Interactions of bile salt micelles and colipase studied through intermolecular nOes

Cyril Dominguez, Corinne Sebban-Kreuzer, Olivier Bornet, Brigitte Kerfelec, Catherine Chapus, Françoise Guerlesquin*

Unité de Bioénergétique et Ingénierie des Protéines, IBSM-CNRS, 31 chemin Joseph Aiguier, 13204 Marseille Cedex 20, France

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Abstract Colipase is a small protein (10 kDa), which acts as a protein cofactor for the pancreatic lipase. Various models of the activated ternary complex (lipase–colipase–bile salt micelles) have been proposed using detergent micelles, but no structural information has been established with bile salt micelles. We have investigated the organization of sodium taurodeoxycholate (NaTDC) micelles and their interactions with pig and horse colipases by homonuclear nuclear magnetic resonance (NMR) spectroscopy. The NMR data supply evidence that the folding of horse colipase is similar to that already described for pig colipase. Intermolecular nuclear Overhauser effects have shown that two conserved aromatic residues interact with NaTDC micelles.

Key words: Colipase; Bile salt; Micelle; Nuclear magnetic resonance; Intermolecular nuclear Overhauser effect

1. Introduction

In intestine, dietary fat hydrolysis results from the combined effect of pancreatic lipase (triacylglyceride esther hydrolase, EC 3.1.1.3), colipase and biliary secretion. One of the well-known functions of colipase is to anchor lipase to the water/lipid interface coated by amphiphilic compounds. In vivo, lipolysis is likely to occur via the formation of an active ternary complex involving lipase, colipase and a mixed micelle. In this complex, colipase plays a central role by binding the lipase, the mixed micelle and the water/lipid interface.

The polypeptide chain of colipase forms four fingers protruding from a compact core held together by a network of five invariant disulphide bridges. The interfacial binding site involving the tyrosine-rich region appears to be located at the tips of the fingers, while the lipase binding site is on the opposite side [1–3]. Colipase is mainly associated to the C-terminal domain of the lipase [4]. In the complex, lipase adopts an active conformation (open flap conformation), and in the active ternary complex, supplementary interactions are created between the lipase flap and residues 13–15 of the colipase [5]. Topologically distinct from the interfacial binding site, the micelle binding site occupies the concave face of the colipase [6].

In the physiological conditions, bile salt micelles are known to be essential for lipolysis. They are involved in the transport of the lipolysis products, in the activation of pancreatic lipase and likely in the catalysis itself. Neutron crystallography gave evidence that the lipase–colipase activation is not interfacial but occurs in the aqueous phase in presence of micelles [7]. In the ternary complex, the micelle interacts more extensively with the colipase than with the lipase. Very limited contacts are observed between the micelle and the 401–412 loop of the C-terminal domain of the lipase. Upon micelle binding, a conformational change corresponding to an 82° rotation of the 70–85 loop occurs in the colipase [7,8]. The protein surfaces involved in the micelle recognition are amphipathic without forming patches of either hydrophilic or hydrophobic amino acid side chains. This observation can be correlated to the finding that colipase is able to form binary complexes with a large variety of micelles [1,9,10]. It has been suggested that in the ternary complex, Tyr55 and Phe52 of pig colipase are directly involved in the micelle binding [7]. Indirect evidence of the involvement of Trp52 of horse colipase has also been reported [11,12].

The porcine colipase three dimensional structure has been solved both by nuclear magnetic resonance (NMR) and X-ray crystallography [2,3]. In order to get more information on the colipase–bile salt micelle interactions, we have undertaken a two dimensional (2D) NMR study of the sodium taurodeoxycholate (NaTDC)–colipase complex. The experiments have been carried out on horse and pig colipases with the aim to compare the interacting site on the two molecules.

2. Materials and methods

2.1. NMR samples

NaTDC was obtained from Sigma. NMR experiments for proton assignment were carried out on two samples, at 1 mM concentration, in 100 mM phosphate buffer, respectively, at pH 4.5 and 7.0. NaTDC micelle organization was observed increasing the NaTDC concentration from 0.1 to 500 mM.

Pancreatic colipases were isolated from porcine and horse pancreas according to the procedures already described [13]. For proton assignments, pig colipase was prepared in the same conditions as previously reported [2] and horse colipase was concentrated at 1 mM in 0.2 M NaCl, 100 mM KH_2PO_4, pH 2.7.

NaTDC–colipase complexes were studied at 1 mM colipase and 16 mM NaTDC concentrations, in 100 mM NaCl, 20 mM phosphate buffer, pH 7.0.

