ELSEVIER

Biophysical Chemistry 116 (2005) 219 – 225

Biophysical Chemistry

http://www.elsevier.com/locate/biophyschem

Incoherent elastic and quasi-elastic neutron scattering investigation of hemoglobin dynamics

Chiara Caronna[a,b], Francesca Natali[a,*], Antonio Cupane[b]

[a]INFM OGG & CRS-SOFT, c/o ILL, 6 rue Jules Horowitz, BP 156-38042 Grenoble Cedex 9, France
[b]Istituto Nazionale per la Fisica della Materia and Dipartimento di Scienze Fisiche ed Astronomiche, Universita' di Palermo, via Archirafi36, I-9023, Palermo, Italy

Received 8 February 2005; accepted 17 February 2005
Available online 23 May 2005

Abstract

In this work we investigate the dynamic properties of hemoglobin in glycerolD_8/D_2O solution using incoherent elastic (ENS) and quasi-elastic (QENS) neutron scattering. Taking advantage of complementary energy resolutions of backscattering spectrometers at ILL (Grenoble), we explore motions in a large space–time window, up to 1 ns and 14 Å; moreover, in order to cover the harmonic and anharmonic protein dynamics regimes, the elastic experiments have been performed over the wide temperature interval of 20–300 K. To study the dependence of the measured dynamics upon the protein quaternary structure, both deoxyhemoglobin (in T quaternary conformation) and carbonmonoxyhemoglobin (in R quaternary conformation) have been investigated.

From the ENS data the mean square displacements of the non-exchangeable hydrogen atoms of the protein and their temperature dependence are obtained. In agreement with previous results on hydrated powders, a dynamical transition at about 220 K is detected. The results show interesting differences between the two hemoglobin quaternary conformations, the T-state protein appearing more rigid and performing faster motions than the R-state one; however, these differences involve motions occurring in the nanosecond time scale and are not detected when only faster atomic motions in the time scale up to 100 ps are investigated.

The QENS results put in evidence a relevant Lorentzian quasi-elastic contribution. Analysis of the dependence of the Elastic Incoherent Structure Factor (EISF) and of the Lorentzian halfwidth upon the momentum transfer suggests that the above quasi-elastic contribution arises from the diffusion inside a confined space, values of confinement radius and local diffusion coefficient being compatible with motions of hydrogen atoms of the amino acid side chains. When averaged over the whole range of momentum transfer the QENS data put in evidence differences between deoxy and carbonmonoxy hemoglobin and confirm the quaternary structure dependence of the protein dynamics in the nanosecond time scale.

Keywords: Protein dynamics; Hemoglobin quaternary structure; Mean square displacement; Dynamical transition

1. Introduction

It is nowadays generally accepted that proteins are dynamic objects and that the dynamic properties are relevant to protein functions. In particular, a variety of techniques (including, e.g., Mössbauer spectroscopy [1], neutron scattering [2], optical absorption spectroscopy [3] and molecular dynamics simulations [4]) have shown a transition, occurring in the range 180−220 K, in the dynamic behavior of hydrated proteins from a low temperature harmonic behavior to an high temperature anharmonic behavior characterized by an increase of atomic mean square displacements well above the "harmonic" hyperbolic cotangent behavior. The onset of anharmonic dynamics has often been deemed necessary for optimal enzyme activity and protein function [5]. On the other hand, motions in proteins are known to exist in a wide range of time and length scales, spanning, e.g., from local atomic

* Corresponding author. Tel.: +33 4 76 20 70 71; fax: +33 4 76 48 39 06.
E-mail address: natali@ill.fr (F. Natali).

0301-4622/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2005.02.004

vibrations (10^{-12} s and 10^{-1} Å time and length scale) to concerted motions of protein segments (10^{-3} s and 10^{1} Å). Therefore, when studying protein dynamics and in particular the transition between harmonic and anharmonic regimes, attention must be paid to the time and length scale probed by the experimental technique used. As an example, for the enzyme glutamate dehydrogenase the dynamical transition has been shown to be highly time scale dependent [6] and enzyme activity has been shown to be unaffected by anharmonic motions taking place in the picosecond time scale.

