NMR Structure of Phospho-Tyrosine Signaling Complexes

Carol Beth Post, Beverly S. Gaul, Elan Zohar Eisenmesser, Michael L. Schneider

Department of Medicinal Chemistry, Purdue University, West Lafayette IN 47907-1333

▼

Abstract: A structural basis for activation and substrate specificity of src tyrosine kinases, and regulation of protein–protein association by tyrosine phosphorylation is described. Lyn, a src-family tyrosine kinase, recognizes and phosphorylates the immunoreceptor tyrosine-based activation motif, ITAM, a critical component in transmembrane signal transduction in hemopoietic cells. The structure of an ITAM peptide substrate bound to an active form of Lyn tyrosine kinase was determined by high-resolution NMR, and a model of the complex was generated using the crystallographic structure of Lck, a closely related Src-family kinase. The results provide a rationale for the conserved ITAM residues and specificity of Lyn, and suggest that substrate plays a role in stabilizing the kinase conformation optimal for catalysis. It is our hope that the Lck-ITAM peptide model complex will be useful in aiding structure-based drug design efforts that target substrate binding determinants in the design. Concerning the regulation of protein–protein association, we report on a complex between erythrocyte band 3 and two glycolytic enzymes, aldolase and glyceraldehyde-3-phosphate dehydrogenase. The formation of this complex is negatively regulated by tyrosine phosphorylation of band 3 by p72syk tyrosine kinase. In red blood cells, this association results in a decrease in glycolysis due to competitive inhibition of the glycolytic enzymes. The structure of band 3 recognized by the glycolytic enzymes was determined by solution NMR, and found to be a loop structure with tyrosine centrally positioned and excluded from intermolecular contact. This *p*hosphorylation *s*ensitive *i*nteraction, or PSI, loop may be the basis of a general mechanism for negative regulation through tyrosine phosphorylation. Med Res Rev, 19, No. 4, 295–305, 1999.

Key words: B-cell signaling; tyrosine phosphorylation; NMR structure; band 3; exchange-transferred NOE

Correspondence to: Carol Post, Dept. of Medicinal Chemistry-Molecular Pharmacology, 1333 RH PH, West Lafayette, IN 47907; e-mail: cpb@stella.bio.purdue.edu

 CCC 0198-6325/99/040295-11

1. INTRODUCTION

Tyrosine phosphorylation of proteins is a fundamental feature to the transduction of extracellular signals into the cell to govern cell growth or death, proliferation, and differentiation. Careful control of protein–protein associations and enzymatic activity through tyrosine phosphorylation at various steps along a signaling pathway is essential to ensure proper cellular function. Phosphorylation of proteins at specific sites can modulate, either positively or negatively, protein–protein associations or subtle changes in the level of enzymatic activity throughout the signaling cascade. Incorrect transduction of signals has been associated with a large number of human diseases, including cancer, artheroschlorosis,[1] and immune–related disease.[2] As such, protein tyrosine kinases, the proteins responsible for phosphorylation in signaling, are of intense interest as targets for drug therapy against these diseases. Knowledge of the structure of these proteins and their complexes is important to drug design efforts by providing a basis for a detailed understanding of the function and specificity of these proteins in the cell, and the underlying physical features controlling function through tyrosine phosphorylation. In this report, we describe two structures of signaling complexes: a src-family tyrosine kinase complex with a natural substrate, and a downstream complex between erythrocyte band 3 and aldolase for which tyrosine phosphorylation negatively mediates complex formation.

2. LYN-SUBSTRATE COMPLEX

An initial step in signaling, following stimulation of the B-cell receptor (BCR), is activation of the src-family tyrosine kinase, Lyn (Fig. 1), to phosphorylate the immunoreceptor tyrosine-based activation motif (ITAM) of the co-receptor Ig-α. The ITAM region is a sequence motif (D/EX7D/EX2YX2L/IX7YX2L/I) defined from only 6 of 26 residues.[3–5] Lyn preferentially phosphorylates the first tyr residue of the ITAM.[6,7] Results of mutational studies indicate that competent signaling through ITAM requires phosphorylation of both tyr residues.[8,9] Nonetheless, the kinase responsible for *in vivo* phosphorylation of the second tyr residue of Ig-α ITAM has not been identified, although blk may serve this role.[10] The doubly phosphorylated ITAM region serves as a docking site for p72syk, and the signal cascade propagates upon phosphorylation and activation of syk to multiple pathways.

