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Pex5p Binding Affinities for Canonical and Noncanonical PTS1 Peptides

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ABSTRACT **The majority of proteins targeted to the peroxisomal lumen contain a C-terminal peroxisomal targeting signal-1 (PTS1) that is bound by the peroxin Pex5p. The PTS1 is generally regarded as a C-terminal tripeptide that adheres to the consensus (S/A/C)(K/R/H)(L/M). Previously, we studied the binding affinity of peptides of the form YQX(-3)X(-2)X(-1) to the peptide-binding domain of human Pex5p (referred to as Pex5p-C). Optimal affinity was found for YQSKL, which bound with an affinity of 200 ± 40 n*M*. To extend this work, we investigated the properties of a peptide containing the last 9 residues of acyl-CoA oxidase (RHYLKPLQSKL) and discovered that it binds to Pex5p-C with a dissociation constant of 1.4 ± 0.4 n*M*, 180 times tighter than YQSKL. Further analysis revealed that the enhanced affinity is primarily due to the presence of leucine in the (-5) position. In addition, a peptide corresponding to the luciferase C-terminus (YKGGKSKL) was found to bind Pex5p-C about 20 times tighter than YQSKL. The majority of this effect results from having lysine in position (-4). Catalase contains a noncanonical PTS1 (-AREKANL). The affinity of YQANL was found to be 3600 ± 400 n*M*. This relatively weak binding is consistent with previous unsuccessful attempts to direct chloramphenicol acetyltransferase to the peroxisome by fusing -ANL to its C-terminus (-GGA-ANL). The peptides YKANL, YEKANL, YREKANL, and YAREKANL all bound Pex5p-C with higher affinities than did YQANL, but the affinities are still lower than peptides that correspond to functional targeting signals in other contexts. Because both catalase and Pex5p are tetramers (as opposed to the monomeric Pex5p-C and the peptides used in our studies), multidentate effects on binding affinity between Pex5p and other oligomeric proteins should be considered. Our study provides direct thermodynamic data revealing that peptide binding to Pex5p-C binding is favored by lysine in the (-4) position and leucine in the (-5) position. Our results suggest that peptides or proteins with optimized residues in the (-4) and/or (-5) positions can bind to Pex5p with affinities that are at least two orders of magnitude greater than that of YQSKL, and that this stabilization can compensates for otherwise weakly binding PTS1s. Proteins 2004;55:856–861.**

Key words: peroxisome; peroxin; specificity; affinity; fluorescence anisotropy; catalase; acyl-CoA oxidase; luciferase

INTRODUCTION

Protein compartmentalization in eukaryotes is crucial for many metabolic processes. The peroxisome is a membrane-enclosed organelle found in essentially all eukaryotes[1] that functions in fatty acid and cholesterol metabolism.[2] The glyoxysome[3] and the glycosome[4] are homologous organelles found in plants and parasites that harbor enzymes for other metabolic pathways. Most proteins to be sorted to the matrix of these organelles have a peroxisomal targeting signal (PTS).[5] The most common targeting signal is the PTS1; this signal occurs precisely at the carboxyl terminus of a protein and has been defined by the consensus sequence -SKL.[6] Sequences that approximate this consensus are present at the terminus of more than 90% of all known peroxisomal matrix proteins.[7]

The importance of peroxisomal assembly is revealed by the existence of a set of clinically characterized peroxisomal biogenesis disorders (PBDs).[7] These PBDs are caused by mutations in genes encoding peroxins, proteins involved in the biosynthesis and assembly of the peroxisome. Among the peroxins that have been associated with PBDs is Pex5p, a protein that functions as the PTS1 receptor.[8] Pex5p consists of two primary domains: an amino-terminal domain involved in tetramerization[9] and other functions, and a carboxyl-terminal PTS1-binding domain. PTS1 recognition by the C-terminal domain of Pex5p is mediated by 6 tetratricopeptide repeat (TPR) motifs. TPRs are 34-amino acid helix–turn–helix motifs that have been shown to be important in mediating protein–protein interactions.[10,11] We determined the crystal structure of a carboxyl-terminal fragment of human Pex5p (hereafter referred to as Pex5p-C) complexed with the peptide YQSKL.[12] The structure is composed of two sets of 3-TPR motifs linked together by a helical hinge. The two TPR arrays make numerous contacts with the peptide. Five conserved asparagine residues interact with the peptide

