Eur Biophys J (2002) 31: 549–553
DOI 10.1007/s00249-002-0252-0

ARTICLE

B.H. Havsteen

Evidence of quasi-linear gas transport through sperm whale myoglobin

Received: 1 March 2002 / Revised: 13 June 2002 / Accepted: 2 August 2002 / Published online: 3 October 2002

Abstract The diffusion of molecular oxygen or its isosteric analogue, carbon monoxide, from the surface of myoglobin to its deeply imbedded haem appears to represent one of the simplest protein functions. Hence, it was chosen for the study of the possible role of a global controlling effect like an attractor. However, whereas the six statistical criteria of the classical non-linear dynamic analysis for the existence of an attractor in myoglobin were fulfilled and invariant to the Fourier transformation, the properties of this attractor were not as simple as anticipated. The parameters were tested and confirmed by alternative approaches, the interpoint distance method of Judd and Fourier transformation. If the diffusion were approximately linear, the order of the attractor would be expected to be near one. However, a clearly higher value, 1.46 ± 0.03, was found, indicating the existence of additional steps. Later, the latter were identified as a 90° rotation of CO followed by a translocation by 0.4 Å to a transient pocket. These additional steps may explain the high number of regulatory factors found, 10 ± 1. The autocorrelation function was damped with a correlation length of at least 20 residues. The Poincaré plot showed a dense domain compatible with the cross-section of a quasi-spherical attractor. The first Lyapunov exponent, λ_1, was clearly positive. The Hurst fractal coefficient was 1.90 ± 0.22, indicating a clear departure from simple linear diffusion.

Keywords Myoglobin · Attractor · Gas transport · Non-linear dynamics · Carbon monoxide

B.H. Havsteen (✉)
Biochemisches Institut, Universität Kiel,
Olshausenstrasse 40, 24098 Kiel, Germany
E-mail: benthavs@worldonline.dk
Fax: +45-45856196

Mailing address: B.H. Havsteen
Abildgaardsvej 49, 2830 Virum, Denmark

Introduction

In many ways, myoglobin may be regarded as a prototype of the haemoproteins. Besides, it was the first protein for which the spatial structure was elucidated. Myoglobin is especially interesting since not only its structure, but also its function, is relatively simple. Yet, some aspects of the mechanism of action of this protein remain still to be clarified. One example is the mechanism by which a gas molecule (e.g. molecular oxygen or its equivalent) makes its way through the compact apoprotein to the haem in the centre. The spatial coordinates derived from high-resolution synchrotron radiation diffraction or NMR do not offer many hints about either the course of the gas transport channel, or the mechanism of its gating. Relevant, reliable information, gained from inhibition binding measurements using isosteric molecules, is available only on the minimal and maximal widths of the channel (for a review see Phillips 1978). Hence, an approach to the problem that can yield new information on the problem is desirable. In this communication, an attempt is made to use the techniques of molecular dynamics to throw new light upon the problem of the regulation of the gas transport channel.

The non-linear dynamics of the transport of carbon monoxide through myoglobin proved to possess the parameters of the lowest order of any of about 10 proteins which have been investigated so far (Havsteen 1989, 1991, 1997, 1999a, 1999b; Isvoran 1997; Isvoran et al. 1999, 2000a, 2000b, 2000c; Isvoran and Morariu 2000a, 2000b; Havsteen, unpublished data): chymotrypsin, egg white lysozyme, human lysozyme, cytochrome *c*, sea hare myoglobin, mitochondrial H^+-ATPase, Ascaris trypsin inhibitor, ferredoxin, antidigoxin antibody, esterolytic abenzyme and the prion protein. This observation supports the hypothesis that molecular dynamics, here represented by a manifestation of non-linear interactions, an attractor, plays an active role in protein function. Consequently, measurements of molecular vibrations

seem to permit predictions on the probable biological function of an unknown protein. In that case, the characterization of a protein that has been expressed from one of the many newly discovered genes might be greatly facilitated.

