Structural investigation of the specific binding of a herpesviral protein to its cellular target

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Abstract:

Herpesvirus saimiri which is able to induce leukemia and lymphoma in New World primates codes for a tyrosine kinase interacting protein (Tip). A 38 amino acids containing Tip fragment turned out to be necessary and sufficient to form a stable complex with the T−cell specific kinase Lck in vitro. This binding region contains a segment that shows a significant sequence similarity to the carboxy−terminal region of several kinases of the Src family (CSKH region) and a proline−rich segment that binds to the SH3 domain of Lck (SH3B) [1]. Circular dichroism (CD) spectroscopy proved that the polyproline helix of Tip is already formed prior to SH3 binding and is conformationally stable, while a helical secondary structure of the CSKH element is only formed after addition of trifluoroethanol (TFE). Binding of Tip to the SH3 domain of Lck was studied by NMR titration experiments using ^{15}N−labeled Tip that was expressed as an ubiquitin−fused protein thus allowing efficient isotopic labelling. Both chemical shift data and hydrogen exchange experiments reveal that the residues carboxy-terminal to the polyproline helix are also involved in SH3 binding. Further, changes in the NOE pattern indicate novel intramolecular contacts in Tip after binding to the LckSH3 domain. Fluorescence spectroscopy shows that Tip binds to the SH3 domains of several Src−kinases (Lck, Hck, Lyn, Src, Fyn, Yes) exhibiting the highest affinities for Lyn, Hck, and Lck.

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          140         150         160         170         180         190
           |           |           |           |           |           |
MiniTip  M VRHKSEDLQS FLEKYPPDFR KPKRDLSATW DPGMPTPPLP PRPANLGERQ A
Tip20W                                   ATW DPGMPTPPLP PRPANLG
Tip13                                        GMPTPPLP PRPAN
                    DYLRS VLEDF                  XPPLP XR
                   CSKH /αI−Helix          SH3B/PPII−Helix (class II)
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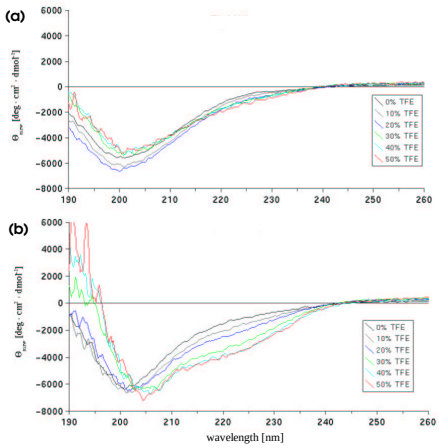
Sequence alignment of the three Tip−peptides investigated − Tip(140−191) referred to as MiniTip, Tip(168−187) referred to as Tip20W and Tip(173−185) referred to as Tip13. The numbering scheme given is derived from full−length Tip. The consensus of the class II polyproline helix type II and the CSKH−region is highlighted in bold. The amino acids are named by the one−letter code.

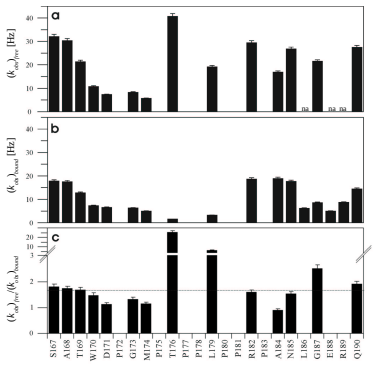
Methods and Results:

CD−spectroscopy

Under the experimental conditions investigated Tip20W shows a pronounced minimum at 203 nm, which is typical of a left−handed polyproline helix of *trans*-imide groups with three residues per one turn (polyproline II, or PPII) [2]. Addition of TFE does not change the spectroscopic properties of Tip20W substantially. Thus the SH3B region, matching the consensus of a class II PPII, forms a stable proline helix even prior to SH3 binding. The same properties are exhibited by MiniTip in the absence of TFE, whereas the stepwise addition of up to 40% of TFE decreases the negative molare elipticity at 222 nm indicating the presence of an α−helical secondary structure. According to this results MiniTip exhibits an intrinsically marginally stable α−helical structure in the CSKH region that can be stabilized by TFE. This finding together with the results of secondary structure prediction suggest that the CSKH−motiv may interact in a helical conformation with the kinase domain of Lck [3].



Far−UV CD spectra of Tip20W (a) and MiniTip (b) upon addition of 10% to 50% TFE collected at pH 6.4 with a starting concentration of 5 µM. CD spectra measured at 0%, 10%, 20%, 30%, 40% and 50% TFE concentration are shown in black, dark gray, light gray, blue, green and red lines, respectively.

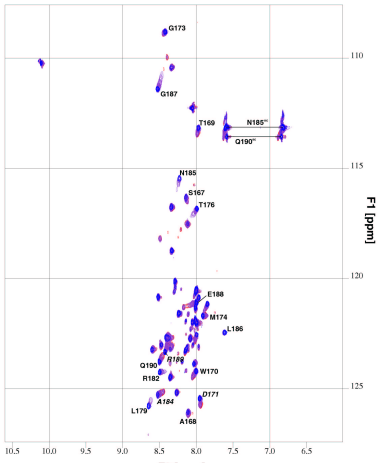


Histogram of the rates of the fast exchanging amides derived from NewMEXICO−FHSQC experiments of the binding region of free MiniTip (a) and MiniTip after addition of a 1.2−molar excess of LckSH3 (b). Error bars represent a standard error of 3%.
(c) Ratio of the rates of the fast exchanging amides of free MiniTip to MiniTip bound to LckSH3. The mean value (with exclusion of T176 and L179) is shown as dashed line. na = not assigned

