Solution structure and mapping of a very weak calcium-binding site of human translationally controlled tumor protein by NMR

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Abstract

Human translationally controlled tumor protein (TCTP) is a growth-related, calcium-binding protein. We determined the solution structure and backbone dynamics of human TCTP, and identified the calcium-binding site of human TCTP using multi-dimensional NMR spectroscopy. The overall structure of human TCTP has a rather rigid well-folded core and a very flexible long loop connected by a short two-strand β-sheet, which shows a conserved fold in the TCTP family. The C-terminal portions of loop Laβ8 and strand β9 and the N-terminal region of strand β8 may form a calcium-binding site in the human TCTP structure, which is largely conserved in the sequence alignment of TCTPs. The Kd value for the calcium binding is 0.022–0.025 M indicating a very weak calcium-binding site.

The translationally controlled tumor protein (TCTP) is a highly conserved protein in most eukaryotes from yeast to human with various biological functions and medical importance, which has been studied for more than 20 years [1]. TCTP is a 20–25 kDa protein abundantly and ubiquitously expressed in cell. In yeast of exponential growth phase, TCTP is one of the top 20 most abundant proteins detected by two-dimensional gel analysis [2]. The mRNA of human TCTP (hTCTP) is one of the top 50 most abundant transcripts in 84,103 unique genes of 19 normal and diseased tissue types [3]. hTCTP gene is one of the top 10 most ubiquitously expressed genes in human by examining 1753 libraries from kinds of tissues [4]. However, TCTP expression levels vary widely with the cell/tissue types and the developmental stages, and are highly regulated in response to a wide range of extracellular signals and cellular conditions [1,5]. TCTP expression is regulated not only at the translational level as indicated by its name, but also at the transcriptional level, which may provide the rapid adaptation of TCTP levels in various conditions [1,6].

The abundance and ubiquity indicate that TCTP may have important primary functions, which are still not understood. However, a large number of cellular and biochemical functions have been found since 1980s. Most of these functions can be classified into two groups. One is growth-related biological functions, including microtubule-binding, anti-apoptosis, tumor-related, proliferation, etc [7–15]. The other is immunity-related, medical importantly functions, in which TCTP was also named as histamine release factor (HRF) [16–21]. TCTP was identified as a
calcium-binding protein first in parasite Trypanosoma brucei in 1992 [22], following in human, rat and several kinds of parasites [23–28]. Functional relationship between TCTP and calcium was reported as TCTP is regulated by calcium at both the transcriptional and post-transcriptional levels [24]. More recently, TCTP was suggested to be involved in the calcium handling of eukaryotic cells [29]. The calcium-binding site was preliminarily identified by constructing several fragments of rat TCTP, which confirmed that TCTP is a novel family of calcium-binding proteins [25].

The first structure of TCTP solved by NMR spectroscopy in 2001 was from Schizosaccharomyces pombe, which revealed significant structural similarities to the human protein Ms4, a guanine nucleotide-free chaperone of Rab GTPases [30]. This finding suggested that TCTP may interact with GTPase as a guanine nucleotide exchange factor (GEF) or guanine nucleotide-free chaperone. Later research found that TCTP is involved in the elongation step of translation by interacting with the large GTPase eEF1A [12]. More recently, Hsu et al. reported that TCTP displays GEF activities and directly associates with Rheb, a small GTPase, and is an essential new component of the tuberous-sclerosis-Rheb pathway [15].

Although the structures of S. pombe TCTP (45.9% identity and 2.3% gap with hTCTP) and Plasmodium knowlesi TCTP (35.4% identity and 4.0% gap with hTCTP) have been reported [30,31], most functional studies of TCTP were based on mammalian TCTPs for which no structure determination was reported. In this research, the solution structure of hTCTP was determined by heteronuclear NMR method. The structure has a rigid well-folded core and a flexible long loop connected by a short two-strand β-sheet. The backbone dynamics of hTCTP indicates that the three parts of hTCTP have different dynamic characteristics in agreement with the structure. The calcium titration experiments and chemical shift mapping analysis determined a very weak calcium-binding site on hTCTP which is conserved in the TCTP family.

