The renaissance of myoglobin: dynamics, structure and oxygen binding control

M. Brunori

Department of Biochemical Sciences, University of Rome 'La Sapienza', P.le A. Moro, 5, I-00185 Rome (Italy), Fax +39(6)4440062

Abstract

Myoglobin as a prototype of a globular protein has been widely employed in advanced studies on the dynamics, folding and evolution of proteins. The advent of fast and super-fast lasers, the expression of site-directed mutants, and the application of advanced analytical and spectroscopic techniques have provided new insights into the structure-function relationships in proteins.

The work carried out by the Rome group has foc-used on the mechanisms of control and discrimination of ligand binding in myoglobin. It is known[1] that bound oxygen is stabilised by H-bonding to the distal His(E7), and that substitution of this residue is generally associated to a large (or very large) increase in the oxygen dissociation rate constant[2]. We have attacked the problem of engineering into the sperm-whale 3-D framework alternative distal residues that would exert a control on bound oxygen even in the absence of His(E7)[3]. Of particular interest in this context is the synthesis of a new mutant[4] which is expected (on the basis of molecular modelling) to make H-bonds with bound oxygen, one with Gln(E7) and the other with Tyr(B10), mimicking therefore the structure of the active site of *Ascaris* Hb[5].

The functional properties of this new myoglobin mutant (see table 1) can be accounted for by the new H-bonding network, and provide a clue to a possible strategy for engineering a distal site suitable for a blood substitute, given that a low oxygen dissociation rate constant (which reduces the rate of autoxidation, see ref. 6) is associated in this mutant to a lowered oxygen association rate constant, thereby moderating oxygen affinity.

Mini-myoglobin has been prepared in our group by limited proteolysis of horse Mb as a model for the central exon of the globin gene in order to test theories on the role of introns in molecular evolution. This myoglobin fragment binds heme stoichiometrically, acquires a high degree (~63%) of α-helical secondary structure and (more importantly) binds oxygen with equilibrium and kinetic parameters very similar (within a factor of 2) to those of native myoglobin (see table 2)[7,8]; therefore it is a valuable model for a possible ancestor of the modern-day oxygen carriers.

Table 1. O_2 Binding data on mutant myoglobins.

		k_{off} (s^{-1})	k_{on} (M^{-1} s^{-1})	$p_{1/2}$ (mmHg O_2)
Wild type	Leu(29) His(64)	17	$1.6 \cdot 10^7$	0.6
Single mutant	Leu(29) Gln(64)	100	$2.0 \cdot 10^7$	1.8
Double mutant	Tyr(29) Gln(64)	1	$1 \cdot 10^6$	0.6

Table 2. Functional properties of horse heart mini-Mb.

	CO	O_2	
	1′ (M^{-1} s^{-1})	k′ (M^{-1} s^{-1})	k (s^{-1})
Mini-Mb	$4.5 \cdot 10^5$	$5 \cdot 10^6$	20
Mb	$5.0 \cdot 10^5$	$1 \cdot 10^7$	11

Laser photolysis of the CO-derivative of Mini-myoglobin, and extensive analysis of its temperature and wavelength dependence[9], have indicated that Mini-myoglobin is likely to be the domain responsible for the ligand-linked conformational transition, while the two polypeptides corresponding to the terminal exons of the globin gene would stabilise the protein by damping the conformational fluctuations of the central exon, a novel view on the relationships between the dynamics at the heme site and the overall mass of the protein.

1 Phillips, S. E. V., J. molec. Biol. *142* (1980) 531.
2 Olson, J. S., Mathews, A. J., Rohlf, R. J., Springer, B. A., Egeberg, K. D., Sligar, S. G., Tame, J., Renand, J. P., and Nagai, K., Nature *336* (1988) 265.
3 Cutruzzolà, F., Travaglini-Allocatelli, C., Ascenzi, P., Bolognesi, M., Sligar, S. G., and Brunori, M., FEBS Lett. *282* (1991) 281.
4 Travaglini-Allocatelli, C., Cutruzzolà, F., Brancaccio, A., Vallone, B., and Brunori, M., FEBS Lett. *352* (1994) 63.
5 De Baere, I., Lin, L., Moens, L., Van Beeumen, J., Crielens, C., Richelle, J., Trotman, C., Finch, J., Gerstein, M., and Perutz, M.F., Proc. natl Acad. Sci. USA *89* (1992) 4638.
6 Brantley, R. E., Smerdan, S. J., Wilkinson, A. J., Singleton, E. W., and Olson, J. S., J. biol. Chem. *268* (1993) 6995.
7 De Sanctis G., Falcioni, G., Giardina, B., Ascoli, F., and Brunori, M., J. molec. Biol. *200* (1988) 725.
8 De Sanctis G., Falcioni, G., Grelloni, F., Desideri, A., Polizio, F., Giardina, B., Ascoli, F., and Brunori, M., J. molec. Biol. *222* (1992) 637.
9 Di Iorio E. E., Yu, W., Calonder, C., Winterhalter, K. H., De Sanctis, G., Falcioni, G., Ascoli, F., Giardina, B., and Brunori, M., Proc. natl Acad. Sci. USA *90* (1993) 2025.