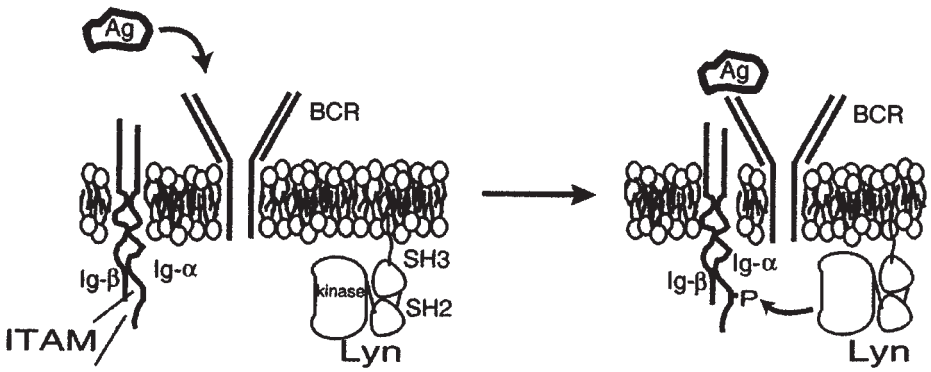

Figure 1. Initial events in B-cell signaling through the B-cell receptor (BCR). Antigen-binding immunoglobulin molecules (Ag) bind BCR to activate Lyn, a src-family tyrosine kinase. Lyn phosphorylates the immunoreceptor tyrosine-based activation motif (ITAM) region of the Ig-α co-receptor. Phosphorylated Ig-α serves as a binding site for other signaling proteins, such as p72syk, to continue the signaling cascade.

Enzymatic activity of src-family kinases is controlled subtly by its own state of phosphorylation and by the competition between inter- and intramolecular binding of the SH2 and SH3 domains. Activation associated with SH2 and SH3 domain interactions is an increase of up to 10 fold in activity, not several orders of magnitude.[11] Src-family kinases have two phosphorylation sites; the inactive form of src kinases is phosphorylated on tyr 527 (c-Src numbering) in the c-terminal tail, while phosphorylation of tyr 416 activates the enzyme. The crystallographic structures of src-family kinases in the inactive form (i.e., singly phosphorylated at tyr 527)[12–14] revealed significant and unanticipated features important to activation of these kinases. While the negative regulatory residue, ptyr 527, on the C-terminal tail binds intramolecularly in the ptyr site of the SH2 domain, as anticipated, this interaction was not found to block access to the active site. A second unexpected finding is the interaction of the linker region between the SH2 and the kinase domains with the SH3 binding site. These domain–domain contacts were suggested to be significant in determining the relative orientation of two lobes of the kinase domain.[12,13]

A. Structure Determination of an ITAM Peptide Bound to Lyn Tyrosine Kinase

The structure of a 12-residue peptide derived from the Ig-αITAM (Ac-DENLYEGLNLDD-NH2) was determined by NMR in combination with restrained molecular dynamics (Gaul, Geahlen, Harrison, Post, to be submitted). Exchange-transferred nuclear Overhauser enhancement (et-NOESY) was used to define distance restraints of ITAM peptide bound to the kinase domain of Lyn (kLyn). Bacterial expression of a glutathione-S-transferase (GST)—kLyn fusion clone yielded sufficient protein (3–4 mg/L culture) for NMR studies. The ITAM peptide (ITP) was chemically synthesized.

The structure of ITP bound in the active site of Lyn was determined from the interproton distances for ligand–ligand proton pairs estimated from the et-NOESY cross-peak intensities. Such use of the et-NOESY experiment requires a fast exchange of the small-molecule peptide between free and bound states[15–17] corresponding typically to a 10 μM dissociation constant. The dissociation constant estimated from the Michaelis Menton constant *KM* is 13 μM (B. Gaul and C. Post, unpublished results), which is appropriate for et-NOESY analysis.

In the et-NOESY approach, distance information of a small molecule ligand bound to a large molecule is obtained as a result of the exchange process, and the exploitation of the dependence of the NOE cross-relaxation on molecular size.[18] The sample is prepared with large molar excess of ligand. Rapid exchange of the peptide between free and bound states results in averaging chemical shift, linewidth, and NOE interaction according to the molar ratio of free and bound peptide. In the free state, the peptide does not give rise to an appreciable NOE intensity because of the fast tumbling rate of the small molecule. When the peptide binds the protein, and thus adopts the slow tumbling rate of the macromolecule, cross-relaxation is an efficient process and NOE crosspeak intensity, reflecting the bound-state structure of the peptide, is observed. The NOE signal is measured from the averaged ligand resonance which is dominated by the free peptide linewidth and chemical shift values. Interproton distances separating ligand protons are estimated from these intensities in a fashion similar to direct NOE intensities. NOE interactions involving either one or two protein protons are not generally measurable in an etNOE experiment due to the broad linewidths of the protein.

The bound ITAM peptide structure was defined from 98 NOE interactions. Importantly, all residues manifested interactions with a residue 2 or more residues apart in sequence. The occurrence of these long-range interactions significantly increases the accuracy of the etNOE structure (Eisenmesser, Zabell, Post, submitted for publication). The convergence of the structures determined by restrained molecular dynamics using both the X-PLOR and CHARMM programs is 2.0 Å overall for 20 conformations selected from consistency with the NMR data and low internal energy. The local structure of both termini is significantly more ordered; after superposition of the main-chain atoms N, Ca, C of either residues 1 to 7 or 8 to 12, the rms value for these main-chain atoms is 0.69

Åand 0.71 Å, respectively. All structures satisfy the NMR distance restraints; the average number per structure of interproton distances exceeding the restraint limits by more than 0.1 Å is 0.55, in excellent agreement with the NMR data.