Materials and methods

Expression and purification of the human TCTP

For expression of hTCTP, the protein was fused with a His-tag at the C-terminus using the vector pET22b. Uniformly 15N-labeled and 15N/13C-double labeled hTCTP were expressed in Escherichia coli BL21(DE3) with M9 minimal medium at 37 °C. When the optical density at 600 nm of the cell culture reached about 0.8, the protein expression was induced with 0.760, 1.150, 1.500 and 2.000 s. The NOE values were calculated from the relaxation delay times for R1 and R2 measurements were set to 17.0, 33.9, 55.9, 67.8, 84.8, 101.8, 118.7, 135.7, 169.6, and 203.5 ms. The relaxation delay time for R1 measurements were set to 0.060, 0.140, 0.240, 0.360, 0.530, 0.760, 1.150, 1.500 and 2.000 s. The NOE values were calculated from the ratios of the peak intensities with and without proton saturation. R1 and R2 relaxation rates were determined using the CurveFit software (http://cpcmnet.columbia.edu/dept/gasbiochem/labs/palmer/software/curvefit.html).

Calcium titration

The samples containing 0.4 mM 15N-labeled hTCTP were dialyzed extensively for removing the residual calcium in the sample solution. The dialysis was made firstly against the sample buffer (20 mM Tris-HCl, 200 mM NaCl, pH 7.8) containing 5 mM EDTA and 5 mM EGTA, and then against the sample buffer without EDTA/EGTA. Small volumes of calcium chloride stock solution were added into the sample to an appropriate ratio. The dissociation constant Kd for Ca2+ interaction with hTCTP was obtained by fitting the observed chemical shifts to the equations:

\[ Y = Y_{\text{free}} + Y_{\text{bound}} + F_{\text{bound}} \times K_d = [Ca][P]/([Ca]+[P]) \]

where Y is the observed chemical shift; Yfree and Ybound are the chemical shifts for Ca2+-free and Ca2+-bound hTCTP; Fbound = [P]/([Ca][P] + [P]) and Fbound = [Ca][P]/([Ca]+[P]) are the fraction of Ca2+-free and Ca2+-bound

NMR spectroscopy

The NMR sample contained 1.0–2.0 mM 15N- or 15N/13C-labeled hTCTP, 200 mM NaCl, 0.01% NaN3, 0.01% 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), and 10% (v/v) D2O in 50 mM phosphate buffer (pH 7.8). All NMR experiments were performed at 308 K on a Bruker DMX 600 MHz spectrometer equipped with a cryo-probe. The 2D 1H–15N HSQC and 3D HNCA, HNCCAB, CBCA(CO)NH, HNCO, HNCACB, HBHA(CBCA)NH, HBHA(CBCA)/CO/NH, H(C)CH-CH-COSY, (H)CCH-COSY, H(C)CH-TOCSY, and (H)CCH-TOCSY experiments were adopted for the resonance assignments. NOE distance constraints were derived from 3D 1H–15N NOESY-HSQC experiment, and two 3D 1H–15N NOESY-HSQC experiments for the aliphatic region and for the aromatic region. The mixing times for 13C–13C TOCSY and 1H–1H NOESY experiments were 12 ms and 80 ms, respectively. All NMR data were processed and analyzed using software FELIX 98.0 (Accelrys Inc.). 1H chemical shifts were referenced to internal DSS. 15N and 13C chemical shifts were referenced to DSS indirectly [32].

Structure calculations

NOEs were manually assigned using software FELIX. A homology-modeling structure generated by MODELLER [33] and yeast TCTP structure (PDB ID: 1H6Q) [30] was also used to assist the NOE assignments for the initial structure calculations. According to the peak volumes, all assigned NOEs were grouped into four classes of distance restraints: 1.8–2.5 Å, 1.8–3.5 Å, 1.8–4.5 Å, and 1.8–5.5 Å. Dihedral angle restraints were obtained using the program TALOS [34]. Hydrogen bond restraints were added according to the regular secondary structure patterns. The structures were calculated using the program CNS version 1.1 [35]. A family of 100 structures was generated, and the 20 structures with lowest energies were selected. Structural analysis and statistics were obtained using programs MOLMOL [36] and PROCHECK-NMR [37]. The atomic coordinates of hTCTP and all restraints have been deposited in the Protein Data Bank under accession ID 2HR9.