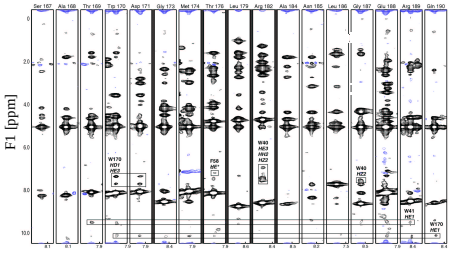
NMR−spectroscopy

Information about those residues of MiniTip involved in LckSH3 binding was obtained from NMR titration experiments. Changes of the chemical shifts in the ^{1}H,^{15}N−HSQC spectrum are also observed for residues outside the PPII−helix, predominantly those carboxy−terminal to the polyproline helix. Exchange of the amide protons of free [$(k_{obs})_{free}$] and LckSH3 bound MiniTip [$(k_{obs})_{bound}$] with water was measured using the ^{15}N-WEX II−FHSQC pulse sequence [4]. The ratio of $(k_{obs})_{free}/(k_{obs})_{bound}$ indicates a significant decrease of the exchange rates for the amide protons of residue T176 and L179 that are part of the proline helix by 25-fold and 6-fold, respectively. In addition, a significant decrease of the exchange rates is observed for G187 and Q190 that are located carboxy-terminal to the polyproline−helix.

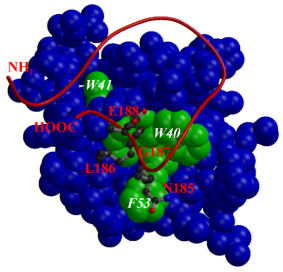
NOE cross peaks in the ^{1}H,^{15}N−NOESY−HSQC spectrum reveal the formation of a lariat−like strukture of MiniTip upon LckSH3 binding. Thereby the lariat is formed by interactions of the residues 169-171 amino−terminal of the proline helix with the carboxy−terminal end (residues 188−190) of the Tip fragment. Additional NOE cross peaks obtained from ω_2−^{15}N−*filtered*−NOSEY spectra reveal intermolecular contacts between the residues 185−188 of MiniTip and residues W40, W41 and F53 of LckSH3 that form a hydrophobic pocket on the surface of the SH3 domain.



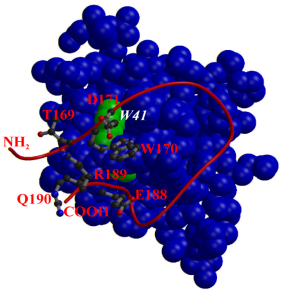
NMR titration experiment showing the changes in the ^{1}H,^{15}N−HSQC spectrum of MiniTip upon gradual addition of LckSH3. The first step of the titration represents a MiniTip to LckSH3 ratio of 0.3 (blue). The resonances of the final step of titration, representing a 5−fold molar excess of MiniTip are shown in red. Resonances are labelled with corresponding sequence positions. Site−chain ('sc') NH_2−resonances for N185 and Q190 are connected.



Strip plot of the ^{1}H,^{15}N−NOESY−HSQC spectrum of the binding region of MiniTip with exception of P172, P175, P177−P178, P180−P181 and P183 (black spacing). Strips are representing a width of 2 ppm in F3 in the individual ^{15}N planes of the three-dimensional data set. Assigned cross peaks corresponding to protons of W170 of MiniTip, W40, W41 and F58 of LckSH3 and different amide protons of MiniTip are highlighted (small boxes). Positive signals are shown in black, negative signals in blue.



MiniTip (backbone representation shown in red) is binding to LckSH3 (space−filled representation shown in blue) via interations to hydrophobic cluster on LckSH3 surface. Residues N185, L186, G187 and E188 of MiniTip (ball−and−stick representation) are interacting with W40, W41 and F53 of LckSH3 (shown in green) .



Lariat−like structure of MiniTip (backbone representation in red) binding to LckSH3 (space−filled representation in blue). Residues involved in the formation of the lariat-like structure of MiniTip are shown in ball−and−stick representation. Residue W41 of LckSH3 exhibiting NOE cross−peaks to T169, W170 and E188, R189 of MiniTip is shown in green.

Fluorescence spectroscopy

Generally, a stronger binding to Src family SH3 domains was observed for Tip20W170L compared to Tip13. The overall differences in binding affinity are less than one order of magnitude. These observations suggest that the additional residues in Tip20W170L dot not represent major determinants of binding affinity, but may contribute to its specificity.

	K_d (µM)	
GST−SH3	Tip20 W170L	Tip13
Lyn	1.88 ± 0.22	9.58 ± 0.95
Hck	3.15 ± 0.31	12.03 ± 0.37
Lck	8.70 ± 0.77	30.13 ± 1.06
Src	20.51 ± 3.10	44.31 ± 1.26
Fyn	50.58 ± 6.62	63.99 ± 5.46
Yes	53.69 ± 5.17	71.59 ± 3.77

Affinity measurements of proline−rich Tip Peptides for GST−tagged SH3 domains. The value were determined from fluorenscence titration experiments. The W170L mutation of Tip20 was used in order to allow an undisturbed detection of the SH3 fluorescence.

References:

[1] Jung, J.U. *et al.* (1995) *J. Biol. Chem.* **270**: 20660−20667
[2] Viguera, A.R. *et al.* (1994) *Biochemistry* **33**: 10925−10933
[3] Sefton, B.M. *et al.* (2000) *Virology* **276**: 339−348
[4] Mori, S. *et al.* (1997) *J. Mag. Res.* **108B**: 94−98