Crystal Structure and Ligand-Binding Studies of a Screened Peptide Complexed with Streptavidin

Patricia C. Weber,* Michael W. Pantoliano, and Leo D. Thompson

Crystallography and Biophysical Chemistry Group, The Du Pont Merck Pharmaceutical Company, Du Pont Experimental Station, P.O. Box 80228, Wilmington, Delaware 19880-0228

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ABSTRACT: The thermodynamic binding parameters and crystal structure for streptavidin–peptide complexes where the peptide sequences were obtained by random screening methods are reported. The affinities between streptavidin and two heptapeptides were determined by titrating calorimetric methods [Phe-Ser-His-Pro-Gln-Asn-Thr, K_a = 7944 (±224) M^{-1}, $\Delta G°$ = –5.32 (±0.01) kcal/mol, and $\Delta H°$ = –19.34 (±0.48) kcal/mol; His-Asp-His-Pro-Gln-Asn-Leu, K_a = 3542 (±146) M^{-1}, $\Delta G°$ = –4.84 (±0.03) kcal/mol, and $\Delta H°$ = –19.00 (±0.64) kcal/mol]. The crystal structure of streptavidin complexed with one of these peptides has been determined at 2.0-Å resolution. The peptide (Phe-Ser-His-Pro-Gln-Asn-Thr) binds in a turn conformation with the histidine, proline, and glutamine side chains oriented inward at the biotin-binding site. A water molecule is immobilized between the histidine and glutamine side chains of the peptide and an aspartic acid side chain of the protein. Although some of the residues that participate in binding biotin also interact with the screened peptide, the peptide adopts an alternate method of utilizing binding determinants in the biotin-binding site of streptavidin.

Random peptide synthesis (Cwirla et al., 1990; Lam et al., 1991) and phage epitope (Scott & Smith, 1990; Devlin et al., 1990) methods provide large screening libraries of compounds potentially useful in pharmaceutical discovery. Although originally envisioned as a means of screening for ligands of proteins that bind peptide effectors or substrates, the combination of rapid detection and retrieval raises the possibility that peptide epitope methods represent a general technology for the generation of new drug leads (Scott & Smith, 1990; Cwirla et al., 1990). In the general case, these peptides could be used in the design of nonpeptide molecules having similar or improved affinities for the macromolecule of interest.

Two screening studies have been carried out to define peptides that specifically bind to streptavidin, a biotin-binding protein secreted from *Streptomyces avidinii* (Green, 1990). Devlin et al. (1990) screened a phage library of nearly 20 million random 15-residue sequences expressed on the phage surface to obtain 20 peptides that detectably bound to streptavidin. Lam et al. (1991) screened a library of random synthetic pentapeptides and found 28 peptides with easily detected binding among at least 2 476 099 sequences tested. Most screened peptides that bind streptavidin share the tripeptide sequence His-Pro-Gln, with occasional substitutions of methionine or asparagine for the glutamine. Little amino acid sequence conservation was observed on either side of the His-Pro-Gln. For example, all possible nonpositively charged residues were found at the amino-terminal side of the histidine. High sequence diversity also occurred adjacent the glutamine where six residues, Ala, Gly, Asn, Phe, Tyr, and Asp, and ten residues, Leu, Thr, Ser, Pro, Arg, Asp, Ala, Asn, Val, and Gly, were found at the next two positions.

The present work was undertaken to determine how amino acids might interact in a protein site that naturally binds a nonpeptide ligand and to examine the feasibility of using peptide sequences to design nonpeptide molecules. The thermodynamic parameters of peptide binding, determined by titrating calorimetry, provide information on the relative enthalpy and entropy contributions to the interaction energy. The crystal structure reveals the types of interactions made between streptavidin and the peptide. These interactions are compared to those made by the natural ligand, biotin, in the same binding pocket.

EXPERIMENTAL PROCEDURES

Ligand-Binding Studies. S. avidinii streptavidin was purchased from Calbiochem (La Jolla, CA) and used without further purification. Streptavidin was dissolved at an initial concentration of 10 mg/mL (w/v) in 50 mM potassium phosphate (pH 7.62) or 50 mM sodium acetate (pH 4.91) and dialyzed against the same buffer for 36 h with three 1-L buffer changes. The streptavidin solution was adjusted to approximately 0.6 mM with dialyzate and filtered through a 0.2-μm filter. Peptides were synthesized on an Applied Biosystems peptide synthesizer and purified to greater than 95% homogeneity by HPLC on an acetonitrile-water gradient using a C18 column. Lyophilized peptide was dissolved in dialyzate to an approximate concentration of 6 mM and filtered through a 0.2-μm filter. Final concentrations of streptavidin and peptide were determined by amino acid composition analysis.