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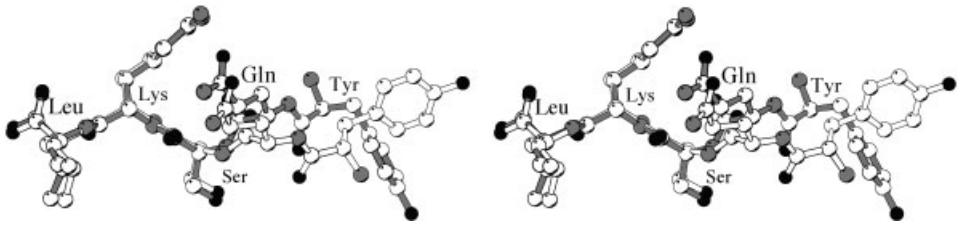

Fig. 1. An overlay of the structures of two YQSKL peptides shown as a stereo pair. The structures of the two PTS1 peptides bound to Pex5p-C observed in the two independent complexes in the asymmetric unit of the Pex5p-C/YQSKL complex[12] are very similar for the Leu, Lys, and Ser residues, but differ substantially for the Gln and Tyr residues. Carbon atoms are shown in white, oxygen in black, and nitrogen in gray.

backbone, anchoring the peptide in the binding pocket. Interactions between Pex5p and the PTS1 peptide side-chain atoms confer a high degree of binding specificity. The L(-1) binding pocket is lined with hydrophobic residues that make van der Waals contacts with the leucine side-chain. The K(-2) binding pocket contains acidic residues that make water-mediated contacts with the ammonium group at the end of the lysine side-chain. The S(-3) binding pocket is highly constrained and likely fits only small side-chains; contacts are made with the S(-3) hydroxyl group, including a water-mediated hydrogen-bonding interaction. The peptide used in these studies had Q in position (-4) and Y in position (-5). The Q residue in position (-4) was chosen based on the sequence of the peroxisomal enzyme acyl-CoA oxidase. The Y residue in position (-5) was selected to provide a chromophore for peptide quantitation. These two residues exist in different conformations in the two complexes within the asymmetric unit in the crystal form that we studied, and no clear binding pockets for these residues are evident (Fig. 1). Moreover, the amino-terminal ends of the peptides lie near a noncrystallographic 4-fold axis that relates 4 molecules of the Pex5p-peptide complexes to one another. Modest interactions between the peptides and both cognate and adjacent molecules in the crystal are present. For this reason, it is not clear if either of the two observed conformations is a good representation of the structure(s) that would be observed with PTS1-containing proteins. For this reason, the structures are not analyzed further.

While -SKL is clearly the consensus for functional PTS1 signals, some variation in these residues can be tolerated without loss of targeting function. These studies have largely been based on the carboxyl-terminus from firefly luciferase, either on the native protein or appended to chloramphenicol acetyltransferase (CAT).[5,13,14] Luciferase ends with the sequence -KGGKSKL. In this context, targeting in human cells has been demonstrated[5,15] using L or M in position (-1), K, R, or H in position (-2), and S, A, or C in position (-3). Other sequences in this context, such as -KGGKSKI, did not function as peroxisome targeting signals.