As far as hemoglobin is concerned, dynamic studies are further complicated by the possible presence of effects linked to the quaternary conformation. Indeed, it is well known that hemoglobin can reversibly adopt two different quaternary conformations: the tense (T) conformation, in the absence of ligands, and the relaxed (R) conformation, stabilized by the presence of ligands such as CO or O_2. The widely different structural and functional properties of the two conformations are well characterized [7,8]. Much less is known about their dynamic properties and about the structure–dynamics–function relationships of the two different quaternary conformations. Low temperature optical absorption spectroscopy has pointed out remarkable differences between the dynamic properties of deoxyhemoglobin (deoxyHb) and carbonmonoxyhemoglobin (HbCO) [9,10]. However, optical spectroscopy is an "instantaneous" technique that probes the local dynamic properties of the active site (i.e. of the heme pocket) which is highly dependent upon the local tertiary structure and in particular upon the presence/absence of the heme ligand. Therefore, information on the dependence of the global protein dynamics upon the time scale investigated and upon the quaternary conformation, as well as on the possible relevance of dynamic properties in determining the functional behavior of the molecule in different quaternary conformations is up to now lacking.

Incoherent neutron scattering from proteins in deuterated solutions, which is primarily sensitive to the non-exchangeable hydrogen atoms of the protein, appears as a technique suited to address the above points [11,12]. Indeed, being biomolecules made up mainly of H (around 50% of the total atom numbers), C, O and N atoms, the incoherent signal arises principally from the H atoms whose incoherent cross section is two orders of magnitudes higher than that of the remaining atoms; moreover, non-exchangeable hydrogen atoms are evenly distributed over the whole protein. On the other hand, the solvent contributions can be subtracted by performing accurate scans on a "blank" sample, i.e. on a sample containing identical quantities of solvent, buffer and salts and in which only the protein is missing. Thus, the incoherent neutron scattering technique (INS), which describes the correlation of a given particle at different times, provides information on the global molecule dynamics. Moreover, by exploiting the different energy resolutions and momentum transfer (Q) ranges of different spectrometers, motions occurring in different time and length scales can be investigated.

In this paper we present the results of an incoherent elastic and quasi-elastic neutron scattering investigation of hemoglobin dynamics. Experiments have been performed on 65% glycerolD_8/D_2O solutions of both deoxyHb and HbCO; the presence of glycerolD_8 as a cryosolvent is necessary in order to span a wide temperature range, covering the harmonic and anharmonic regimes, without the appearance of undesired ice Bragg reflections. Two different spectrometers at ILL (Grenoble) have been used: IN13 with an energy resolution of ~8 μeV (FWHM) and a Q range of ~1.1–5.0 $Å^{-1}$ and IN16 with an energy resolution of ~0.9 μeV (FWHM) and a Q range of ~0.4–1.9 $Å^{-1}$. This enables to explore motions occurring in the time scale up to 100 ps and 1 ns, respectively, and in the length scale from ~1 Å to ~14 Å. Aim of the work is to investigate the dynamic properties of hemoglobin in solution in the widest possible time and length intervals and to put in evidence the possible presence of effects linked to the quaternary structure of the protein.

2. Materials and methods

2.1. Samples

Hemoglobin was prepared from the blood of a single healthy individual as already described elsewhere [13]; it was stored in liquid nitrogen under the oxy form at a concentration of about 10% by weight. To prepare samples for the experiments, an appropriate amount was thawed and deuterated by first concentrating to about 30% by weight and then re-diluting to 10% with D_2O (Euro-isotope, Grenoble, France, purity >99.8%). This procedure was repeated several times, to reach a final D_2O/H_2O concentration greater than 98:1. Samples were then mixed with glycerolD_8 (Euro-isotope, Grenoble, France, purity >99.8%) in a 1:2 proportion and with phosphate buffer 1 M (pD=7) to have a final buffer concentration of 10^{-2} M. Use of glycerolD_8 as a cryoprotectant allows us to expand the explored temperature range towards low T values (T<273 K), normally limited by the presence of ice Bragg reflections.

To prepare deoxy Hb, the sample was equilibrated with N_2 by gently stirring under nitrogen atmosphere for about 15 min and then deoxygenated with 5×10^{-2} M sodium dithionite. To prepare HbCO, exactly the same procedure was followed, using CO as equilibration gas.

The pD of the samples was measured immediately after preparation and found to be around 6.7 for both samples.

2.2. Neutron scattering experiments

Neutron scattering experiments have been performed on two high-resolution backscattering spectrometers, which