Influence of Temperature on the Topological Features of Inner Cavities in Cytoglobin

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Abstract

Cytoglobin (Cygb) is a novel member of the globin family in man, but there is no clear evidence about its biological function. Cygb exhibits a highly complex ligand rebinding kinetics, which agrees with the structural plasticity of the inner cavities and tunnels found in the protein matrix. In this work we have examined the effect of temperature on the topological features of Cygb. To this end, the structural and dynamical properties of human Cygb are compared with those determined for the Antarctic fish Chaenocephalus aceratus. The results support a distinct temperature-dependence of the topological features in the two proteins, suggesting different adaptations to cold and warm environments.

I. Introduction

Cytoglobin (Cygb) is a fourth member of the globin family discovered in man [1]. Human Cygb consists of 190 amino acids, showing extensions of about 20 amino acids at both C- and N-termini with respect to standard globins. The amino acid sequence fits well into the conserved globin fold pattern, covering helices from A to H, and key residues such as the proximal (F8) and distal (E7) histidines, and phenylalanine CD1 at the CD corner. Along with neuroglobin, Cygb is a bis-histidyl hexacoordinated globin. In the absence of exogenous ligands, the sixth heme iron coordination site is occupied by the distal HisE7 residue in both ferric and ferrous forms. Binding of exogenous ligands is possible only with concomitant displacement of the distal HisE7, a regulatory mechanism that has been suggested to require a substantial conformational change.

The crystal structure of bis-histidyl hexacoordinated Cygb shows an extended apolar protein matrix cavity, which differs from the topology of inner cavities found in other hemoglobins [2], thus raising questions about the functional role of Cygb in cells. Besides a role as scavenger of nitric oxide or reactive oxygen species, oxygen storage and sensor protein [3, 4], a potential implication in cancer as a tumour suppressor gene has been suggested [5].

The internal volume and the topological nature of inner cavities are largely dependent on the coordination state of Cygb [6]. In particular, a substantial change in both protein dynamics and inner cavities is observed upon transition from the CO liganded to the pentacoordinated and bis-histidyl hexacoordinated species. This agrees with the complex kinetic pattern derived from carbon monoxide rebinding assays, which suggests the involvement of distinct reaction intermediates, reflecting rebinding from temporary docking sites, second order recombination, and formation (and dissociation) of a bis-histidyl heme hexacoordinated reaction intermediate. These findings are also consistent with the expected versatility of the molecular activity of this protein.

In this work our interest is focused on the temperature dependence of the topological features of inner cavities of Cygb. Specifically, our aim is to ascertain the impact of temperature on the protein dynamics and the number and nature of inner cavities. To this end, we want to determine the structural, dynamical and topological properties of human Cygb with the protein in the Antarctic fish *Chaenocephalus aceratus*. The results obtained from molecular simulations will be complemented with data derived from flash photolysis experiments to discuss the impact of temperature on the ligand migration properties of these two proteins.

II. Methods

The structural model of *C. aceratus* Cygb (caCygb) was built up using SWISSMODEL taking advantage of the X-ray structures human Cygb (hCygb) and the significant sequence similarity between the two proteins (around 55% sequence identity). Then, the protein structures were refined using the protocol outlined in previous studies [6], and molecular dynamics simulations were carried out for both hCygb and caCygb at three temperatures (10, 25 and 40 degrees). For hCygb simulations the X-ray structures 3AG0 and 4B3W were used as templates in order to account for the conformational change of residue Trp151. Production runs were extended up to 1.5 μs using Amber12 and the parm99SBildn force field. Essential dynamics was used to analyse the dynamical behaviour of the protein. The topology of inner cavities were examined by using the MDpocket program. The analysis was performed for a series of 5000 snapshots taken in 250 ns windows during the last half of the trajectory.

III. RESULTS

Previous studies performed for hCygb at 298 K revealed that the oxygenated protein contains a big cavity. In the simulation started from the X-ray structure 4B3W there was no clear passage from the protein interior to the bulk solvent. However, the trajectory started from X-ray structure 3AG0 showed a well defined path that connects the primary docking site to the bulk solvent, passing through residues in loop AB and the final segment of helix G. The analysis also suggested the existence of a secondary path that involves the migration through distinct pocket sites and the exit via a passage between helix A and loop EF. Remarkably, the integrity of

these paths is lost when simulations at 283 and 313 K are examined (data not shown). In contrast to the preceding findings, simulations performed for caCygb reveal a well shaped path through the protein matrix at 283 K, which is nevertheless disrupted when the temperature is raised at 298 and 313 K (Fig. 1).