Methods

The data employed in this analysis were obtained from the Protein Data Bank at the Rutgers University College of Science and Biology (http://www.rcsb.org/pdb/downloading.html) under the codes 1A6N (deoxymyoglobin) and 1AJG (carboxymyoglobin). It was submitted by Vojtechovsky et al. (1998) and by Teng et al. (1994), who obtained their data from the sperm whale (*Physeter catoden*) variety of myoglobin. Although the structure of the latter is slightly different from human myoglobin, the channel regulation mechanism is expected to be the same for the two species, owing to evolutionary pressures. Another source of uncertainty is the fact that the crystals of Mb were formed in a buffer at pH 6.5, whereas the crystallization of COMb took place at pH 6.0, both at 40 K. Hence, Mb would carry a negative net charge of ~20 at room temperature, whereas the one of COMb would be ~18. The corresponding conformations would presumably remain fixed during the cooling procedure to 40 K. The question of whether this problem would disturb the non-linear dynamic analysis appreciably, can probably be answered negatively. The reason is that the analysis is focused upon the conformational change accompanying the ligand binding, which is assumed to reflect the channel opening to an appreciable extent. Presumably, the latter is only dependent upon electrostatic charges to a minor extent. This argument also applies to the molecular vibrations.

The mathematical methods that are necessary for the evaluation of the parameters of a non-linear singularity like an attractor are: the saturating order of the correlation integral at increasing imbedding dimension, the correlation length as reflected by the autocorrelation function, the Poincaré plot, the first Lyapunov exponent, the power plot and the Hurst fractal coefficient. They have been described previously (Northrup et al. 1980; Havsteen 1989, 1991; Afinrud et al. 1999).

The results of the classical attractor analysis were tested and confirmed by the following recent alternative procedures: the Judd method of interpoint distances and a repeat of the classical analysis upon the Fourier transformed data (Judd 1992, 1994; Ikeguchi and Aihara 1997). The Fourier transformation was performed manually to the limit of the experimental error and, in addition, with the use of the MATLAB program. The two approaches yielded essentially the same results.

The question of the influence of crystal defects on the validity of the temperature factors has been examined by Northrup et al. (1980) in the case of another, in many ways similar, haemoprotein, cytochrome *c*. They found that the errors from this source would normally be minor and that they could be eliminated by the use of several crystals.

Results

The distribution of the difference in temperature factor along the main chain between myoglobin (Mb) and carboxymyoglobin (COMb) is shown in Fig. 1. The position of the histidine residue that is linked to iron in ferrous haem is marked with an arrow. It is notable that most of the conformational change accompanying the binding of the CO molecule occurs in two adjacent segments (from about residue 25 to about residue 60 and from about residue 70 to about residue 110), of which

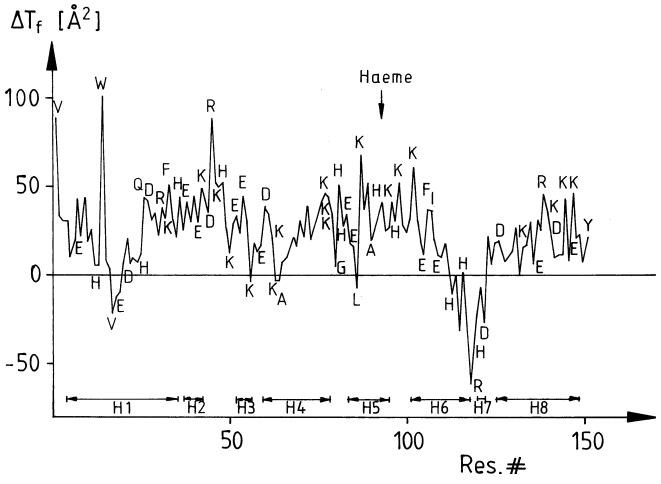

Fig. 1a, b The changes in temperature factors along the peptide chain of myoglobin upon the binding of CO. **a** Mb (*upper trace*, *heavy line*) and COMb (*lower trace*, *light line*). **b** Difference between the traces of Mb and COMb. The helical segments are marked and the amino acids are identified by the one-letter code. The position of the binding of haem is marked

the centre of the latter contains the haem-bound histidine residue. Since the temperature factor diminishes upon the binding of the gas molecule, it represents a contraction (largely) over an extended part of the molecule. The same was the case when the enzymes examined, e.g. chymotrypsin (Havsteen 1989), bound a specific substrate (or a specific inhibitor).