Measurement and analysis of 15N relaxation parameters

Heteronuclear steady-state 1H–15N NOE, 15N-transverse relaxation rate (R2), and 15N-longitudinal relaxation rate (R1) were measured using standard pulse sequences. The steady-state 1H–15N NOE spectra were acquired with and without 1H saturation in an interleaved manner. The relaxation delay times for R2 measurements were set to 17.0, 33.9, 55.9, 67.8, 84.8, 101.8, 118.7, 135.7, 169.6, and 203.5 ms. The relaxation delay times for R1 measurements were set to 0.060, 0.140, 0.240, 0.360, 0.530, 0.760, 1.150, 1.500 and 2.000 s. The NOE values were calculated from the ratios of the peak intensities with and without proton saturation. R1 and R2 relaxation rates were determined using the CurveFit software (http://cpcmnet.columbia.edu/dept/gasbiochem/labs/palmer/software/curvefit.html).
hTCTP, respectively; \([\text{Ca}], [\text{P}], \) and \([\text{CaP}]\) represent the concentrations of free \(\text{Ca}^{2+}\), free hTCTP, and \(\text{Ca}^{2+}\)-bound hTCTP in the sample, respectively.

**Results and discussion**

**Resonance assignments**

To improve the solubility and stability of hTCTP for NMR experiments, we have optimized the pH, salt concentration, and temperature for sample solution. The optimized solution condition was 50 mM phosphate buffer (pH 7.8) with 200 mM NaCl at 308 K.

Nearly complete (more than 95%) backbone and side-chain assignments for \(^1H\), \(^{15}N\), and \(^{13}C\) resonances of hTCTP were achieved. Assignments for the \(^1H\), \(^{15}N\), and \(^{13}C\) resonances of hTCTP have been deposited in the BioMagResBank under an Accession No. 15243. The assignments of amide resonances \(\left(^1H_N\right)\) and \(\left(^{15}N\right)\) are represented in the 2D \(^1H\)-\(^{15}N\) HSQC spectrum of hTCTP (Fig. 1). Vast majority cross peaks in the 2D \(^1H\)-\(^{15}N\) HSQC spectrum are well distributed with relatively strong signals except those cross peaks for residues in the flexible loop, which indicates a stable and ordered structure of hTCTP in solution, since the well formed structure can protect the amide protons from the hydrogen exchange at high pH (pH 7.8) of the sample.

**Structure description and comparison**

The solution structures of hTCTP were generated on the basis of determined NOE distance constraints, identified hydrogen bonds, and dihedral angle restraints. A superposition of 20 hTCTP structures is shown in Fig. 2a, illustrating...
the high quality of the determined structures. PROCHECK-
NMR analysis indicates that the NMR structures of hTCTP
have a good backbone conformational regularity for 81.2%
of all residues in the most favored regions. Detailed structure
statistics and PROCHECK-NMR analysis are given in
Table 1.

The overall fold of hTCTP consists of three \( \alpha \)-helices
and eleven \( \beta \)-strands (Fig. 2b). Eleven \( \beta \)-strands form three
\( \beta \)-sheets in hTCTP: \( \beta \)-sheet A (strands \( \beta \)1, \( \beta \)2, and \( \beta \)11), \( \beta \)-sheet B (\( \beta \)3, \( \beta \)4, \( \beta \)10, \( \beta \)9, \( \beta \)8, and \( \beta \)7), and \( \beta \)-sheet C (\( \beta \)5 and
\( \beta \)6). The \( \beta \)-sheet A ties up the N- and C-terminal regions
of hTCTP and forms a “\( \beta \)-barrel” structure with the \( \beta \)-sheet
B. A short helix \( \alpha \)1 is an insertion in the “\( \beta \)-barrel”
structure. Two longer helices \( \alpha \)2 and \( \alpha \)3 run anti-parallel to each
other forming an \( \alpha \)-hairpin structure with a short loop
L_22_23, which is packed onto the “\( \beta \)-barrel” in hTCTP.
Thus, hTCTP has a well-folded core structure containing
the “\( \beta \)-barrel” and \( \alpha \)-hairpin structural regions. Residues T39-V66 form a long loop (L_55_96) linking strands \( \beta \)5 and
\( \beta \)6. This overall fold is in agreement with the homology
to TCTP structures \[30,31\]. The tertiary packing of the regular
secondary structures in the core of hTCTP is mainly
attributed to the hydrophobic interactions. There are two
main hydrophobic cores in the hTCTP structure, which
are approximately located on the two sides of the \( \beta \)-sheet
B (Fig. 2c). One hydrophobic core is formed by residues in
the \( \beta \)-sheets A, B, and C, helix \( \alpha \)1, and loops L_125_133, L_133_136, and L_147_151. The other hydrophobic core is formed by
the \( \beta \)-sheet B, helices \( \alpha \)2 and \( \alpha \)3, loops L_112_110, L_133_136,
and L_147_151. This indicates that the \( \beta \)-sheets A and B are packed tightly with three helices and loops except
the long loop L_55_96 by hydrophobic packing interactions
between them, generating a very stable core. However,
the \( \beta \)-sheet C is probably less stable in the tertiary packing
of hTCTP, since only the N- and C-terminal residues M35
and V70 of the strands \( \beta \)5 and \( \beta \)6, respectively, have long-range
hydrophobic interactions with the core.