2.2. NMR experiments

All NMR spectra were recorded on a 500 MHz DRX Bruker spectrometer at 310 K. NaTDC micelle organization and NaTDC–coli-

*Corresponding author. Fax: (33)-4-91 16 45 78.
E-mail: guerlesq@ibsm.cnrs-mrs.fr

Abbreviations: CMC, critical micellar concentration; NaTDC, sodium taurodeoxycholate; NMR, nuclear magnetic resonance; nOe, nuclear Overhauser effect; NOESY, nuclear Overhauser effect spectroscopy; TOCSY, total correlation spectroscopy

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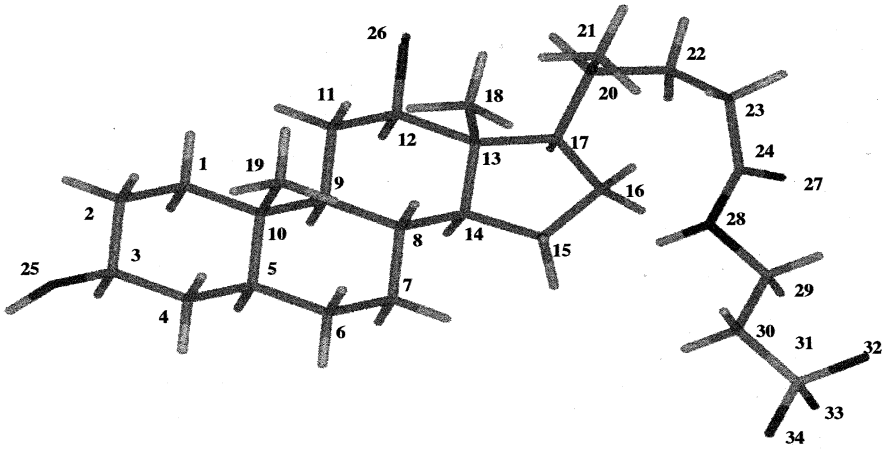

Fig. 1. NaTDC molecular structure.

pase complex formation have been studied using one dimensional (1D) NMR spectra. Double quantum-filtered correlation spectroscopy [14], Clean total correlation spectroscopy (TOCSY) [15] and nuclear Overhauser effect (nOe) spectroscopy (NOESY) [16] spectra were acquired in a phase-sensitive mode using States-time-proportional phase incrementation quadrature detection [17]. NaTDC proton assignments were obtained from 2D NMR spectra: TOCSY (80 ms spin lock), COSY and NOESY (100 ms and 200 ms mixing time). For colipase proton assignments, TOCSY (80 ms spin lock) and NOESY (100 ms and 200 ms) spectra were recorded. Intermolecular nOes were monitored by NOESY (200 ms) experiments performed on the NaTDC–colipase complexes. Spectra were recorded with 2 K complex points in the directly acquired dimension and 512 complex points in the indirectly detected dimension, with 64 transients per t1 increment over a spectral width of 6000 Hz in both dimensions. Water suppression was achieved using presaturation during the relaxation delay (1.3 s), and during the mixing time in the case of NOESY experiments. Data were processed using the program XWINNMR (Bruker). Time domain data were multiplied by a sine function and zero-filled to give a final matrix size of 2 K×1 K (real) points after Fourier transformation. A fifth-order polynomial baseline correction in both dimensions was applied. Proton chemical shifts were calibrated relative to H_2O at 310 K at 4.66 ppm.

3. Results

3.1. Proton assignment of NaTDC

Fig. 1 shows the molecular structure of NaTDC. The proton assignment has been obtained using COSY, TOCSY and NOESY experiments. Proton chemical shifts are found very similar to those of deoxycholic acid [18]. Additional proton resonances occur at NH28 (7.89 ppm), $CH_2$29 (3.63 ppm) and $CH_2$30 (3.14 ppm).

3.2. Formation of NaTDC micelles

NMR is an interesting tool to study micelle formation. Two parameters are notably affected when considering micelle for-

