Multiangular method for analysing molecular geometry from nuclear Overhauser effect results

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A multiangular method, as an extension of a triangular method, has been developed in order to analyse the local conformation of a molecule in an atomic resolution from nuclear Overhauser effect results. When there is a rigid part in the molecule, and the nuclear Overhauser effect signals are observed between several spins attributed to the rigid part of the molecule and the target spin to be analysed, the geometrical probability density of the target spin can be found by the multiangulation method, using distances between spin pairs. The spin density is illustrated by a set of isograms similar to electron density maps from X-ray crystallographic analyses. The molecular model building is performed based upon the isograms. An application to the conformation analysis of transferred nuclear magnetic resonance results of NAD$^+$, which binds to lactase dehydrogenace from Thermus caldophilus *GK24, is described.*

Keywords: molecular structure analysis, multiangulation, nuclear Overhauser effect, molecular modelling

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Recently, distance geometry analyses have been successfully applied to estimate the tertiary structures of short peptide chains and small proteins from nuclear Overhauser effect (NOE) results[1-4]. However, the distance geometry analysis needs a perfect assignment of nuclear magnetic resonance (NMR) signals to the respective spins, and moreover several different structures have been predicted depending upon the initial conformation and the degree of the restriction in the procedures of the calculation[1-4]. These have made it difficult to investigate the structures of large molecular weight proteins.

On the other hand, a recently developed technique, transferred nuclear Overhauser effect (TRNOE), can reveal the conformation of a fast exchanging ligand[5-7,10] and the local conformation of the enzyme around the active site[8-10]. For this technique, the larger molecular weight of the enzyme is generally the more preferable[5].

In this paper, a new method is presented for analysing NOE results, especially TRNOE results, in order to investigate the local structure of the ligand bound to the enzyme and that of the enzyme itself.

METHOD

First, it was assumed that the spins $A_1, A_2, \ldots, A_m$ are on a rigid structure such as an aromatic ring in the molecule, and $a_1, a_2, \ldots, a_n$, are the spins the structure of which are unknown. When the NOE measurements give the ratio of distances $\{|\vec{r}_{Ai} - \vec{r}_{aj}|/|\vec{r}_{Ai} - \vec{r}_{Ai'}|\}$, the absolute distance values $\{|\vec{r}_{Ai} - \vec{r}_{aj}|\}$ are determined from the geometry $\{\vec{r}_{Ai}\}$ of the assumed rigid structures.

In this way, a possible region for spins $\{a_j\}$ can be estimated by an extended triangular method, known as the multiangular method, based upon the rigid geometry $\{\vec{r}_{Ai}\}$.

In the results of NOE and TRNOE measurements, the distance between the spin pairs had some degree of unavoidable uncertainty, due to the spin diffusion, the atomic fluctuation of the molecule and experimental errors. It is assumed here that the distance distribution is described by a Gaussian distribution:

$$\exp(-(|\vec{r}_{Ai} - \vec{r}_{aj}| - d_{ij})^2/2\sigma_{ij}^2) \quad (1)$$

Where d_{ij} is the average distance between A_i and a_j, and σ_{ij} is its deviation.

When both d_{ij} and σ_{ij} were observed, randomly selected points were first generated in the neighbourhood of $\{\vec{r}_{Ai}\}$, obeying the following restrictions.

1) It should be positioned in a shell of a sphere, the centre of which is $\vec{r}_{Ai}$, and should be distributed around the radius d_{ij} following equation (1).

2) When only a lag signal[7] between A_i and a_j is observed, it should be positioned in a sphere shell, the centre of which is $\vec{r}_{Ai}$ and the radius is assumed to be ranged between 3.5 Å and 7.5 Å.

3) When no NOE signal between A_i and a_j is observed, a_j should be further than 4Å from A_i. For a special case with a small correlation time, no NOE was observed for a spin pair even if the two spins are positioned in the neighbourhood. Such a case is outside the scope of this paper as only large molecular weight proteins are described.