The structure of bound ITAM substrate to kLyn, shown in Figure 2, is an irregular helical structure for the main chain. The side chains are directed radially outward from the main chain, although to a degree less than found in a regular α-helix. The etNOESY data reveal few main-chain–main-chain hydrogen bonds.

B. Model for the ITAM Substrate-kLck Complex

The ITAM peptide was docked onto the surface of the kinase domain using the known crystallographic structure of an activated src-family kinase domain, kLck.[19] Two other kinase structures were used as templates to initially dock the ITAM structure on the surface of kLck. Crystallographic coordinates are known for peptide inhibitor complexes of cyclic-AMP dependent protein kinase (cAPK)[20] and a peptide substrate analogue bound to insulin receptor kinase (IRK).[21] Individual NMR structures were initially positioned by superposition of the main-chain atoms of the phosphoryl-acceptor residue, and manually manipulated using the QUANTA graphics program to remove strong steric clashes. Each of the 20 models was subjected to molecular dynamics including the NMR distance restraints. Inclusion of the NMR distance restraints is critical for docking a flexible peptide the size of the ITAM peptide.

The cAPK peptide inhibitor binds in the cleft between the N-terminal and C-terminal lobes of the kinase domain. On the other hand, the IRK peptide contacts primarily the surface of the C-terminal lobe, with only the acceptor tyrosine contacting residues in the active site at the interface of the lobes. Of the two binding modes, the docking strongly favors the binding mode which occurs in the cleft at the interface between the two lobes. The factors that support the cleft binding mode are (1) better agreement with the NMR distance restraints, (2) significantly better convergence after restrained molecular dynamics of the ITAM peptide to a consistent orientation of binding, (3) good steric complementarity, (4) extensive intermolecular hydrogen bonding, and (5) orientation of the acceptor tyrosine in a catalytically competent conformation. The distinction in structural conver-

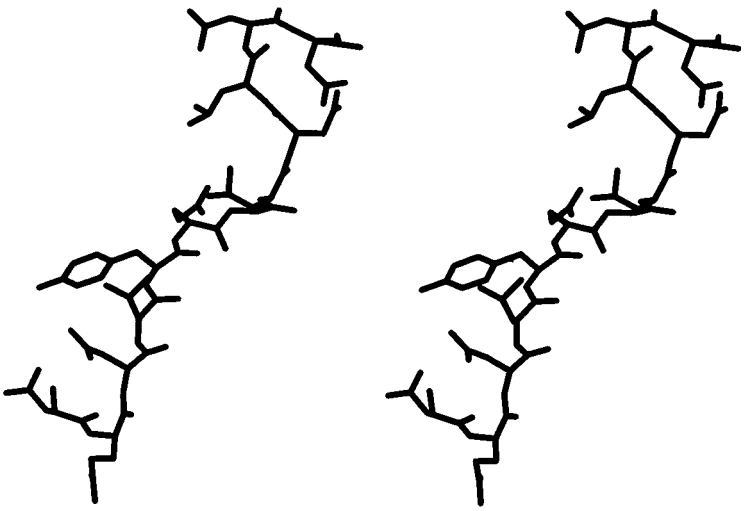

Figure 2. Stereoview of the 12-residue peptide substrate (DENLYEGLNLDD) derived from the Ig-α ITAM. The structure is that of the substrate when bound to activated kLck as determined by the etNOESY method.

gence between the two potential binding modes strongly supports binding in the cleft between the two subdomains, similar to cAPK inhibitor binding. The ITAM peptides based on the cAPK template have a lower NOE restraint energy (average value is 0.6 kcal/mol), while those based on the IRK template have an average value equal to 4.0 kcal/mol. In addition, the ITAM peptides based on the cAPK template are displaced several angstroms on the kLck surface, while those based on the IRK template remain within 1 to 2 Å for the main chain. Given these factors, hereafter we consider only the cleft model of the Lyn-ITAM substrate complex.

Restrained molecular dynamics and energy minimization optimize the binding interactions with respect to the energy. In the resulting model complex, shown in Figure 3, ITAM is well positioned for catalysis. ITAM fits between the N- and C-lobes of the kinase domain, and the acceptor tyrosine interacts well with active site residues. Figure 3 includes the ITAM substrate docked to the apo form of activated kLck, plus the superposition of ATP (not included in the docking procedure) obtained by overlaying coordinates from the cAPK-ATP complex structure.[20] The acceptor tyrosine makes strong hydrogen bonding interactions with the catalytic base residue Asp 364 (386 in scr numbering). Moreover, the orientation of the tyrosine hydroxyl group with the γ-phosphoryl group of ATP is nearly ideal for nucleophilic attack. It should be emphasized that this nearly ideal ITAM substrate—ATP orientation is a result of ITAM substrate-enzyme interactions governing the docking of the substrate. Thus, the activated kinase structure and the bound structure of the ITAM peptide appear sufficient for a catalytically reasonable binding of ITAM, without dependence on interactions with ATP.