Whereas Fig. 1 shows no indication that the conformational change detected is associated with the opening or closing of the gas transport channel, which would be surprising since it is known to be too fast for kinetic measurements with conventional techniques, Fig. 2 yields information on this problem that appears to be valuable. This figure shows the saturation of the correlation integral with increasing imbedding dimension. The saturation value that is identical with the order of a putative attractor was found by fitting the data set to a hyperbola. The justification for this procedure was that a hyperbola fitted the data better than any other function tested. The result was an order of the attractor of 1.46 ± 0.03, which is well below the maximal permissible figure (Isovoran et al. 1999) for the number of data points available ($d < 2 \ln N$, where N is the number of data points, here 151) but near the dimension of 1 expected for this transport process. The abscissa value at the saturation, which indicates the number of regulating factors of the attractor, is 10 ± 1. This figure is usual for a protein of the given size. The regulating factors may include intrinsic parameters as the temperature, local pH values, the ionic strength and the dielectric constant.

In Fig. 3 the dependence of the autocorrelation function upon the distance in space to neighbouring residues is shown. The curve shows the damping expected from the influence of an attractor. A hyperbola was fitted to the data. The correlation length was about 20 amino acid residues.

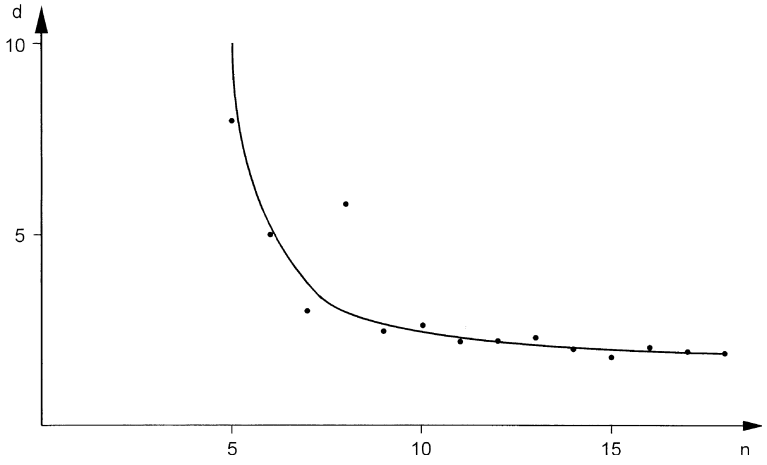

Fig. 2 The dependence of the correlation integral, d, of the putative attractor associated with the transport of gas molecules from the surface of myoglobin to haem upon the number of phase space coordinates, m

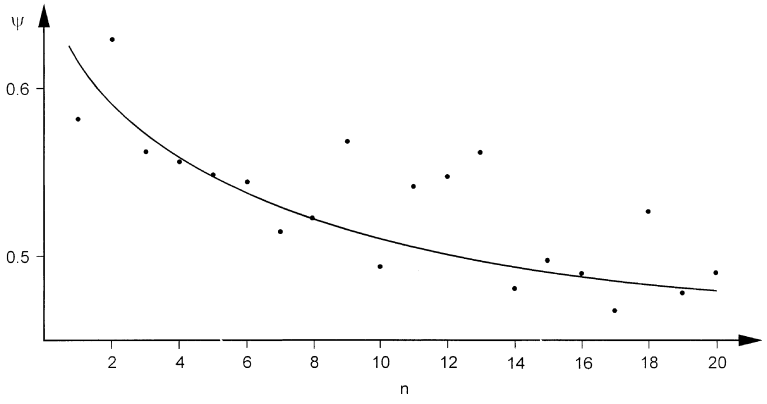

Fig. 3 The dependence of the autocorrelation function, Ψ, upon the distance of interaction, m. Correlation length: ~20