Not all known PTS1s conform to the canonical PTS1 consensus. For example, human catalase, a homotetrameric heme-containing protein that is normally localized to the peroxisomal matrix, ends with the sequence -ANL. Purdue and Lazarow[16] showed that fusion of the last 9 amino acids of catalase (-LAAREKANL) to CAT targeted the protein to the peroxisomal matrix. However, simply fusing the tripeptide -ANL-COO^- to the end of CAT (which ends in -WQGGAT) did not result in peroxisomal import. Thus, additional sequence features prior to the last 3 amino acids are important for targeting in this context.

We recently used a fluorescence-based ligand-binding assay to analyze the effects of sequence variation among the last 3 amino acids of YQSKL on the solution-binding affinity for Pex5p-C.[17] The parent peptide, YQSKL, bound to Pex5p-C with a dissociation constant of 200 $\pm$ 30 n*M*. We examined a set of 12 additional peptides with single amino acid substitutions in one of the last three positions. The results of this study revealed that those peptides that bound with affinities within 1.8 kcal/mol of the parent contained C-terminal tripeptide sequences that had been shown to function as targeting signals in human cells, whereas no peptide that bound outside this range contained a tripeptide sequence that corresponded to a known, functional targeting signal.

Here we report the study of binding of longer peptides corresponding the known functional PTS1 sequences from peroxisomal acyl-CoA oxidase, luciferase, and catalase to Pex5p-C. These peptides reveal a large range of affinities for binding to Pex5p-C. Most remarkably, the acyl-CoA oxidase peptide was found to bind to Pex5p-C approximately 180 times more tightly than YQSKL. These observations underscore the importance of residues prior to the final 3 amino acids in determining binding affinity. Studies of additional peptides revealed that variations in either position (-4) or (-5) effects can result in differences of more than an order a magnitude in affinity. These results highlight the need to extend the definition of a PTS1 to include residues upstream of the -SKL sequence.[18,19]

METHODS

Protein Expression and Purification

Pex5p-C, a 41 kDa fragment of human Pex5p comprising amino acids 235–602, was expressed and purified as previously described,[12] with the following modifications: Complete protease inhibitor cocktail (Roche) was added to the cell lysis buffer at one tenth the final concentration; cells were lysed using a French pressure cell operated at 12,000 psi; the C4 butyl sepharose column was washed with 10 column volumes of buffer A [50 m*M* Tris-HCl, 2 m*M* ethylenediaminetetraacetic acid (EDTA), 5 m*M* NaF, 5 m*M* benzamidine HCl, 1 m*M* dithiothreitol (DTT)]

supplemented with 0.25 *M* ammonium sulfate; and salt was excluded in the acid precipitation steps. Pex5p-C concentration was determined by UV spectroscopy using an extinction coefficient of $\epsilon_{280} = 51{,}960\ M^{-1}\ \mathrm{cm}^{-1}$.

Peptide Synthesis

All peptides were synthesized by solid phase methods on a MilliGen/Biosearch 9050 Peptide Synthesizer using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry with O-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TBTU) and 1-hydroxybenzotriazole (HOBt) activation. Peptides were quantitated using $\epsilon_{274} = 1400\ M^{-1}\ \mathrm{cm}^{-1}$ per tyrosine residue. Peptide identity and purity were confirmed by matrix-assisted laser desorption/ionization time-of-flight (MALDI/TOF) mass spectrometry on a Applied Biosystems Voyager-DE STR Biospectrometry Workstation. Lissamine-labeled YQSKL was prepared by conjugation of lissamine sulfonyl chloride (Molecular Probes) to the peptidyl N-terminus in the presence of diisopropylethylamine as described.[12,20]