The first tyrosine residue of the Ig-α ITAM is the major site phosphorylated by Lyn upon activation of the signaling pathway in B-cells, while the second tyrosine is less extensively phosphorylated.[6,7] The sequence for both tyrosine residues of Ig-α ITAM is the hallmark YXXL, but the two

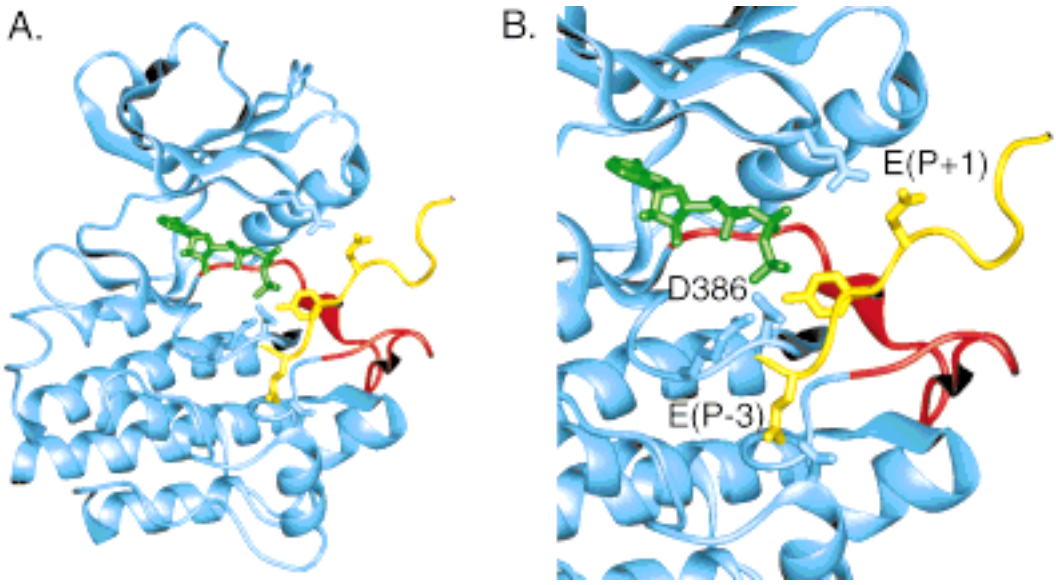

Figure 3. Model of the ITAM substrate-kLck complex. The ITAM peptide (yellow), with the conformation determined by NMR, was docked to the crystallographic structure of activated kinase domain (blue) of kLck[19] based on the template structure of the cAPK-peptide inhibitor complex[20] (see text for details). The conformation of the activation loop (red) is well defined in this phosphorylated, active form of kLck. ATP (green) coordinates from a binary complex of cAPK were overlaid on the kLck-ITAM model to illustrate the good alignment of the ITAM substrate for catalysis. ATP was not present during the docking calculation. (A) The full kinase domain shown with the N-lobe on top and the C-lobe on bottom. (B) Expanded view of the active site region. The catalytic base is D386 (c-src numbering). E(P−3) and E(P+1) of ITAM, conserved residues of Ig-α, contact the C- or N-lobes, respectively.

differ at the P−3 and P+2 positions; these positions around the two tyrosine residues are either Glu or Cys, and either Gly or Asp, respectively. The ITAM-kLck model complex offers an explanation for the distinction in activity by Lyn between the first and second tyrosine sites. The carboxylate group of Glu (P−3) mψakes several hydrogen bonds with the kinase which could not be reproduced by Cys. Moreover, the main-chain dihedral angle values of Gly (P+2) correspond to the region of ϕ, ψ space uniquely favored by glycine. To the extent that these two features are important for binding, they provide the basis for preferential phosphorylation of the first ITAM tyrosine.

C. Recognition and Activation by ITAM Substrate

ITAM binding in the cleft between the two lobes of the kinase catalytic domain implies a role of stabilization of the active form of the enzyme. Based on crystallographic results from numerous free and ligated forms of tyrosine kinases, activation appears to depend in part on the orientation of the N- and C-lobes.[22] (The N- and C-lobes of the kinase domain are the top and bottom halves of kLck as viewed in Fig. 3.) Specifically, the degree of openness between the lobes is thought to influence active site accessibility and positioning of catalytic residues. While the exact consequence of the relative orientation of the two lobes is not fully understood, the differences observed crystallographically strongly implicate the coupling of lobe–lobe displacement with enzymatic activity. Bound ITAM modeled here displays multiple contacts with both lobes of the catalytic domain. These contacts involve extensive interaction with the activation segment of the C-lobe, and the nucleotide-binding loop, the β3-αC loop, and the N-terminus of αC in the N-lobe. Such bridging of the lobes would serve to stabilize the orientation appropriate for optimal catalysis.