Figure 1 shows 1000 points for the H1′ atom of nico-

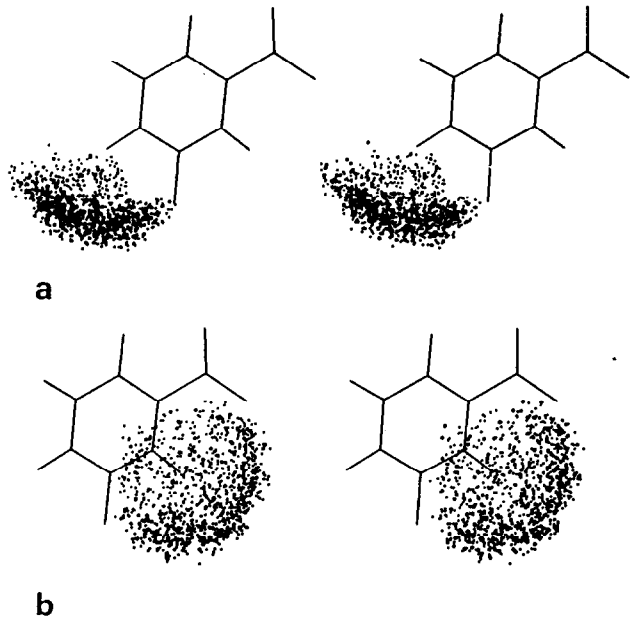

Figure 1. The possible regions of H1′ spin of NAD⁺ bound to LDH from Thermus caldophilus *GK24, indicated by 1000 points, which support the observed distance relations in Table 1, by stereo pairs (a) when the allosteric cofactor FBP does not bind to LHD, and (b) when FBP binds to LDH*

tinamide ribose of NAD^+ bound to LDH from *Thermus caldophilus* GK24. All the points were generated by the procedure described above with the distance relation shown in Table 1[7].

The next step was to generate small cubes dividing the whole space. The number of points included in each cube is then assigned to the centre of the cube $\vec{R}_k$, as the probability density of each spin a_j, normalized by the average number of points in all cubes. That is, this probability density $P_j(\vec{R}_k)$ is the ratio to the density of completely random distribution without any distance restriction. It is evident that $\{P_j(\vec{R}_k)\}$ can be calculated directly from equation (1) and the above three restrictions without the first step of points generation mathematically. From $\{P_j(\vec{R}_k)\}$, a set of isograms can be displayed together with the original structure of the rigid part of the molecule, as shown in Figure 2. When values of $\{a_j\}$ are well assigned, a model building procedure based upon these density isograms can be performed using a standard method[11,12]. Here, the process of the model optimization will be briefly outlined. Following the method of Diamond[13], a target function T is defined in the volume V as follows:

$$T = \sum_j \int \{P_j^{\text{meas}}(\vec{r}) - P_j^{\text{calc}}(\vec{r})\}^2 \mathrm{d}r^3/V$$

$$= \sum_j \sum_k \{P_j^{\text{meas}}(\vec{r}_k) - P^{\text{calc}}(\vec{r}_k)\}^2 \qquad (2)$$

Here, $P_j^{\text{meas}}(\vec{r}_k)$ is the observed probability density defined above, and $P_j^{\text{calc}}(\vec{r}_k)$ is the calculated density, assuming the next expression:

$$P_j^{\text{calc}} = \frac{\exp(-3|\vec{r}_k - \vec{r}_j^{\,c}|^2/2q_j^2)}{<\exp(-3|\vec{r}_k - \vec{r}_j^{\,c}|^2/2q_j^2)>_{\text{ave}}} \qquad (3)$$

where, $\vec{r}_j^{\,c}$ is the position of atom, to which jth spin is attributed, calculated by a model built temporally with several geometrical variables; bond lengths, bond angles and torsion angles. q_j is the effective radius of the atom[13] and $< >_{\text{ave}}$ is the average value of the jth atom. In order to obtain an optimum conformation, the target function T is minimized and a set of the geometrical variables determined.

RESULTS

Figures 1 and 2 indicate the possible regions of H1′ spin of the ribose in NAD^+. The notations of the atoms are indicated in Figures 4(a) and 4(b). In Figure 1(a) and Figure 2(a), NAD^+ binds to the LDH from *Thermus caldophilus* GK24 without an allosteric cofactor FBP[7]. On the other hand, in Figure 1(b) and Figure 2(b), NAD^+ binds to the LDH and FBP is attached. It is clearly seen, that for the system with free FBP and bound FBP, the positions of H1′ spin are in entirely opposite directions[7]. Using all the relationships in Table 1, the positions of H2′ and H3′ spins are also given as in Figures 3(a) and 3(b) for free and bound FBP, respectively. Assuming either C2′-endo or C3′-endo conformation for the ribose, the variable χ (the torsion angle of O4′-C1′-N1-C2) has been determined by a minimization of the target function T (equation (2)). The optimized conformations for two cases, free FBP and bound FBP, are illustrated in Figures 4(a) and 4(b), respectively. In each case, the value of T was much smaller in the C3′-endo conformation than in the C2′-endo conformation, indicating that C3′-endo is the preferred conformation. The distances between spin pairs for each optimized structure agreed fairly well with the observed distances, as shown in Table 1.