Streptavidin solutions were titrated by addition of 18 × 15 μL aliquots of ligand solution at 26 °C at 5-min intervals in a MicroCal Omega titration calorimeter (Northampton, MA) (Wiseman et al., 1989). K_a, $\Delta H°$, and n (stoichiometry per subunit) were obtained through nonlinear least-squares fit of the observed reaction heat for each titration step. Measurements were corrected for heats of dilution of ligand into buffer alone.

Structural Studies. The crystalline peptide–protein complex was prepared by adding a 1000-fold molar excess of lyophilized peptide to a 4-μL droplet containing an apo-streptavidin crystal and allowing equilibration for 24 h. X-ray diffraction data were collected using a Siemens imaging proportional counter and reduced to integrated intensities using XENGEN data reduction software (Howard et al., 1987). Ninety-two percent of the 15 804 possible reflections to 2.0-Å resolution, each measured an average of 4.5 times, merged with a scaling R of 0.069. The crystal belongs to the

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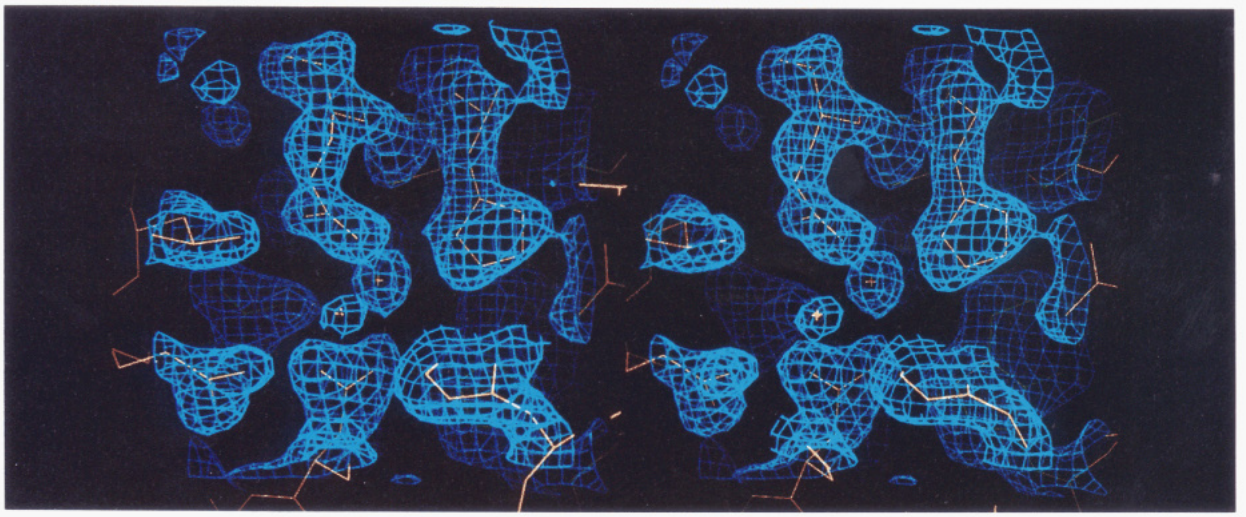

FIGURE 1: Stereoscopic views of electron density corresponding to residues His-Pro-Gln of the screened peptide ligand bound to streptavidin. Contours show the $(2F_o - F_c)\alpha_{calc}$ electron density calculated using data from 10- to 2.0-Å resolution.

orthorhombic space group $I222$ (a = 95.2 Å, b = 106.6 Å, c = 47.9 Å) and contains two streptavidin–peptide complexes per crystallographic asymmetric unit. Crystallographic refinement was initiated using the refined apostreptavidin structure, previously determined at 1.94-Å resolution (Weber et al., 1992).

Positive density in a 2.5-Å resolution $(F_o - F_c)\alpha_{calc}$ difference map revealed the structure of the His-Pro-Gln portion of the peptide ligand. The structure was refined using both simulated annealing (Brünger, 1990) and restrained least-squares methods (Hendrickson & Konnert, 1980). The final model includes 1771 protein atoms, 243 water molecules, and 3 residues of the heptapeptide ligand. The root mean square deviations from ideality are 0.025 Å in bond distances and 0.039 Å in angle distances in the final model. The crystallographic R-value is 0.179 and 15 592 reflections greater than the background signal between 10- and 2.0-Å resolution.