Phosphorylation of tyr 394 (416 in src numbering) in the activation segment positively regulates kinase activity. The model for substrate binding in the cleft provides a rational for the influence of the phosphorylation state on substrate binding, while a mode of binding primarily involving the C-lobe would present a less direct dependence between phosphorylation of tyr 394 and substrate binding. As modeled, ITAM recognizes the form of the enzyme in which the activation segment is phosphorylated, while binding appears inhibited in the absence of phosphorylation. Overlaying the active kLck and the inactive src kinase structures demonstrates a strong steric clash between ITAM substrate and the activation segment in the unphosphorylated state.

3. PSI LOOP IN BAND 3-GLYCOLYTIC ENZYME COMPLEXES

In addition to enzymatic activation and regulation of the association of signaling proteins, tyrosine phosphorylation is key in regulating protein–protein interactions among downstream complexes. Phosphorylation mediates, in either a positive or negative manner, the formation of protein complexes other than those involving protein kinases, adapter proteins, phosphatases or other signaling proteins. One such downstream complex occurs in erythrocytes. Band 3, a substrate of p72syk, associates with glycolytic enzymes and localizes them to the membrane[23, 24] (Fig. 4). The glycolytic enzymes aldolase and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) bind the unphosphorylated form of band 3 and are reversibly inactivated upon binding. Phosphorylation by syk[25] releases the enzymes, and glycolysis levels in the cell increase.[26] That is, tyrosine phosphorylation is a negative control mechanism in the case of band 3-glycolytic enzyme association. The levels of glycolytic intermediates can be varied 30-fold by altering the level of band 3-enzyme association through phosphorylation of band 3.[27] Nonetheless, the physiological role that tyrosine phosphorylation plays in maintaining proper cellular functioning in erythrocytes remains elusive.

Crosspeak intensities were measured in the et-NOESY experiment on a 15-residue peptide from the N-terminus of band in the presence of either aldolase[28] or G3PDH.[29] The band 3 peptide (B3P)

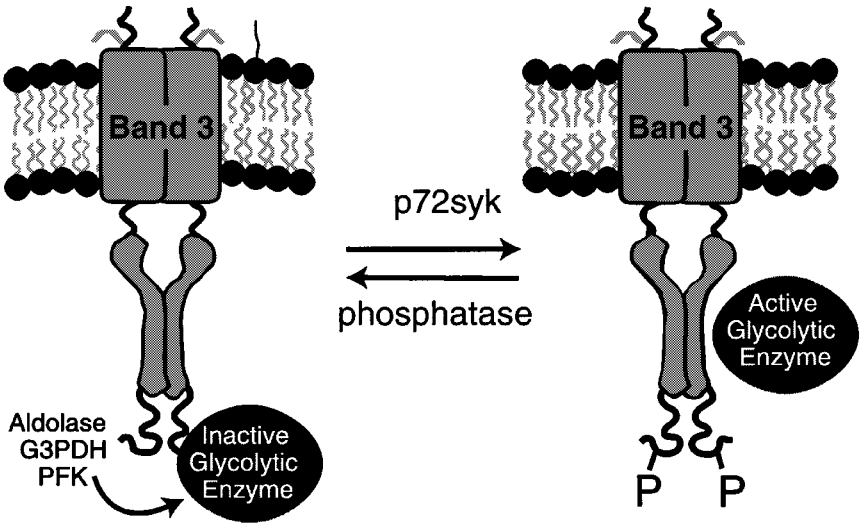

Figure 4. Schematic drawing of erythrocyte band 3 phosphorylation by p72syk. Unphosphorylated band 3 binds and inhibits the glycolytic enzymes, aldolase, glyceraldehyde-3-phosphate dehydrogenase, G3PDH, and phosphofructo kinase, PFK. Phosphorylation of band 3 disrupts the band 3-glycolytic enzyme complex, reversing inhibition.

(MEELQDDYEDMMEEN-NH2) contains tyr 8, one of two phosphorylation sites involved in binding and regulating activity of glycolytic enzymes. Distance restraints derived from the etNOESY intensities were used in restrained molecular dynamics to determine the bound-state structure of band 3 peptide in the unphosphorylated form. A characteristic pattern of NOE intensities was observed for both the aldolase and G3PDH complexes; there are numerous intensities between the aromatic ring protons of tyr 8 and side-chain protons of leu 4 and met 12.