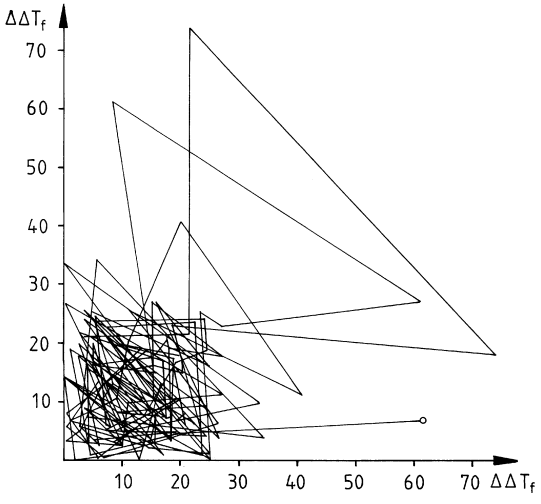

Fig. 4 Poincaré plot of successive pairs of temperature factors (ΔT values) upon the binding of CO to myoglobin. The plot may be regarded as a cross-section of the putative attractor

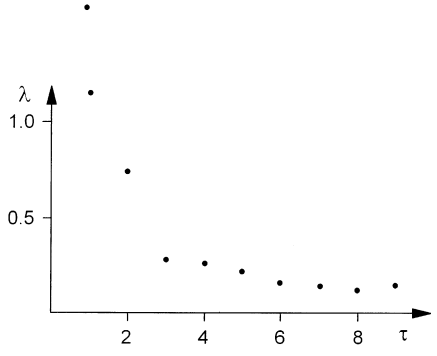

Fig. 5 Plot to test the positivity of the first Lyapunov exponent, λ_1, of the putative attractor associated with the transport of CO through myoglobin to haem. The exponent characterizes the convergence of two arbitrarily chosen, almost parallel trajectories approaching the attractor

Figure 4 shows the Poincaré plot of the change in the vibrations of sequential pairs of neighbouring amino acid residues upon the conformational change that accompanies the binding of carbon monoxide. It may be regarded as a representation of a trajectory of a constituent particle, e.g. during the folding process. The figure shows a particularly dense zone that would be expected from the presence of an attractor. Although experience teaches that many proteins contain dense regions, this case is striking because only a few loose residues are flapping at the surface of the myoglobin. However, their neighbourhood is restrained powerfully by forces that may be largely due to an attractor.

The Poincaré plot was used to construct a plot (Fig. 5) for the evaluation of the first Lyapunov exponent, λ_1. The procedure begins in a region in which two almost parallel trajectories appear to converge. The

distance at their closest approach, P_0, is measured and this calculation is repeated at every step τ along the trajectories. The extrapolation to an infinite τ value of the plot according to the equation defining λ (Havsteen 1991) yields a positive Lyapunov exponent of 0.082 ± 0.004, which is a criterion for the existence of a strange attractor.

The double logarithmic plot of the dependence of the power of the vibrations upon the frequency (see Fig. 6) shows a linearity that indicates the presence of only one attractor. However, the Fourier-transformed data show indications of multifractality (see Table 1). The value of 1.90 ± 0.22 for the Hurst coefficient of fractality was obtained from the slope of the $1/f$ plot. This value is not much higher than the dimension of the attractor (1.46 ± 0.03) found by extrapolation of Fig. 2 to an infinite number of phase space coordinates.

A comparison of the parameters obtained by the three methods of analysis is shown in Table 1.