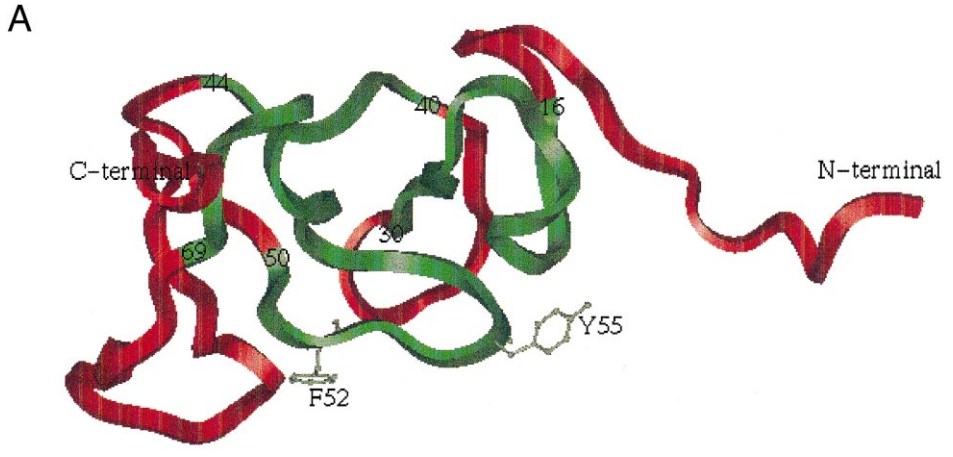

Fig. 2. A: Pig colipase structure [2] (pdb: 1pcn). In green, residues assigned in horse colipase; in red, unassigned residues in horse colipase. Amino acids are numbered according to B. B: Sequences of pig (a) and horse (b) colipases. Residues are colored according to A.

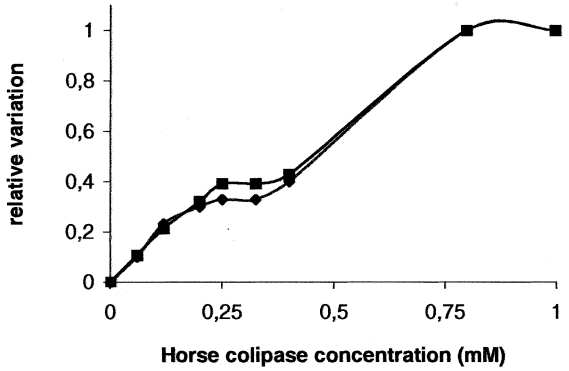

Fig. 3. Titration curve of the complex formation between NaTDC micelles and horse colipase. Relative chemical shift (■) and line-width (♦) variations of H18 resonance are represented as a function of colipase concentration. The maximum of chemical shift was 0.021 ppm and the maximum of line-width variations was 8 Hz. The experiments were done at 4 mM NaTDC concentration, pH 7.0 and 310 K.

mation, i.e. the chemical shift and the line-width. The chemical shift is sensitive to chemical changes around protons and significant variations may be used to observe a complex formation. The line-width of NaTDC protons is correlated to the correlation time of the molecule. When the NaTDC concentration is increased from 0.5 to 1 mM, no effect on these two parameters was observed. From 1 to 16 mM NaTDC concentration, the line-width of all the protons was linearly affected (from 11 to 13.5 Hz); moreover, the chemical shifts of some of the protons are found linearly shifted up to 0.05 ppm. These data are in agreement with previous data reporting that the critical micellar concentration (CMC) of NaTDC is 1 mM [10].

3.3. Proton assignment of horse colipase

Proton assignment of pig colipase has already been reported at pH 4.7 and with a temperature of 310 K [2]. TOCSY and NOESY spectra were carried out to obtain the proton assignment at pH 7.0.

NMR experiments on horse colipase were carried out at pH 4.7, but at this pH, the protein aggregation induces line broadening. The NMR experiments were, thus, carried out at pH 2.7 in the presence of 0.1 M NaCl. Sequential and long-range nOes lead to an unambiguous assignment of three parts of the protein (Fig. 2, in green), found in the structured core of the protein. The loops (Fig. 2, in red) were more difficult to assign due to proton resonance overlaps and a few long-range nOes were assigned. A similar result was reported for pig colipase [2]. In summary, pig and horse colipases have a similar folding which involves a structured core and large unstructured loops which are probably involved in interactions with lipase, bile salts micelles and lipids.

3.4. Intermolecular nOe

To investigate the interaction between the colipase and the bile salt micelles, 1D titration experiments were carried out on NaTDC micelles, increasing the colipase concentration (Fig. 3). Below the CMC of NaTDC (1 mM), the effect of colipase on the NaTDC 1D spectrum was not observed. At 4 mM NaTDC concentration, the presence of colipase affects the chemical shifts and the line-width of NaTDC resonances. The increase of the NaTDC line-width is associated to the loss of resolution in the colipase spectrum, indicating that there is a complex formation. These results indicate that colipase interacts only with NaTDC micelles and not with soluble bile salts. The stoichiometry of the complex formation is difficult to access because it is not definitively known how many NaTDC molecules are present in a micelle molecule. Considering that a micelle contains 16 NaTDC molecules, one can expect a 1:1 ratio (one colipase per micelle) at physiological conditions. When increasing the colipase/micelle ratio, a second slope of the titration curve suggests a higher stoichiometry (3:1), which is in agreement with the neutron diffraction data [19].