The band 3 structure defined by the etNOE data is a loop conformation which folds around the tyrosine side chain and is stabilized by nonpolar interactions of the triad leu 4, tyr 8, and met 12, and by a hydrogen bond between the side chains of tyr 8 and asp 10. This *p*hosphorylation *s*ensitive *i*nteraction (PSI) loop is recognized by both aldolase[28] and G3PDH.[29] The internal structure of the PSI loop is well defined from the NMR data, while the terminal residues are more disordered. This variation in the degree of structural order is consistent with the NMR linewidths of B3P in exchange with aldolase. Thus, the conformational heterogeneity found after restrained molecular dynamics is a reasonable reflection of conformational mobility present in solution.

The PSI loop was docked to the active site of aldolase.[28] The stoichiometry of binding and competitive binding between B3P and the aldolase substrate indicate the active site for B3P binding. The modeled complex, shown in Figure 5, has the PSI loop positioned into the β-barrel of aldolase in proximity to the active site residues and other highly conserved regions of aldolase. Binding is primarily electrostatic in nature as illustrated by the blue surface, which corresponds to electro-positive potential in Figure 5, being in close contact with asp 6 and asp 7 of B3P. Tyr 8 makes no intermolecular contacts, unlike tyrosine binding where the phosphorylated form is the recognized state for SH2 domains and PTB domains. A number of intermolecular hydrogen bonding interactions are found. Furthermore, there is high steric complementarity between the PSI and the aldolase active site pocket.

When tyr 8 of human erythrocyte band 3 is phosphorylated, aldolase inhibition is disrupted and full catalytic activity returns.[30] The mechanism for negative control of the association between band 3 and the glycolytic enzymes is suggested from the model for the B3P-aldolase complex to be an intramolecular negative repulsion of the band 3 phosphoryl group with acidic band 3 residues distant

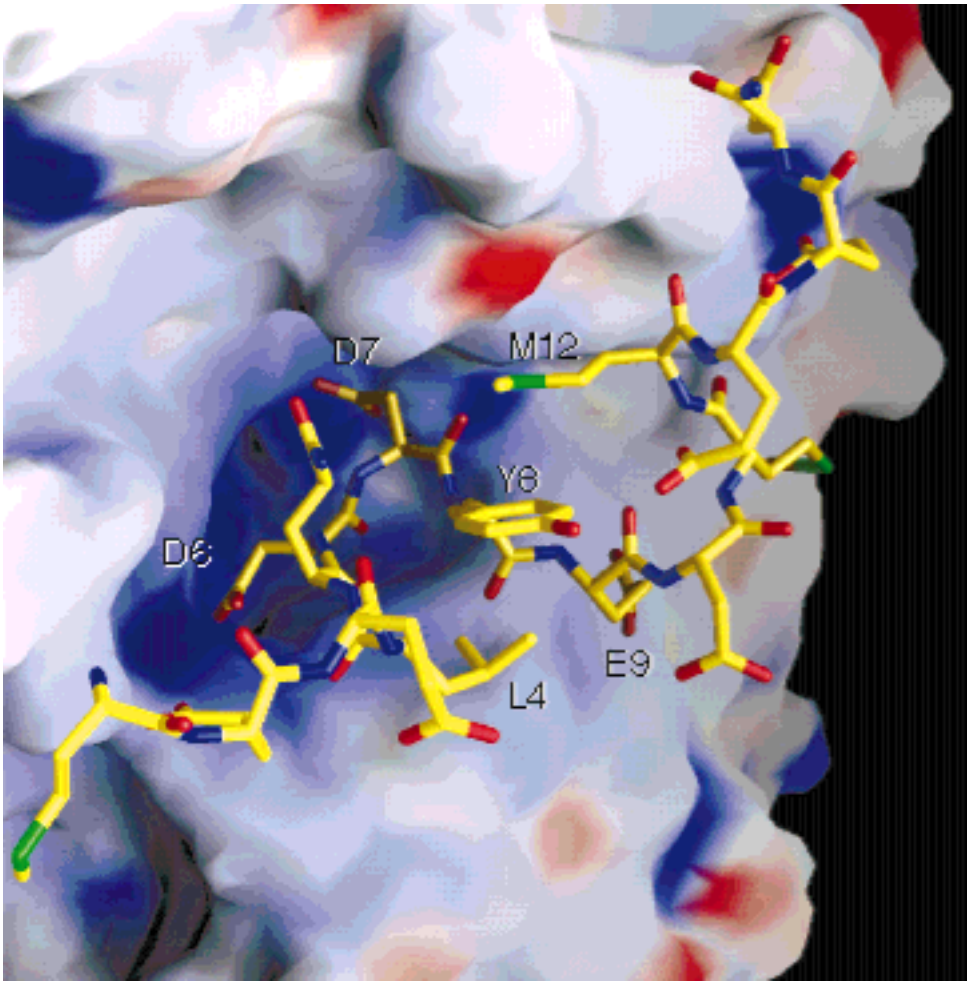

Figure 5. The PSI loop of band 3 modeled in the active site of aldolase. The bound-state structure of the PSI was determined by NMR. The aldolase structure from x-ray crystallography is represented by a GRASP[34] colored according to the electrostatic potential where blue is positive potential and red is negative potential. The electrostatically positive pocket of aldolase complements both electrostatically and sterically the PSI loop. Labeled residues of the PSI loop are important for intramolecular stabilization (L4, Y8, M12) or intermolecular interactions (D6, D7, E9). (Reprinted with permission of The American Chemical Society. This image originally appeared in Biochemistry 1995:34;16581.)