Discussion

Myoglobin is a suitable object for the investigation of new principles of protein regulation, because many supportive data on this protein are available from numerous studies (Phillips 1978). One of the key questions under current investigation is the possibility of the presence of one or more attractors in proteins. If it can be answered positively, then it is probable that the properties of the attractor reflect aspects of the nature of the physiological function of the protein. Such information would facilitate the identification of the many proteins which have been discovered and expressed as a result of the completion of the human genome project. The approach to this analysis is a study of the molecular vibrations of the protein after crystallization and diffraction analysis or solution of the spatial structure using NMR spectra. The atomic vibrations are revealed by the temperature factors from the diffraction measurements or by the RMS deviation of the atomic positions from the NMR analysis. (Isvoran 2000) has shown that these two methods of observation are equivalent.

The application of the standard statistical methods of non-linear dynamics for the identification of an attractor in a flexible macromolecule revealed that all of the six criteria available were satisfied. Besides, the order of the attractor found, 1.46 ± 0.03, is close to the expected value of 1. Since the value found is clearly higher than 1, a process in addition to the linear transport of the gas

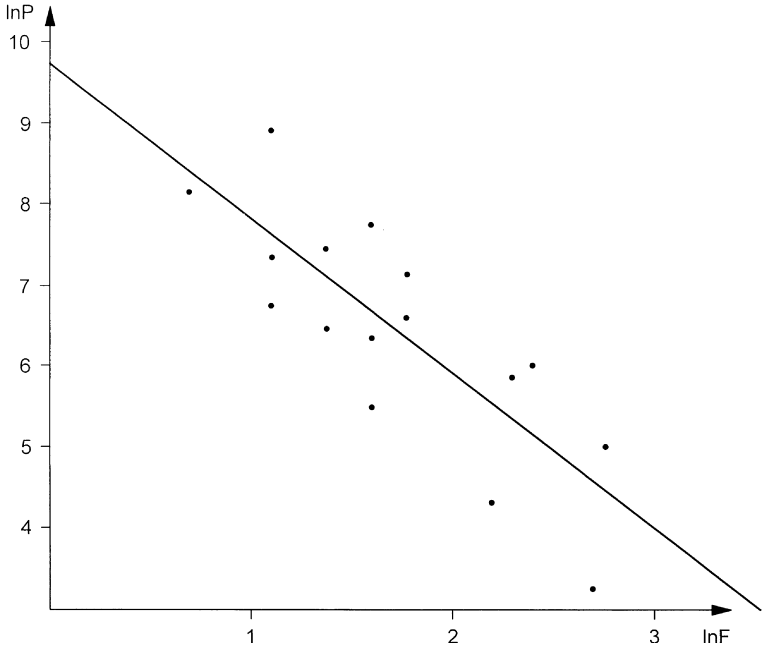

Fig. 6 $1/f$ plot for the determination of the Hurst fractal dimension of the conformational change associated with the binding of CO to myoglobin. This parameter is equal to the numerical value of the slope. Ordinate: power (expressed as $(\Delta T_f)^2$); abscissa: frequency

Table 1 Comparison between the values of the parameters characterizing the putative attractor before and after the Fourier transformation (FT) as well as by the Judd method

Before	FT	After FT	Judd method[a]
Order	1.46 ± 0.03	1.98 ± 0.09	2.20 ± 0.09
Controlling factors	10 ± 1	12 ± 2	10 ± 2
Correlation length	101 ± 1	23 ± 14	10 ± 3
Lyapunov exponent, λ_1	0.082 ± 0.004	0.115 ± 0.004	0.399 ± 0.012
Hurst fractal coefficient	1.90 ± 0.22		2.29 ± 0.04
Low frequency		0.64 ± 0.01	
High frequency		2.22 ± 0.05	

[a]Method of interpoint distance

molecule between haem and the surface must be taken into consideration. Such a process, the rotation of the carbon monoxide molecule by 90° with a translocation of 0.4 Å prior to its release from haem, has recently been observed by Anfinrud et al. (1999), who used 100 ps laser pulses to perturb the equilibrium between Mb + CO and MbCO. These additional steps also explain the high number of regulatory factors found. The long correlation length of about 20 amino acid residues seems to reflect the high resiliency of the dense, hydrophobic matrix.