Up to date, several structures of TCTP from various
organisms have been solved by NMR spectroscopy and X-
ray crystallography. The first TCTP structure from S. pombe
(sTCTP) was solved by NMR method, which revealed a
MSS4 fold (PDB ID: 1H6Q, 1H7Y) \[30\]. Crystal structure
of P. knowlesi TCTP (pTCTP) was solved in a structural
genomics project (PDB ID: 1TXJ) \[31\]. Crystal structure
of human TCTP was also determined and deposited (PDB
ID: 1YZ1) by Cavarelli et al. without a publication yet.

For human TCTP, the present determined NMR structures
and the crystal structures (PDB ID: 1YZ1) are very
similar. The RMSD value of C_\text{\alpha} atoms aligned by SSM
server \[38\] is about 1.0 Å between the NMR and crystal
structures (Fig. 3a). The most significant difference is obtained
for the \( \beta \)-sheet C which is not well determined in the crystal
structures. In 20 NMR structures, the strands in the \( \beta \)-sheet
C have four residues in average, while in the crystal
structure, two of four monomers have two-residue strands in
the \( \beta \)-sheet C and the other two monomers have none. Some
minor conformational differences between solution and crystal
structures were also observed for loops as expected.

The RMSD value of C_\text{\alpha} atoms for pTCTP and sTCTP
solution structures is 1.60 Å. The similar \( \beta \)-sheet C containing
4–5 residues strands is observed for sTCTP (Figs. 3b
and 4a). The RMSD value of C_\text{\alpha} atoms for hTCTP solution
structure and pTCTP crystal structure is about 1.59 Å. The
crystal structure of pTCTP has two additional 3_10-helices
formed by the residues at sequence positions 23–29 which
are not contained in the sequence of hTCTP (Figs. 3c
and 4b). The strands of the \( \beta \)-sheet C in pTCTP is also shorter
than those in hTCTP solution structures.

Because of the highly conserved sequences of TCTPs
(Fig. 4), the overall structures of TCTPs are very similar,
implying the functional conservation of TCTPs in various
organisms.

### Backbone dynamics

The amide \(^1\text{H}–15\text{N}\) NOE for 145 out of the 167 non-proline residues

<table>
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<th>Parameter</th>
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</tr>
<tr>
<td>Hydrogen bonds</td>
<td>58</td>
</tr>
</tbody>
</table>

| Number of dihedral angle restraints | |
| \( \phi \) angle | 111 |
| \( \psi \) angle | 111 |

| CNS energies (kcal/mol) | |
| \( E_{\text{total}} \) | 119.20 ± 2.44 |
| \( E_{\text{Bond}} \) | 3.97 ± 0.16 |
| \( E_{\text{Angle}} \) | 65.40 ± 0.90 |
| \( E_{\text{Improp}} \) | 4.60 ± 0.26 |
| \( E_{\text{Vdw}} \) | 36.57 ± 1.43 |
| \( E_{\text{NOE}} \) | 8.50 ± 0.54 |
| \( E_{\text{Cdyn}} \) | 0.15 ± 0.03 |

| Number of restraint violations (maximum violation) | |
| Distance restraint violations >0.2 Å | 0 (0.107 Å) |
| Dihedral angle restraints >5° | 0 |

| PROCHECK-NMR Ramachandran map analysis (%) | |
| Most favored regions | 81.2 |
| Additional allowed regions | 16.7 |
| Generously allowed regions | 1.1 |
| Disallowed regions | 1.1 |

| RMS deviations (Å) (residue 1–38, 67–172) | |
| Backbone heavy atoms | 0.33 ± 0.06 |
| All heavy atoms | 0.77 ± 0.04 |

| RMS deviations (Å) (secondary structure residues) | |
| Backbone heavy atoms | 0.30 ± 0.06 |
| All heavy atoms | 0.75 ± 0.04 |