to the phospho-tyrosine site (Fig. 6). In particular, asp 10 is spatially near the position that would be occupied by a phosphoryl group on tyr 8. As such, the PSI loop conformation is not consistent with phosphorylation of tyr 8. The electrostatic repulsion is born out by calculations of the internal nonbonded energy for the phosphoryl group shown in Table I. The calculations also show that there is no steric conflict associated with phosphorylation, in contrast to the binding of isocitrate dehydrogenase which involves direct conflict with substrate binding by a phosphorylated active site serine.[31,32] The intramolecular nature of the destabilization suggests that a PSI loop mechanism for negatively mediating protein–protein interaction might be a general one.

Given the potential generality of the PSI-loop based mechanism for negative regulation of protein–protein interactions through tyrosine phosphorylation, we have examined other syk substrates for a possible PSI loop structure. Indeed, we find a potential PSI loop which may play a signaling role in B-cells. The control of microtubulin polymerization depends on binding of the microtubulin

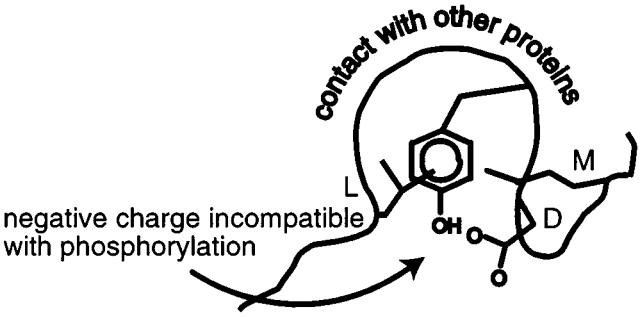

Figure 6. Schematic drawing of the PSI loop mechanism for tyrosine phosphorylation mediating against protein–protein association. The tyrosine side chain is buried within the PSI loop by leucine (P−4) and methione (P+4). Aspartic acid (P+2) hydrogen bonds to the tyrosine. Phosphorylation of tyrosine, while not sterically excluded, destabilizes the PSI loop by electrostatic repulsion involving acidic residues distant in sequence but near in space.

associated protein called tau. Tau is needed to promote microtubule formation and associates with monomeric tubulin at a region that includes tyr 432, a site shown to be phosphorylated by syk.[33] Phosphorylation of tubulin mediates against binding by tau and against the formation of microtubulin. Moreover, the sequence of tubulin surrounding tyr 432 (MAALEKDYEEVGVDS) includes the motif characterizing a PSI loop. This sequence motif, LXXXYXD/EXZ, where Z corresponds to a hydrophobic amino acid, includes the leu at the P-3 position and the hydrophobic amino acid to form a hydrophobic triad, and the acidic residue at the P+2 position to provide electrostatic repulsion in the phosphorylated state, destabilizing the PSI loop conformation and mediating against a complex involving recognition of the PSI loop (Fig. 6). We predict that tau recognizes a PSI loop upon binding tubulin.

4. CONCLUSION

Peptide-protein models of two protein–protein complexes involved in signaling were described. Substrate recognition and enzymatic activation related to binding of an ITAM substrate to the src-family kinase, Lyn, depend on both amino acids defining the ITAM sequence motif and on the intervening amino acids. The structure of a natural substrate bound to a src-family kinase offers potential to facilitate structure-based drug design efforts with improved specificity over the known inhibitors which are ATP analogues. From a complex of nonsignaling proteins, but also regulated through tyrosine phosphorylation, a PSI loop conformation was found. In the complex of B3P with either aldolase or G3PDH, the PSI loop folds around the tyrosine residue, rather than have the tyrosine side chain extended and accessible for intermolecular contact. Phosphorylation of this tyr would destabilize the PSI loop, and thus disrupt formation of the band 3 complex. The structural similarity of B3P bound to aldolase and G3PDH underscores the importance of the PSI loop in phosphotyrosine regulation of protein–protein interactions, and potential general use of this structural motif for negative control through tyrosine phosphorylation.

REFERENCES

1. Levitzki A, Gazit A. Science 1995;267:1782–1788.
2. Bolen JB, Brugge JS. Annu Rev Immunol 1997;15:371–404.
3. Reth M. Nature 1989;338:383–384.