In the model building procedure, an interactive graphic display is useful in helping to determine the initial conformation for the minimization of the target function. In the present study, a 3D raster graphic system (Seiko GR-3424) was used, and the model structure of the nicotinamide ring and the isograms of the probability density shown on the same screen. Using the optional dials, the torsion angle χ has been rotated, so that H1′, H2′ and H3′ atoms are found simultaneously inside the respective 3D isograms with the values 10 times larger than the average density. Starting from this initial conformation, the optimization has been readily performed after a small number of iterative calculations.

DISCUSSION

A number of methods have been proposed which only use distance information between spin pairs to identify local conformations[14–16]. However, all of the procedures rely on an operator intuitively moving a molecular model simultaneously to fit many distance restrictions, using an expensive interactive graphic display system. The method proposed here does not need any human intuition to obtain the probability density of the unknown spins. In principle, the optimum structural parameters and their uncertainties have been given uniquely by the minimization of T in equation (2). At present, the uncertainty is mainly due to the following:

- there are only few spin pairs to determine the possible area of the target spin from the multiangular method.
- The effect of spin diffusion is too large to determine precisely the distance value, and

Table 1. Distance relationships (d_{ij}(Å)) between spin pairs of NAD^+ bound to LDH (from *Thermus caldophilus* GK24)

a) Free FBP	H1'[a]		H2'		H3'	
	Observed[b]	Optimized[c]	Observed	Optimized	Observed	Optimized
H2	lag	3.4	lag	3.8	2.6 (σ = 0.2)	2.7
H6	2.3 (σ = 0.2)	2.4	2.5 (σ = 0.2)	2.8	lag	4.5
H4	none[d]	5.7	none	5.6	none	5.8
H5	none	4.6	none	4.6	none	5.8
b) Bound FBP	H1' Observed	H1' Optimized[e]	H2' Observed	H2' Optimized	H3' Observed	H3' Optimized
H2	2.1 (σ = 0.2)	2.4	lag	4.4	Lag	4.8
H6	lag	3.5	2.1 (σ = 0.2)	1.8	2.3 (σ = 0.2)	2.0
H4	none	5.7	none	5.7	none	5.8
H5	none	5.3	none	4.1	none	4.1

[a]The notations of atoms are indicated in Figures 4(a) and (b)
[b]Observed values by TRNOE measurements[7]
[c]Calculated values for the optimized conformation without FBP given by the present method (C3'endo-ribose and χ_{opt} = 17° with the uncertainty of ($\Delta\chi$) = ±20°)
[d]No obvious NOE signals were observed for this spin pair
[e]Calculated values for the optimized conformation with FBP (C3'endo-ribose and χ_{opt} = −77° with $\Delta\chi$ = ±22°)

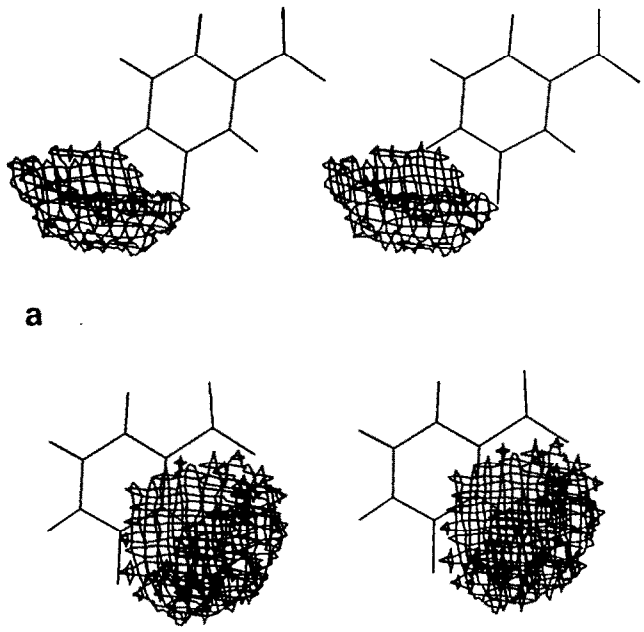

Figure 2. The region indicated in Figures 1(a) and 1(b) are quantified and illustrated by stereo pair isograms. Their values are 10 times larger than the average probability density (a) when FBP does not bind to LDH, and (b) when FBP binds to LDH