Fig. 2. Solution structure of human TCTP. (a) Stereo view of 20 lowest energy structures. (b) Ribbon representation of hTCTP structure. (c) Two hydrophobic cores on the two sides of sheet (b) (green). The heavy-atom side-chains of all hydrophobic residues in hTCTP are shown as red ball-stick model. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 3. Comparison of TCTP structures. (a) Crystal (red) and NMR (blue) structures of hTCTP. (b) hTCTP (blue) and Schizosaccharomyces pombe TCTP (yellow) NMR structures. (c) hTCTP NMR structure (blue) and Plasmodium knowlesi TCTP crystal structure (magenta). The additional $3_{10}$-helices of $P$. knowlesi TCTP are in green. The right figure is the top view of the left one. The long flexible loops of all structures are not shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
Fig. 4. Sequence alignments of TCTPs. The sequences were aligned using SSM [38] or CLUSTAL W [43], and plotted using Jalview [44]. Residues are shaded according the conservations in the alignments. (a) Structure-based sequence alignment of hTCTP and sTCTP using SSM. (b) Structure-based sequence alignment of hTCTP and pTCTP using SSM. Residues forming the additional 310-helices in pTCTP are boxed. (c) Sequence alignment of TCTPs from various organisms. (d) Sequence alignment of hTCTP and non-calcium-binding TCTPs. Residues of the calcium-binding site are indicated by dashed boxes in figures (c) and (d).
of hTCTP are shown in Fig. 5. Residues generating overlapped cross peaks and residues G49-A54 and G59-T65 having very low signal intensities in the spectra were neglected in the analysis. \(^{1}H-^{15}N\) NOE values spread in a narrow range with an average value of 0.79 ± 0.08 for residues of the core region. However, residues in the loop of residues T39-V66 have \(^{1}H-^{15}N\) NOE values <0.5 with an average value of −0.11 ± 0.49. \(^{1}H-^{15}N\) NOE values for residues in the strands \(\beta 5\) and \(\beta 6\) decreased slightly from the N- to the C-terminus and significantly from the C- to the N-terminus, respectively. An average \(^{1}H-^{15}N\) NOE value of 0.67 ± 0.12 for residues in the strands \(\beta 5\) and \(\beta 6\) from the \(\beta\)-sheet C is obtained, which is lower than the average value for the core region. This clearly indicates that hTCTP has a rather rigid well-folded core and a very flexible loop \(L_{\beta 5\beta 6}\), and the rigid to flexible transitions occur in the \(\beta\)-sheet C between these two structural regions. The analysis of \(R_1\) and \(R_2\) values also indicates the same conclusion. Therefore, the dynamic structure of hTCTP is divided to two structural regions by the \(\beta\)-sheet C: a stable rigid core and a highly flexible loop.

**Determination of calcium-binding site**

Binding of \(Ca^{2+}\) with hTCTP was detected by the \(Ca^{2+}\) titration which was monitored using 2D \(^{1}H-^{15}N\) HSQC spectra. Cross peaks in the 2D HSQC spectra shifted upon the binding of hTCTP with \(Ca^{2+}\). Cross peaks for residues Y132 and Y151 at the C-termini of loop \(L_{a3\beta 8}\) and strand \(\beta 9\), respectively, shifted largely in the 2D HSQC spectra during the titration (Figs. 6a and 7a). These two residues are spatially close to each other in the hTCTP structure. In general, calcium ion is coordinated by oxygen atoms. However, the shifts of cross peaks with increasing of the \(Ca^{2+}\) concentration in the present titration experiments are provided by the amide proton (\(^1HN\)) and nitrogen (\(^{15}N\)) resonances in the 2D HSQC spectra. In this case, the changes in the chemical shifts of amide group resonances (\(\delta ^{1}HN\) and \(\delta ^{15}N\)) are the consequences of the changes in hydrogen bonds, charges and/or conformations of peripheral residues caused by the calcium binding. Y132 showed downfield shifts of amide group resonances having a slope (\(\delta ^{15}N/\delta ^{1}HN\)) of 12.8, which suggested a shortening
hydrogen bond between corresponding residues [39]. In three of four conformers of hTCTP crystal structures, $^1$HN of Y132 forms a hydrogen bond with backbone oxygen of F129. Thus, the chemical shift changes of amide group resonances for Y132 may be due to strengthening the hydrogen bond to backbone oxygen of F129 as a result of conformational changes induced by binding of Ca$^{2+}$. Thus, Y132 is not directly involved in the Ca$^{2+}$ binding. For Y151, the much larger $^1$HN chemical shift changes than the $^{15}$N chemical shift changes (the slope is 1.7) were observed. The $^1$HN of Y151 is hydrogen bonded to side chain O$_d$ of D150 in some conformers of hTCTP solution (four of 20) and crystal (three of four) structures. It is probably that the side chain O$_d$ (O$_{d1}$ and O$_{d2}$) of D150 is directly involved in the Ca$^{2+}$ association, causing the environment charge changes for the amide proton of Y151. The side chain $^1$H$_{d1}$ and $^1$H$_{d2}$ ($^1$H$_{e1}$ and $^1$H$_{e2}$) chemical shifts of N131 and Q133, respectively, showed also relatively large changes upon binding of Ca$^{2+}$ (Figs. 6a and 7a). This can be attributed for the Ca$^{2+}$ association with O$_{d1}$ and O$_{d2}$ of N131 and Q133, respectively. Residues N131, Q133, and D150 are located in a structural region formed by the C-terminal portions of loop L$_{38}$ and strand β9 and the N-terminal region of strand β8 (Fig. 6b). Thus, side chain O$_{d1}$, O$_{e1}$, and O$_{d}$ of N131, Q133, and D150, respectively, may form a distorted coordinating site for the Ca$^{2+}$ association, in which the nearby backbone oxygen atoms of N131 and L149 could be also involved according to hTCTP structure (Fig. 6b).