RESULTS

The His-Pro-Gln portion of the heptapeptide Phe-Ser-His-Pro-Gln-Asn-Thr is most clearly defined in electron density maps (Figure 1). Weaker density is found for the flanking Ser and Asn residues. Lower levels of discontinuous electron density can be seen for the terminal residues, suggesting that they interact with surface streptavidin residues. Conformational flexibility of the amino terminus is consistent with observed sequence diversity in residues amino terminal to the conserved histidine where any residue except His, Lys, or Arg was found among the screened peptides (Devlin et al., 1990; Lam et al., 1991). Exclusion of positively charged residues at this position may reflect electrostatic repulsion with a nearby lysine, residue 121 from a symmetry-related subunit of the streptavidin tetramer.

The ordered segment of the peptide binds in a compact conformation where the histidine and glutamine side chains curl around to form a loop closed by hydrogen bonding to a water molecule. Both the peptide histidine and glutamine side chains form hydrogen bonds with the immobilized water molecule (Figures 2 and 3). The loop formed by the peptide and water molecule is attached to the protein via hydrogen-bonding interactions between the water and an aspartic acid, Asp128, between the peptide glutamine and Ser27, and between the peptide histidine and a side-chain oxygen from Thr90 (Figures 2 and 3). The isotropic temperature factors of the glutamine and histidine side chains of the peptide are lower than for their backbone atoms and the entire proline residue, indicating that the extended hydrogen-bonding network fixes the peptide in place at the biotin-binding site.

Structural searches of the Brookhaven Protein Data Bank (Bernstein et al., 1977) were undertaken to determine how characteristic the features of the screened peptide are among known protein structures. A single occurrence of His-Pro-Gln was located in a β-sheet of aspartate transcarbamylase (1AT1; Gouaux & Lipscomb, 1990), and one His-Pro-Met sequence was found at an α-helix amino terminus in citrate synthase (5CSC; Weigand et al., 1984). These examples have both backbone and side-chain conformations different from those of the screened peptide His-Pro-Gln sequence, although the conformation of the screened peptide is energetically allowed and is observed in different sequence contexts in globular proteins. Searches for His-Pro-X, His-X-Gln, and X-Pro-Gln substructures (Finzel et al., 1989) identified several sequences whose backbone conformations for either the His-Pro or Pro-Gln segment were similar to that of the screened peptide. None of the His-X-Gln sequences possessed similar backbone conformations or incorporated a water bridging the His and Gln side chains. Consequently, the specific stuctural determinants observed for the bound screened peptide appear to be relatively uncommon among globular protein structures.

It is interesting to compare the binding interactions made between the screened peptide and streptavidin with those of the natural streptavidin ligand, biotin. Although the peptide proline side chain and the biotin tetrahydrothiophan ring and valeric acid aliphatic chain occupy roughly corresponding locations in the hydrophobic binding pocket, there is little atom-for-atom correspondence between the ligands (Figure 2). When biotin binds to streptavidin, a flexible loop (residues 47–51) becomes ordered as it folds over to bury the ligand (Weber et al., 1989). This loop remains disordered in the peptide complex, and Ala46 and Ser45 move outward to accommodate the larger volume of the peptide in the binding site. Both the peptide and biotin make hydrogen-bonding interactions with streptavidin residues Asp128, Thr90, and Ser27. Asp128 and Ser27 are involved in a hydrogen bond network through the biotin ureido ring, and Thr90 interacts with the biotin sulfur atom (Figure 2b). In the case of the peptide, these residues participate in an analogous network that extends through the histidine and glutamine side chains and incorporates a water molecule that bridges the peptide histidine and glutamine side chains (Figures 2 and 3). An