4. Reth M. Annu Rev Immunol 1992;10:97–121.
5. Cambier JC, Pleiman CM, Clark MR. Annu Rev Immunol 1994;12:457–486.
6. Nagai K, Takata M, Yamamura H, Kurosaki T. J Biol Chem 1995;240:6824–6829.
7. Johnson SA, Pleiman CM, Pao L, Schneringer J, Hippen K, Cambier JC. J of Immun 1995;155: 4596–603.
8. Romeo, C, Amiot M, Seed B. Cell 1992;68:889–897.
9. Letourneur F, Klausner RD. Science 1992;255:79–82.
10. Cambier JC. J of Immunology 1995;3281–3285.
11. Moarefi I, LaFevre-Bernt M, Sicheri F, Huse M, Lee C-H, Kuriyan J, Miller WT. Nature 1997;385:650–653.
12. Sicheri F, Moarefi I, Kuriyan J. Nature 1997;385:602–609.
13. Xu W, Harrison SC, Eck MJ. Nature 1997;385:595–602.
14. Williams J-C, Weijland A, Gonfloni S, Thompson A, Courtneidge S-A, Superti-Furga G, Wierenga RK. J Mol Biol 1997;274:757–775.
15. Post CB, Schneider ML. In: Rao D, Kemple M. editors. NMR as a structural tool for macromolecules: Current status and future directions. New York: Plenum Press; 1996.
16. Zheng J, Post CB. J Phys Chem 1996;100:2675–2680.
17. Ni F. Progress in NMR Specroscopy 1994;26:517–606.
18. Campbell AP, Sykes BD. Annu Rev Biophys Struct 1993;22:99–122.
19. Yamaguchi H, Hendrickson WA. Nature 1996;385:484–489.
20. Knighton DR, Zheng J, Ten Eyck LF, Xuong N-H, Taylor SS, Sowadski JM. Science 1991;253:414–423.
21. Hubbard SR. EMBO J 1997;16:5572–5581.
22. Williams JC, Wierenga RK, Saraste M. TIBS 1998;23:179–184.
23. Low PS. Biochem Biophys Acta 1986;864:145–167.
24. Rogalski AA, Steck TL, Waseem A. J Biol Chem 1989;264:6438–6446.
25. Harrison ML, Isaacson CC, Burg DL, Geahlen RL, Low PS. J Biol Chem 1994;269:955–959.
26. Harrison ML, Rathinavelu P, Arese P, Geahlen RL, Low PS. J Biol Chem 1991;266:4106–4111.
27. Low PS, Rathiavelu PR, Harrison ML. J Biol Chem 1993;268:14627–14631.
28. Schneider ML, Post CB. Biochemistry 1995;34:16574–16584.
29. Eisenmesser EZ, Post C. Biochemistry 1998;37:867–877.
30. Low PS, Allen DP, Zioncheck TF, Chari P, Willardson BM, Geahlen RL, Harrison ML. J Biol Chem 1987;262:4592–4596.
31. Hurley JH, Dean AM, Sohl JL, Koshland Jr. DE, Stroud RM. Science 1990;249:1012–1016
32. Dean AM, Koshland Jr. DE. Science 1990;139:1044–1046.
33. Peters JD, Furlong MT, Asai DJ, Harrison ML, Geahlen RL. J Biol Chem 1996;271:4755–4762.
34. Nicholls A, Sharp KA, Honig B. Proteins 1991;11:281–296.

Carol Beth Post *is an Associate Professor in the Department of Medicinal Chemistry at Purdue University where she holds an NIH Research Career Development Award. She received her Ph.D. in Chemistry from University of California at San Diego, LaJolla, CA, where she worked on biophysical properties of DNA working with Professor Bruno Zimm. She received an NIH postdoctoral fellowship to work in the laboratories of Dr. Martin Karplus at Harvard University, Cambridge, MA, and Dr. Christopher Dodson at Oxford University, Cambridge, England, on the picosecond timescale internal motions of proteins investigated by molecular dynamics and NMR relaxation methods. Her recent research interests are activation and recognition of signaling proteins, function of viral proteins, and solution NMR methods of protein structure determination involving both theoretical and experimental methods.*

Beverly S. Gaul *attended the University of Northern Iowa, Cedar Falls, IA, under a full-tuition scholarship, and received a B.S. degree in Chemistry in 1992. She obtained a Ph.D. degree in the Department of Medicinal Chemistry at Purdue University in 1998. She is an NMR research associate at the University of Minnesota, where she continues her research in NMR spectroscopy and protein structure and function.*

Elan Zohar Eisenmesser *graduated for the State University of New York, Stony Brook, with a B.S. in Astronomy and Physics in 1991. After spending some time in Israel, he entered the graduate program at Purdue Uni-*

versity and received his Ph.D. in Biological Sciences in 1998. He is currently a postdoctoral associate at the National Cancer Institute working in the laboratory of Dr. Andrew Byrd where he continues his interest in NMR study of proteins related to human disease.

Michael L. Schneider *obtained a B.S. in Chemistry from the University of Texas at San Antonio, San Antonio, TX, in 1990. He entered the graduated program in the Department of Medicinal Chemistry, Purdue University, and obtained a Ph.D. in 1997. His research interests included protein ligand interactions, and protein structure of toxins investigated by NMR spectroscopy.*