The previous study to identify the calcium-binding site using rat TCTP fragments suggested that calcium-binding sites were in the region of residues 81–112 [25]. However, the TCTP fragment was constructed by removing residues 113–172 which are involved in the formation of helix α3, strands β8, β9, β10, and β11, and loops between them. Deletion of these residues destroys the β-sheets A and B and the α2–α3 helix-hairpin, making an unfolded TCTP. Thus, the early determined calcium-binding region is not for the natively folded TCTP.

In the titration experiment, the significant changes in chemical shifts of proton and nitrogen resonances for hTCTP were observed only at the calcium/protein ratio higher than 10. Based on the assumption of TCTP:Ca$^{2+}$ = 1:1 binding mode, a dissociation constant $K_d$ value of 0.022–0.025 M was obtained by fitting the chemical shift changes of amide group resonances for Y132 (Fig. 7b). This implies that the calcium-binding affinity is very low. On the other hand, all in vitro calcium-binding experiments previously reported were performed by $^{45}$Ca overlay for a high sensitivity [22–28]. Most of these reports did not give a quantitative affinity of calcium to TCTP, except T. brucei TCTP showed a high affinity with a $K_d$ value of 8 μM [22]. The hTCTP was identified as a calcium-binding protein by $^{45}$Ca overlay in 1997, and studies of the binding site and binding affinity were undertaken [23]. However, no such report is seen until now. These literature reports might just pick up the weak calcium-binding site of TCTP as identified in

![Calcium-binding site of human TCTP.](image_url)
the present study except the non-native fragment of rat TCTP, while the affinity was not reported perhaps because of the weakness.

The biological significance for the very weak calcium-binding affinity determined in vitro is not clear. Members of TCTP family probably have a conserved primary function, since the sequences of TCTPs from various organisms are much conserved. However, TCTP also has some organism-specific functions and regulations, such as the histamine release activity and Plk phosphorylation regulation [40] which should only conserved in mammals. According to the sequence alignment of TCTPs, the residues in the calcium-binding site deduced in this study are highly conserved (Fig. 4c). Most TCTP homologues have Glx or Asx residues at the positions corresponding to N131, Q132, and D150 of hTCTP. This suggests the conservation of the calcium-binding activity in most of TCTP-containing organisms. However, four TCTPs from ixodid ticks [41] and shrimp [42] having the conserved calcium-binding residues (Fig. 4d) were reported without calcium-binding activities in vitro. One possible explanation is that the conformation of the structural region for calcium-binding may be different in these four TCTPs, causing the loss of the calcium-binding activity.

Fig. 7. Titration of calcium ion monitored by measuring $^1$H and $^{15}$N chemical shift changes in 2D $^1$H- $^{15}$N HSQC spectra. (a) Differences in chemical shifts of resonances for hTCTP in the absence of calcium from those in the presence of calcium at calcium to protein ratio of 80:1. (b) $^1$H and $^{15}$N Chemical shift changes with increasing of Ca$^{2+}$ concentrations. Curves and $K_d$ values are the curve-fitting results.
Thus, a postulation can be made that the calcium-binding activities of TCTPs may be regulated by structural variations in the calcium-binding region, and there may be the post-translational modifications of TCTP to enhance the calcium-binding affinity in vivo.

Acknowledgments

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