Fluorescence Anisotropy Titrations and Data Analysis

Anisotropy assays were carried out as previously described.[17] Determination of the fraction of fluorophore bound (f_B) for a given anisotropy value (r) and quantum yield ratio (Q) was computed with the expression

$$f_B = \frac{r - r_{free}}{(r_{bound} - r)Q + (r - r_{free})}$$

given the anisotropy of the free (r_{free}) and bound (r_{bound}) fluorophore.[21] The quantum yield ratio is the ratio of the intensities of bound to free fluorophore. The intensity of free fluorophore was determined experimentally, and that of the bound fluorophore was calculated by monitoring lissamine fluorescence intensity as Pex5p-C was titrated into a solution containing lissamine-labeled YQSKL. A quadratic equation describing a single-site binding phenomenon was fitted to the data using Kaleidagraph (Synergy Software). Using this method, lissamine-labeled YQSKL was found to bind to Pex5p-C with a dissociation constant of 16 ± 1 n*M*. Lissamine-labeled YKSKL (used in competition experiments with YKGGQSKL, YKGYKSKL, and YKGLKSKL) bound to Pex5p-C with a dissociation constant of 2.7 ± 0.1 n*M*.

Competitive titration curves (f_B plotted as a function of unlabeled ligand concentration, $L_{unlabeled}$) were calculated and fitted to the experimental data using a modified version of a previously described[17] curve-fitting algorithm written in Mathematica (Wolfram Research). The algorithm searches through a range of dissociation constants of the unlabeled peptide ($K_{unlabeled}$) for the receptor and finds the best fit to the observed data. The algorithm was modified so that simulated binding curves could be fit to multiple titration data sets using global estimates for the total concentration of fluorescent ligand ($L_{labeled}$), the dissociation constant for the labeled peptide ($K_{labeled}$), r_{bound}, and r_{free}.

During our attempts to fit the data globally, we discovered that the quality of the fit of the simulated curve (measured as the square root of the sum of the squares of the residuals) to certain data sets was extremely sensitive to r_{bound}. The best-fit r_{bound} value was 0.299 for titrations with RYHLKPLQSKL and YLQSKL, 0.29 for YLKSKL, 0.319 for YKGGKSKL, and 0.309 for all others. This sensitivity arises for the titration curves of tight-binding peptides. Competitive titration curves were fit to all of the data (with the exception of the YKGGQSKL, YKGYKSKL, and YKGLKSKL titrations, *vide infra*) using $L_{labeled}$ = 225 n*M*, $K_{labeled}$ = 16 n*M*, Q = 0.68, and individual r_{free} values measured at the beginning of each experiment. Back titrations with YKGGQSKL, YKGYKSKL, and YKGLKSKL were carried out using lissamine-labeled YKSKL; simulated curves were fit to these data using $L_{labeled}$ = 174 n*M*, $K_{labeled}$ = 2.8 n*M*, Q = 0.65, and r_{bound} = 0.325.

Errors in the $K_{unlabeled}$ values were estimated by computationally determining the values of $K_{unlabeled}$ that doubled the square root of the sum of the squares of the residuals. The reported errors associated with $\Delta\Delta G°$ were obtained through propagation of the errors associated with the corresponding $K_{labeled}$ values.

RESULTS

Recently, we described the impact of varying residues in the peptide model PTS1 peptide YQ-X(-3)X(-2)X(-1) on the affinity of peptide binding to Pex5p-C.[17] The YQSKL model peptide was chosen because of its relative simplicity, ease of quantitation, and use in our structural studies. However, there are some disadvantages to using this peptide. While YQSKL binds to Pex5p-C with moderately high affinity, no known peroxisomal protein ends with this pentapeptide sequence, nor has this pentapeptide been used in any *in vivo* peroxisome targeting studies, making its biological significance difficult to assess. To address this shortcoming, we studied the binding of Pex5p-C to longer peptides from proteins known to be targeted to peroxisomes.