Comparison between the residues that shape the inner tunnel in the two proteins suggest a seemingly key difference, which is the mutation of Met30 in hCygb to Ser in caCygb. This change leads to the formation of a stable hydrogen-bond interaction between the Ser hydroxyl group and the indole NH unit of Trp151. This interaction fixes the orientation of Trp151, which in turn favors the formation of the tunnel in the interior of caCygb, especially at low temperatures. In contrast, the presence of Met instead of Ser in hCygb leads to an increased fluctuation of Trp151, thus contributing to blur the tunnel.

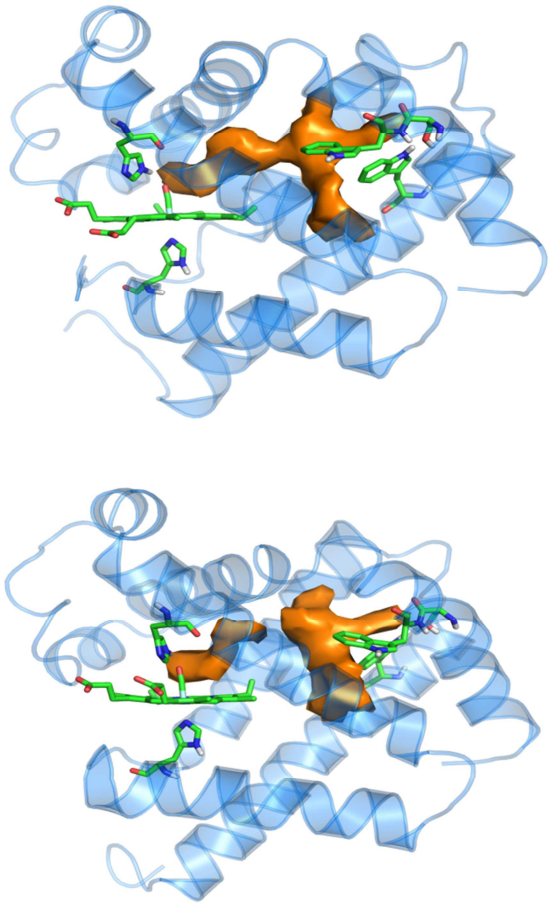

Fig. 1. Representation of the inner tunnel found in caCygb (corresponding to a frequency level of 50% as determined from MDpocket calculations; shown in orange) at (top) 283 and (bottom) 298 K. The protein backbone is shown in blue and the oxygenated haem is shown as green sticks, together with the distal and proxima histidine residues.

An additional factor appears to be the distinct flexibility of the residues around the tunnel. Several analyses performed for different layers of residues suggest that the residues shaping the tunnel are less flexible in caCygb than in hCygb. Thus, it seems that they are pre-configured through van der Waals and hydrogen-bonded interactions to properly shape the tunnel at low temperature in caCygb, whereas the increased flexibility afforded by temperature compromises the integrity of the tunnel. In contrast, the enhanced flexibility in hCygb seems to favor the formation of interactions between Trp151 and Ile114 or alternatively Ser111, thus favoring a proper arrangement for the formation of the tunnel at higher temperature.

IV. CONCLUSION

The results supports a different temperature sensitivity of both caCygb and hCygb. The former contains an inner tunnel at 283 K, whereas in the latter it appears at 298 K. In the two cases the tunnel is blurred upon increase of the temperature to 313 K. Comparison of the two proteins points out that the Met30→Ser mutation might be a key factor in shaping the features of the tunnel in the interior of the proteins. Thus, the balance between the flexibility of residues in layers around the tunnel and the stability of specific interactions between residues seems to be critical for preserving the nature of the inner tunnel. Future studies will then attempt to determine the integrity of the topological features of inner cavities by introducing the corresponding mutation in the two proteins.

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