This evidence, together with similar results from the analysis of several other proteins of different nature, e.g. enzymes, antibodies, an oxidation-reduction catalyst, protein inhibitors and transport proteins, supports the hypothesis that attractors are present in many proteins, in which they participate in the biological function. If this is upheld by additional evidence, a radically new principle of the mechanism of protein action may have been discovered. The latter would represent the contribution of purely physical effects, e.g. to enzyme catalysis, as opposed to the classical, chemical explanation (acid-base catalysis, nucleophilic catalysis, etc.).

Acknowledgements Much help from Mr. A. Kraemer with computer problems and the competent draftwork by Mrs. A. Krohn are gratefully acknowledged.

References

Anfinrud P, de Vivie-Riedle R, Engel V (1999) Ultrafast detection and control of molecular dynamics. Proc Natl Acad Sci USA 96:8328–8329

Havsteen BH (1989) A new principle of enzyme catalysis. J Theor Biol 140:101–127

Havsteen BH (1991) A stochastic attractor participates in chymotryptic catalysis. A new facet of enzyme catalysis. J Theor Biol 151:557–571

Havsteen BH (1997) Attractor control of the binding of digoxin to a specific antibody. J Theor Biol 189:367–376

Havsteen BH (1999a) An esterolytic antibody with vibrational properties of a catalytic H-chain and a non-catalytic L-chain. J Theor Biol 196:377–387

Havsteen BH (1999b) Attractor control of the redox reactions of bovine cytochrome *c*. J Protein Chem 18:791–799

Ikeguchi T, Aihara K (1997) Estimating correlation dimensions of biological time series with a reliable method. J Intel Fuzzy Syst 5:33–52

Isvoran A (1997) Analysis of the appearance of ordered dynamic structure in ferredoxine. Anal Univ Timisoare Ser Phys 36:117–121

Isvoran A (2000) Molecular dynamics of some biopolymers. Doctoral Dissertation, Dept. of Physics, Univ. of Timisoara, Romania

Isvoran A, Morariu VV (2000a) Comparison of the behavior of sea hare myoglobin, when it forms two different complexes. Chaos Solitons Fractals (in press)

Isvoran A, Morariu VV (2000b) Analysis of the non-linear behavior of Ascaris trypsin inhibitor from NMR data. Chaos Solitons Fractals (in press)

Isvoran A, Morariu VV, Negreanu-Maior A (1999) Attractor control of the molecular vibrations of human lysozyme upon substrate binding. Int J Chaos Theory Applic 4:13–22

Isvoran A, Dolha D, Morariu VV (2000a) Attractor control of the stability of lysozymes. Int J Chaos Theory Applic (in press)

Isvoran A, Unipan L, Morariu VV (2000b) Chaos in enzymic activity of egg white lysozyme. Rom Biophys J (in press)

Isvoran A, Unipan L, Licz A, Morariu VV (2000c) Determination of the fractal dimension of the lysozyme backbone for three different organisms. Chaos Solitons Fractals 12:757–760

Judd K (1992) An improved estimation of dimension and some comments on providing confidence intervals. Physica D 56:216–228

Judd K (1994) Estimating dimension from small samples. Physica D 71:421–429

Northrup SH, Pear MR, McCammon JA, Karplus M, Takano T (1980) Internal mobility of ferrocytochrome *c*. Nature 287:659–660

Phillips SEV (1978) Structure of oxymyoglobin. Nature 273:247–248

Teng TY, Srajer V, Moffat K (1994) Photolysis-induced structural changes in single crystals of carbon monoxide. Nat Struct Biol 1:701

Vojtechovsky J, Berendsen J, Chu K, Schlichting I, Sweet RM (1998) Implications for the mechanism of ligand discrimination and identification of substrates derived from crystal structures of myoglobin-ligand complexes at atomic resolution. Code 1AJG, Protein Data Bank, Rutgers University