better than that obtained by STANDARD. To attain the precision provided by COMBINE, the number of trial structures to be generated using the STANDARD protocol would be, on average, five times greater.

The narrowing of the overall ranges of the dihedral angles provided by COMBINE did not introduce an additional bias, since the accuracy of the structures determined by the COMBINE procedure was always lower than that obtained by STANDARD. Moreover, in contrast to the precision, the higher accuracy achieved

by COMBINE cannot be reached by STANDARD even by increasing the number of randomly chosen trial structures. The observed improvement in the accuracy suggests that COMBINE estimates the overall ranges of the dihedral angles not only more precisely but also more accurately, in comparison with the STANDARD protocol.

To better evaluate the quality of the structure determination, the distribution of the values of mean squared error for the individual structures obtained by the different procedures was analyzed. MSE of each structure was estimated as the square of the corresponding RMSD between the calculated structure and the ‘‘gold standard’’. According to the MSE values, all structures were divided into four categories: excellent, very good, good and fair. The structures with MSE values of less than 0.5 for all heavy atoms were assigned as excellent; the structures with the value of MSE within the interval of 0.5–0.8 as very good; those within the interval 0.8–1.25 as good; and the structures with the value of MSE greater than 1.25 as fair. For the backbone atoms, the corresponding categories were determined by the following MSE values: excellent—less than 0.2; very good—within the interval 0.2–0.3; good—0.3–0.55; and fair—greater than 0.55. The number of structures determined by the STANDARD and COMBINE procedures that fell in these categories is shown in Table 4.

Table 3
Overall precision of structures reproduced by STANDARD and COMBINE protocols: average and standard deviation (in parentheses) of the RMSDs between accepted conformers and the average structure

Protein	All heavy atoms			Backbone atoms		
	STD50	STD250	COMB50	STD50	STD250	COMB50
APPT	0.46 (0.07)	0.46 (0.04)	0.44 (0.06)	0.18 (0.04)	0.15 (0.05)	0.15 (0.05)
Crambin	0.60 (0.13)	0.39 (0.08)	0.35 (0.06)	0.41 (0.12)	0.26 (0.08)	0.24 (0.07)
BPTI	0.48 (0.06)	0.44 (0.05)	0.45 (0.04)	0.23 (0.04)	0.19 (0.03)	0.18 (0.05)

Table 4
Number of the structures categorized by values of MSE

Quality of structure	All heavy atoms			Backbone atoms		
	STD50	STD250	COMB50	STD50	STD250	COMB50
<i>APPT</i>						
Excellent	0	0	0	0	0	0
Very good	0	0	6	0	0	0
Good	16	12	14	16	15	19
Fair	4	8	0	4	5	1
<i>Crambin</i>						
Excellent	1	5	10	1	2	5
Very good	7	15	10	3	3	7
Good	11	0	0	13	15	8
Fair	1	0	0	3	0	0
<i>BPTI</i>						
Excellent	0	0	0	0	0	1
Very good	1	0	7	7	6	19
Good	19	20	13	13	14	0
Fair	0	0	0	0	0	0
<i>Total</i>						
Excellent	1	5	10	1	2	6
Very good	8	15	23	10	9	26
Good	46	32	27	42	44	27
Fair	5	8	0	7	5	1

As seen in Table 4, most of the structures determined by both procedures fall in the good category. However, for all heavy and backbone atoms the distribution of the structures produced by the COMBINE procedure was significantly shifted to the very good and the excellent categories. In contrast, STANDARD produced significantly lower amounts of the very good and the excellent structures; the fair structures were mostly produced by STANDARD but not by COMBINE.

It is likely that a careful narrowing of the overall ranges of the dihedral angles, provided by COMBINE, helps the process of the target function minimization avoid some “false” local minima. In fact, we found that the values of the target function are poorly correlated with the corresponding values of the MSE; the structures with the lowest values of target function are not necessarily the ones closest to the X-ray structure or vice versa. Thus the values of target function per se cannot serve as an ideal characteristics of goodness of the structure. On the other hand, our calculations

clearly showed that STANDARD produces a bigger number of structures with poor MSE in comparison with the COMBINE procedure. It is likely that COMBINE rejects the structures with poor value of MSE (i.e. the structures corresponding to the “false” local minima of target function) better than STANDARD does.

Recently, relative populations of the torsional angles derived from PDB were used to define a conformational database potential [19] implemented as a new term in the simulated annealing refinement by X-PLOR [10]. The knowledge-based distribution for dihedral angles in proteins was noted as a useful addition for the refinement of NMR structures that provides improvements in the physicochemical reasonableness of dihedral angles and the overall packing, but increases the precision only slightly [19]. At the same time, our computational experiments showed improved accuracy and precision of structure calculations when the NMR data were used in conjunction with the probability density function for the torsional angles derived from PDB.

The COMBINE approach and the method of the conformational database potential are based on a common idea: to restrict sampling of dihedral angles during structure calculations to those that are known as physically realizable. However, an implementation of this idea by two approaches is different. In contrast to conformational database potential, COMBINE uses the preliminary clustered mixture distribution from the conformational database and selects only those conformational clusters that are consistent with the given set of NMR data. It allows COMBINE to decrease informational “noise” and increase the “signal/noise” ratio. COMBINE acts directly on allowed ranges for torsion angles and restricts the ranges before structure determination increasing the number of stereospecific assignments of β -protons. By increasing accuracy and precision of input data, COMBINE provides improved precision and accuracy of output data, i.e. better quality of NMR protein structures.

4. Conclusion

The combined use of knowledge-based and NMR information significantly improves the quality and efficiency of protein structure determination in solution. The computational experiments presented in this paper clearly showed that the use of allowed ranges for torsion angles obtained by the COMBINE procedure as input data for the DIANA program provides a higher precision and accuracy of 3D protein structures reproduced from NMR constraints. The COMBINE procedure may be incorporated into any protocol using as input data the allowed ranges of torsion angles consistent with a given set of NMR constraints. Since the COMBINE procedure proved to be effective, reliable and robust, it may be recommended for general use in 3D structure determination of proteins and peptides from NMR data.

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