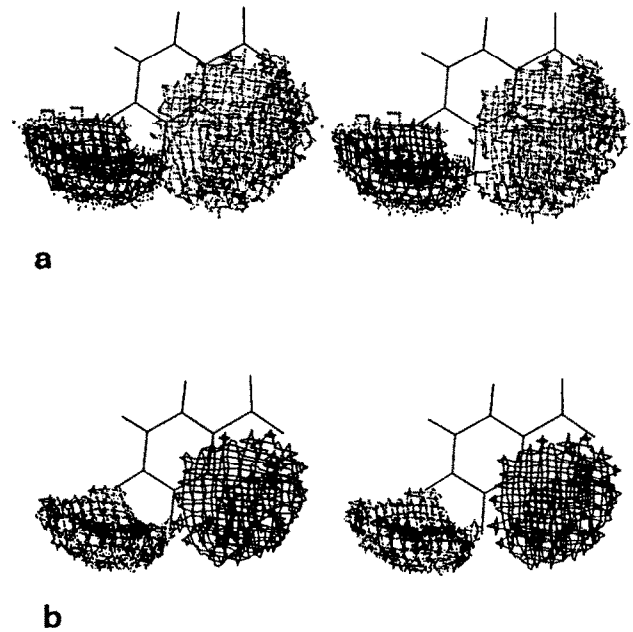

Figure 3. The probability densities of H1'(———), H2'(- - - -) and H3'(·····) of the same ribose group as those in Figures 1 and 2 by stereo pairs. The values of the isograms are all 10 times larger than the average values. (a) when FBP does not bind to LDH, and (b) when FBP binds to LDH

- the amplitude of the fluctuation of atoms is large.

In the present study, the first two effects have up till now had a serious effect, but they are artificial and may be reduced by using synthetic probe molecules designed to bind the enzyme, and by measuring time resolved NOE or TRNOE signals. In this way, it is possible to correctly observe the spin positions which are affected only by the third effect, namely, fluctuation.

From the spread of the probability density isograms, not only isotropic fluctuation but also anisotropic fluctuation can be investigated. This is one of the advantages of the present method, in contrast to the distance geometry, where one rigid structure is normally assumed[17].

Another advantage of the multiangulation used together with TRNOE is that, even if the resonance peaks in the NMR signal are not assigned to the specific

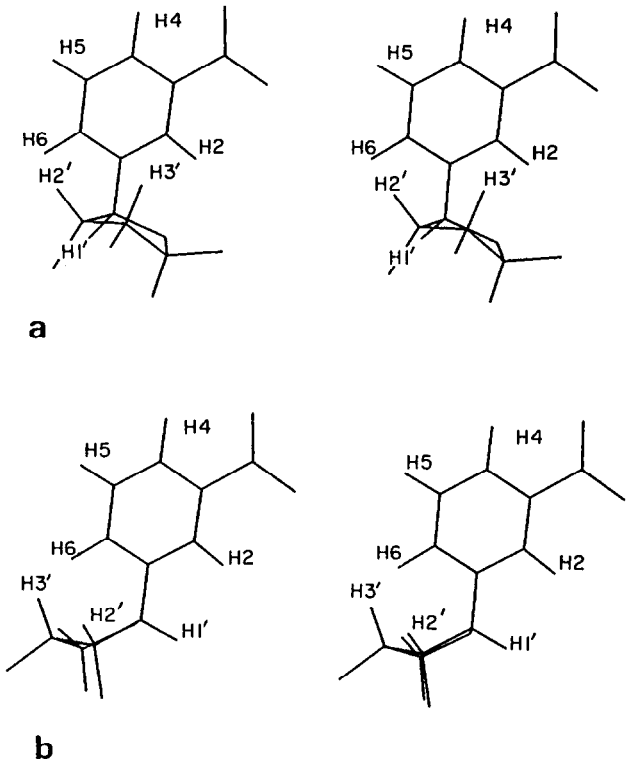

Figure 4. The stereo pair conformations of nicotinamide–ribose optimized by minimization procedures of the target functions T *(equation (2)) (a) when FBP does not bind to LDH with* $\chi_{opt} = 17°$*, and (b) when FBP binds to LDH with* $\chi_{opt} = -77°$

spins, we may calculate the probability density for the target peaks where TRNOE signals are obtained. Also, even if the peak assignment is impossible due to large molecular weight, the chemical group (e.g. aromatic, methylene, methyl etc.) to which the unknown spin is attributed, is readily identified from the chemical shift value. From the biochemical and pharmaceutical point of view, this information may be valuable.

However, when the 'target spin' is incorporated into an unknown, polymorphic structure it is difficult to find the probability density using the method presented here. Several improvements should be necessary.

The combined use of TRNOE and multiangulation may be a useful technique in the analysis of the conformation of drugs, which bind to large receptors. Moreover, using rigid probe molecules, which bind specifically to receptors, it may be possible to investigate the tertiary structure of the receptor binding site including the fluctuation effect. The space resolution is not so good as those produced by X-ray analyses, but this approach may be effective enough for drug design and protein engineering.

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