Intermolecular contacts of horse and pig colipases with NaTDC micelles were observed using NOESY experiments. Fig. 4 shows the NOESY experiments for horse colipase and pig colipase in presence of stoichiometric NaTDC micelles. In both experiments, colipase intra-nOes are poorly observed, whereas NaTDC intra-nOes are clearly observable. Moreover, the fast exchange between monomer and micelles of NaTDC allows us to evidence intermolecular nOes between colipase and micellar NaTDC (right and central panels). nOes involving aromatic residue protons of the colipase are easily assigned due to a good resolution on this part of the spectrum. Intense nOes between H18, H19 and H20 NaTDC protons were found with Phe52 ring protons in pig colipase and Trp52 ring protons in horse colipase. nOes with the conserved Tyr55 are stronger for pig colipase than for horse colipase,

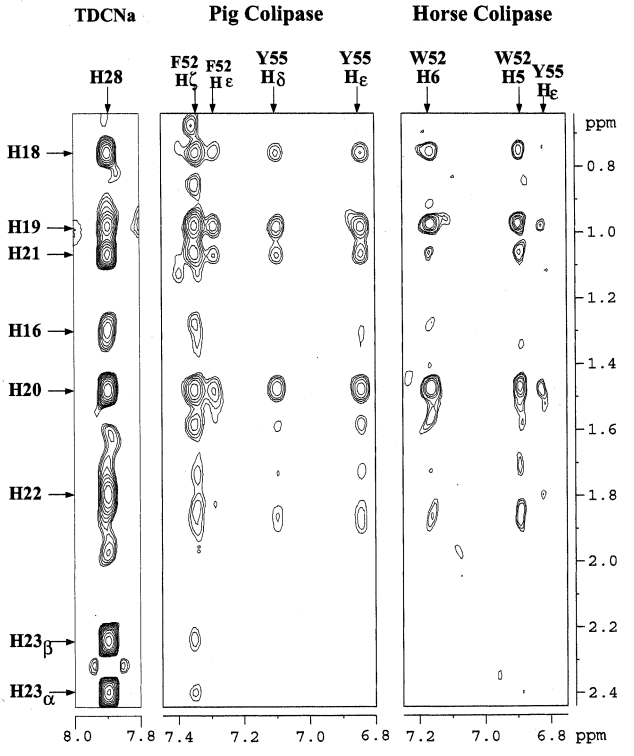

Fig. 4. NOESY experiments (200 ms) recorded on NaTDC micelles in the presence of pig and horse colipases at 16 mM NaTDC concentration, 1 mM colipase concentration, pH 7.0 and 310 K. Correlations of NaTDC protons with NaTDC H28 are indicated in the left panel, correlations of NaTDC protons with F52 and Y55 ring protons of pig colipase are indicated in the central panel and correlations of NaTDC protons with W52 and Y55 ring protons of horse colipase are indicated in the right panel.

probably corresponding to a different orientation of this loop at the interaction interfaces. These variations may be correlated to specific affinity of the two colipases to bile salts.

4. Discussion

NMR is one of the most powerful techniques to study solution complexes of proteins and the interaction with their physiological partners (protein, DNA or lipids). The lipase–colipase complexes have been extensively studied through X-ray crystallography [3,5–7]. Neutron diffraction has shown using detergent micelles that the colipase–bile salt interactions are the driving force of the lipase activation in the ternary complex. We have demonstrated using homonuclear 2D NMR that bile salt micelles are complexed to colipase in absence of lipase. Intermolecular nOes gave evidence that the two aromatic residues (Tyr55 and Trp or Phe52) of colipases are in close approach to the bile salt micelles. The role of aromatic residues at the micelle–colipase interface has been underlined by many authors [9–12]. In particular photo-CIDNP experiments [10] have shown the implication of two aromatic residues at the colipase/micelle interacting surface. This interaction has also been shown by neutron diffraction in the ternary complex [7]; in this model, the 70 to 85 loop of colipase adopts a new helical conformation when it is in complex with micelles. It is not possible to observe this kind of conformational changes from our homonuclear NMR data, however, the labelling of horse colipase would allow to study the structural changes of the protein upon interaction with bile salt micelles, increasing the sensitivity and the resolution of the method using heteronuclear NMR spectra.

The present approach demonstrates that solution studies of protein–micelle complexes are possible when the exchange between monomers and NaTDC micelles is rapid at the NMR time scale. This approach is particularly interesting to map the interacting site of colipase with various bile salts and to study the complex formation with mixed micelles, which correspond to physiological systems.

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