A peptide containing the C-terminal 9 amino acids of rat peroxisomal acyl-CoA oxidase (RYHLKPLQSKL) was synthesized and characterized. The first 2 amino acids were included to aid in peptide quantitation. Pex5p-C was titrated to near saturation with lissamine-labeled YQSKL, and unlabeled RYHLKPLQSKL was added to displace the labeled peptide. The acyl-CoA oxidase peptide competed very effectively; curve fitting revealed that this peptide bound Pex5p-C with a dissociation constant of 1.4 ± 0.4 n*M*, 180 times tighter than YQSKL (Fig. 2, Table I). Unlabeled YQSKL was found to bind Pex5p-C with a dissociation constant of 250 ± 80 n*M*, within error of the previously reported value.[17]

Similar experiments were performed with octameric peptides derived from luciferase and catalase. A peptide containing the C-terminal 7 amino acids of luciferase, YKGGKSKL, bound Pex5p-C with a dissocation constant of 15 ± 1 n*M*, 10-fold weaker than RYHLKPLQSKL. In contrast, the catalase peptide, YAREKANL, bound with a dissociation constant of 1100 ± 200 n*M*, about 800-fold

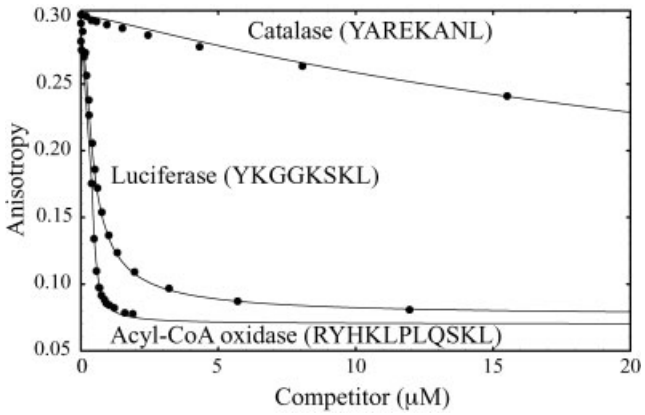

Fig. 2. Binding of peptides to Pex5p-C monitored by a competitive fluorescence anisotropy assay. Binding data and fits are shown for three peptides displacing lissamine-labeled YQSKL from Pex5p-C. These peptides bind with dissociation constants of 1.4 ± 0.4 n*M* (acyl-CoA oxidase), 15 ± 1 n*M* (luciferase), and 1100 ± 200 n*M* (catalase).

weaker than RYHLKPLQSKL. This weaker binding might have been anticipated given that the catalase peptide ends in -ANL rather than SKL. However, the catalase peptide binds 3-fold more tightly than does YQANL, supporting the argument that residues upstream of -ANL in the catalase peptide promote binding and give rise to import in vivo.[16]

To probe the upstream residues that are responsible for these observed binding affinities, a series of truncated peptides and sequence variants were synthesized, and their binding to Pex5p-C was examined using the competitive fluorescence anisotropy assay. Particular attention was paid to the (-4) and (-5) residues, since they are found in a position where they may readily contact Pex5p-C based on the crystal structure. Residues further upstream are likely to extend out of the binding pocket. The peptides studied and their binding affinities are summarized in Table I.

DISCUSSION

Acyl-CoA Oxidase Peptides

The most likely source of the 180-fold increase in affinity observed for the acyl-CoA oxidase peptide (RYHLKPLQSKL) compared with YQSKL is the L residue in position (-5) based on two observations. First, the (-5) residue is positioned near the PTS1 binding pocket and could easily interact with Pex5p. Second, a previous yeast 2-hybrid study involving a random library of hexadecapeptides revealed a significant preference for L and V in position (-5).[18] To test the effect of small hydrophobic residues in position (-5), the binding of Y**L**QSKL and Y**Y**QSKL to Pex5p-C was examined. These were bound by Pex5p-C with dissociation constants of 9 ± 3 n*M* and 90 ± 30 n*M*, respectively. Thus, replacing Y by L results in a 10-fold increase in affinity for Pex5p-C and provides additional evidence that the (-5) binding pocket of Pex5p-C prefers relatively small hydrophobic residues. This substitution accounts for most, but not all, of the difference in affinity between the acyl-CoA oxidase peptide and YQSKL. The fact that **Y**LQSKL binds roughly 6-fold weaker than RYHLK**P**LQSKL results from the effect of having a Y in position (-6) (vs P) and/or sequence effects further upstream.

Luciferase Peptides

Since many studies of PTS1 function *in vivo* have been examined in the context of the C-terminus of firefly luciferase, we examined the ability of a peptide corresponding to this sequence to bind to Pex5p-C. This peptide has the sequence YKGGKSKL, where the N-terminal Y was added to aid in peptide quantitation. This peptide bound Pex5p-C with a dissociation constant of 15 ± 1 n*M*, 17-fold tighter than that for YQSKL. Positions (-4) and (-5) were examined to determine the source of this higher affinity. To examine the importance of the residue in position (-4), the ability of the peptide Y**K**SKL to bind Pex5p-C was compared with that of Y**Q**SKL. The dissociation constant for YKSKL was found to be 60 ± 10 n*M*, more than 4-fold tighter than that for YQSKL. A larger enhancement in affinity was observed when YKGG**K**SKL was compared to YKGG**Q**SKL. To examine the importance of the residue in position (-5), the dissociation constants for the peptides YKG**Y**KSKL and YKG**L**KSKL were determined, revealing values of 9 ± 1 n*M* and 1.3 ± 0.2 n*M*, respectively. Thus, the G residue in position (-5) reduces the affinity of the luciferase peptide compared with peptides with Y or L in this position. The affinity of YKGLKSKL bound with an affinity within error of that measured for YLKSKL (1.5 ± 0.4 n*M*) indicating that effects upstream of position (-5) are more subtle.

In vivo assays have shown that changing the (-1) position of the luciferase PTS1 to I disrupts peroxisomal targeting, while substitution with M does not significantly affect targeting efficiency. We synthesized YKGGKSKM and YKGGKSKI and measured their affinities for Pex5p-C. YKGGKSKM bound Pex5p-C with a dissociation constant of 250 ± 10 n*M*, whereas YKGGKSKI bound with a dissociation constant of 600 ± 30 n*M*. Thus, the peptide corresponding to a functional targeting signal bound 2.4-fold more tightly than did the peptide corresponding to a nonfunctional signal.

Catalase Peptides

Not all peroxisomal proteins bear the PTS1 tripeptide consensus (S/A/C)(K/R/H)(L/M). The best-studied example is catalase, which ends with -LAAREKANL. We examined the affinity of YQANL for Pex5p-C, revealing a dissociation constant of 3600 ± 400 n*M*. The K in position (-4) of the catalase PTS1 may enhance the affinity for Pex5p-C. We examined the affinity of YKANL for Pex5p-C and found it to be 1200 ± 100 n*M*, demonstrating a 3-fold increase in affinity of K versus Q in position (-4). With an appreciation for the effects at the (-4) and (-5) positions, we synthesized a series of peptides corresponding to various lengths of the catalase C-terminus: YAREKANL, YREKANL, and YEKANL. YEKANL bound Pex5p-C significantly weaker than did YQSKL. The effects at the (-5) position were tested by replacing E in Y**E**KANL with L to give Y**L**KANL. This

TABLE I. Dissociation Constants and Binding Free Energies for Peptides to Pex5p-C

Protein family	Peptide sequence	K_d (n*M*)	ΔΔG° (kcal/mol)
Acyl-CoA oxidase	[a]RYHLKPLQSKL	1.4 ± 0.4	0
	YYQSKL	90 ± 30	2.5 ± 0.3
	[a]YLQSKL	9 ± 3	1.1 ± 0.3
	[a]YLKSKL	1.5 ± 0.4	0.0 ± 0.3
	YQSKL	250 ± 80	3.1 ± 0.3
Luciferase	[a]YKGGKSKL	15 ± 1	1.4 ± 0.2
	YKGGKSKM	250 ± 10	3.0 ± 0.2
	YKGGKSKI	600 ± 30	3.6 ± 0.2
	[b]YKGYKSKL	9 ± 1	1.1 ± 0.2
	[b]YKGLKSKL	1.3 ± 0.2	0.0 ± 0.2
	[b]YKGGQSKL	500 ± 100	3.6 ± 0.2
	YKSKL	60 ± 10	2.2 ± 0.2
Catalase	YAREKANL	1100 ± 200	3.9 ± 0.2
	YREKANL	700 ± 200	3.7 ± 0.2
	YEKANL	1900 ± 100	4.2 ± 0.2
	YLKANL	27 ± 7	1.7 ± 0.2
	YKANL	1200 ± 100	4.0 ± 0.2
	YQANL	3600 ± 400	4.6 ± 0.2

[a]Titrations fit using varying r_{bound} values (see Methods).
[b]Liss-YKSKL served as labeled peptide.

peptide bound Pex5p-C 70 times tighter than did YEKANL, suggesting that the E in position (-5) has a deleterious effect on Pex5p-C binding, especially compared with the favorable L. YREKANL and YAREKANL bound Pex5p-C weaker than YEKANL with K_d values of 700 ± 200 n*M* and 1100 ± 200 n*M*, respectively. These binding affinities are lower than that for the peptide YKGGKSKI, a sequence that corresponds to a non-functional signal for the monomeric luciferase and CAT proteins.[5] Given that catalase is a tetramer and full-length Pex5p is a tetramer, a multidentate interaction between catalase and Pex5p may allow these monodentate interactions between the catalase peptide and Pex5p-C to result in sufficient binding avidity to achieve peroxisomal targeting.[22] Further studies between full-length Pex5p and oligomeric peroxisomal matrix proteins will be required to address such issues.

CONCLUSIONS

We have examined the binding affinities of a set of peptides corresponding to C-terminal sequences that have been shown to function as peroxisomal targeting signals. Interestingly, a range of roughly 1000-fold in binding affinities was observed. The sequence from acyl-CoA oxidase bound with the highest affinity, while that from catalase bound with the lowest affinity. Consistent with this difference, studies of peroxisome targeting in cells from a patient with peroxisomal biogenesis disorder caused by a mutation in Pex5p (S563W) revealed that acyl-CoA oxidase is targeted, whereas catalase is not.[23] This mutation lies at the interface between the two sets of 3 TPR domains and may interfere of complete closure of the Pex5p structure upon peptide binding. This may reduce peptide-binding affinity without any other functional consequences. Further studies of the effect of this mutation on Pex5p binding affinities are in progress.

We have demonstrated that over a 100-fold variation in binding affinity of peptides to Pex5p-C can be obtained by modifications at the (-4) and (-5) positions. Effects of this magnitude are likely to affect peroxisomal targeting, and suggest that preferred binding sites for these side-chains may be present. These observations indicate that full definition of the effects of substitutions at positions (-4) and (-5), as well as those further upstream, will be required to define more fully the sequence requirements for functional PTS1-mediated import. In addition, while inclusion of the amino-terminal domain of Pex5p does not appear to have any significant effect on PTS1-peptide binding, further studies involving intact Pex5p with complete protein targets will be required to elucidate fully the relationships between the affinity of Pex5p-protein interactions and peroxisomal protein targeting.

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REFERENCES

1. Lazarow PB, Fujiki Y. Biogenesis of peroxisomes. Annu Rev Cell Biol 1985;1:489–530.
2. van den Bosch H, Schutgens RBH, Wanders RJA, Tager JM. Biochemistry of peroxisomes. Annu Rev Biochem 1992;61:157–197.
3. Huang AHC, Trelease RN, Moore TS Jr. Plant peroxisomes. New York: Academic Press; 1983.
4. Opperdoes FR. Compartmentation of carbohydrate metabolism in trypanosomes. Annu Rev Microbiol 1987;41:127–151.
5. Gould SJ, Keller GA, Hosken N, Wilkinson J, Subramani S. A conserved tripeptide sorts proteins to peroxisomes. J Cell Biol 1989;108:1657–1664.
6. Subramani S. Protein import into peroxisomes and biogenesis of the organelle. Annu Rev Cell Biol 1993;9:445–478.
7. Gould SJ, Raymond GV, Valle D. The peroxisome biogenesis disorders. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors.

The metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 2001. p 3181–3217.
8. Dodt G, Braverman N, Wong C, Moser A, Moser HW, Watkins P, Valle D, Gould SJ. Mutations in the PTS1 receptor gene, PXR1, define complementation group 2 of the peroxisome biogenesis disorders. Nat Genet 1995;9:115–125.
9. Schliebs W, Saidowsky J, Agianian B, Dodt G, Herberg FW, Kunau WH. Recombinant human peroxisomal targeting signal receptor PEX5. J Biol Chem 1999;274:5666–5673.
10. Lamb JR, Tugendreich S, Heiter P. Tetratricopeptide repeat interactions: to TPR or not to TPR? Trends Biochem Sci 1995;20: 257–259.
11. Blatch GL, Lässle M. The tetratricopeptide repeat: a structural motif mediating protein–protein interactions. Bioessays 1999;21: 932–939.
12. Gatto GJ Jr., Geisbrecht BV, Gould SJ, Berg JM. Peroxisomal targeting signal recognition by the TPR domains of human PEX5. Nat Struct Biol 2000;7:1091–1095.
13. Gould SJ, Keller GA, Subramani S. Identification of a peroxisomal targeting signal at the carboxy terminus of firefly luciferase. J Cell Biol 1987;105:2923–2931.
14. Gould SJ, Keller GA, Subramani S. Identification of peroxisomal targeting signals located at the carboxy terminus of four peroxisomal proteins. J. Cell Biol 1988;107:897–905.
15. Swinkels BW, Gould SJ, Subramani S. Targeting efficiencies of various permutations of the consensus C-terminal tripeptide peroxisomal targeting signal. FEBS Lett 1992;305:133–136.
16. Purdue PE, Lazarow PB. Targeting of human catalase to peroxisomes is dependent upon a novel COOH-terminal peroxisomal targeting signal. J Cell Biol 1996;134:849–862.
17. Gatto GJ Jr, Maynard EL, Guerrerio AL, Geisbrecht BV, Gould SJ, Berg JM. Correlating structure and affinity for PEX5:PTS1 complexes. Biochemistry 2003;42:1660–1666.
18. Lametschwandtner G, Brocard C, Fransen M, Van Veldhoven P, Berger J, Hartig A. The difference in recognition of terminal tripeptides as peroxisomal targeting signal 1 between yeast and human is due to different affinities of their receptor PEX5P to the cognate signal and to residues adjacent to it. J Biol Chem 1998;273:33635–33643.
19. Neuberger G, Maurer-Stroh S, Eisenhaber B, Hartig A, Eisenhaber F. Motif refinement of the peroxisomal targeting signal 1 and evaluation of taxon-specific differences. J Mol Biol 2003;328: 567–579.
20. Godwin HA, Berg JM. A fluorescent zinc probe based on metal-induced peptide folding. J Am Chem Soc 1996;118:6514–6515.
21. Lakowicz JR. Principles of fluorescent spectroscopy. New York: Plenum Press; 1983.
22. Gould SJ, Collins CS. Peroxisomal-protein import: Is it really that complex? Nat Rev Mol Cell Biol 2002;3:382–389.
23. Shimozawa N, Zhang Z, Suzuki Y, Imamura A, Tsukamoto T, Osumi T, Fujiki Y, Orii T, Barth PG, Wanders RJ, Kondo N. Functional heterogeneity of C-terminal peroxisome targeting signal 1 in PEX5-defective patients. Biochem Biophys Res Commun 1999;262:504–508.