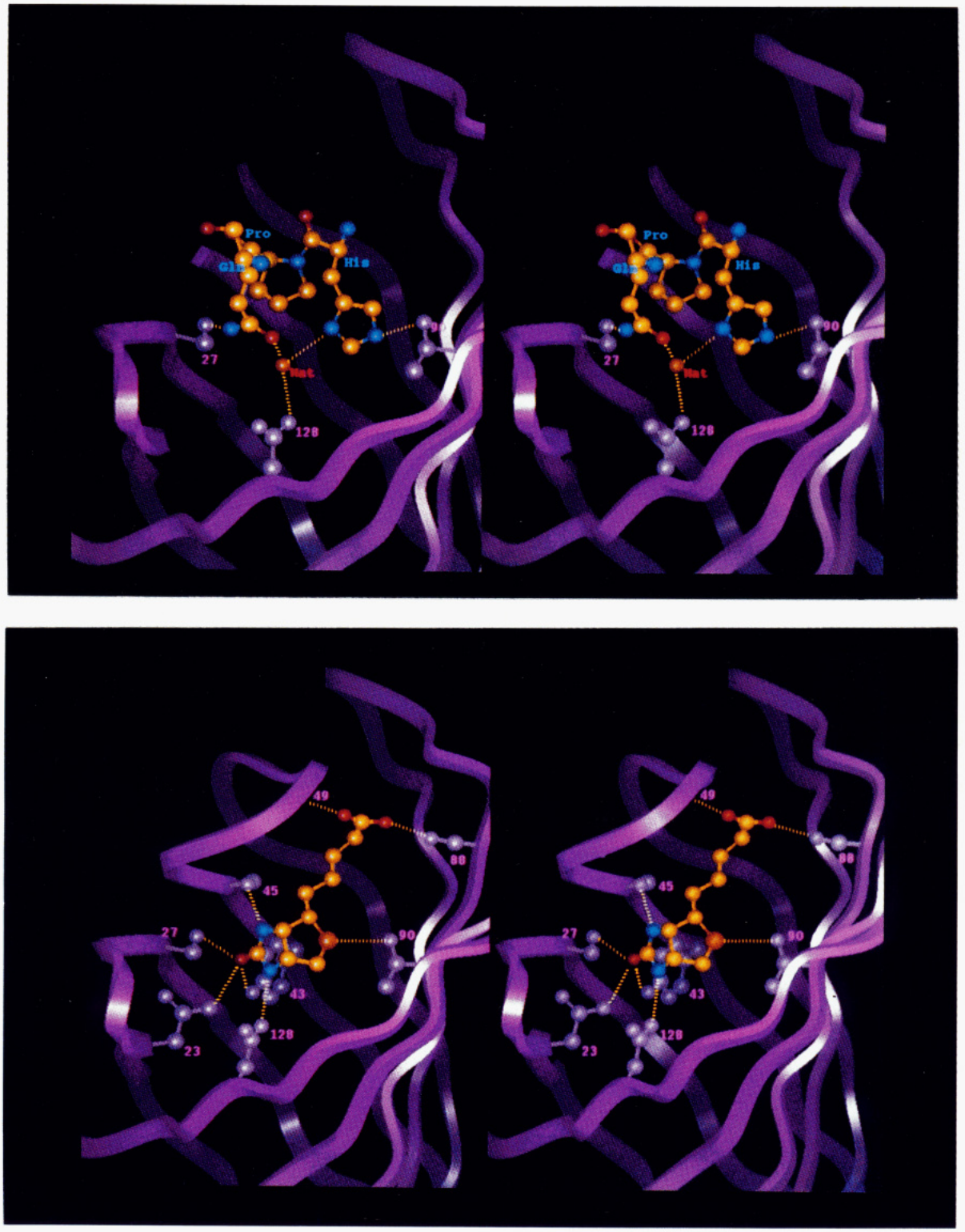

FIGURE 2: Stereoviews of the streptavidin–peptide (a, top) and streptavidin–biotin (b, bottom) complexes. Ligand to protein hydrogen bonds and electrostatic interactions are dashed. Disordered residues 47–52 in the streptavidin–peptide complex are indicated by a break in the streptavidin chain in the upper left corner of (a). Corresponding residues in the streptavidin–biotin complex (b) are ordered in the electron density maps.

important contributor to biotin binding is the polarizability of the ureido group which allows the ureido oxygen to participate as an acceptor of three hydrogen bonds from Ser27, Tyr43, and Asn23 (Weber et al., 1989, 1992). This arrangement is not present in the peptide interaction where no hydrogen bond acceptor occupies a position corresponding to that of the ureido oxygen.

Relatively large heats of reaction were observed when streptavidin was titrated with peptide (Figure 4). These were fitted to generate the thermodynamic parameters of binding (Figure 4, Table I). Both heptapeptides formed a relatively weak 1:1 complex with streptavidin subunits. The association constants differed by about a factor of 2 for the two heptapeptides, Phe-Ser-His-Pro-Gln-Asn-Thr and His-Asp-His-Pro-Gln-Asn-Leu, which contained four of seven residues (His-Pro-Gln-Asn) in common. The electron density maps indicated that peptide residues at both sides of the His-Pro-Gln segment interact with the streptavidin surface, so that the

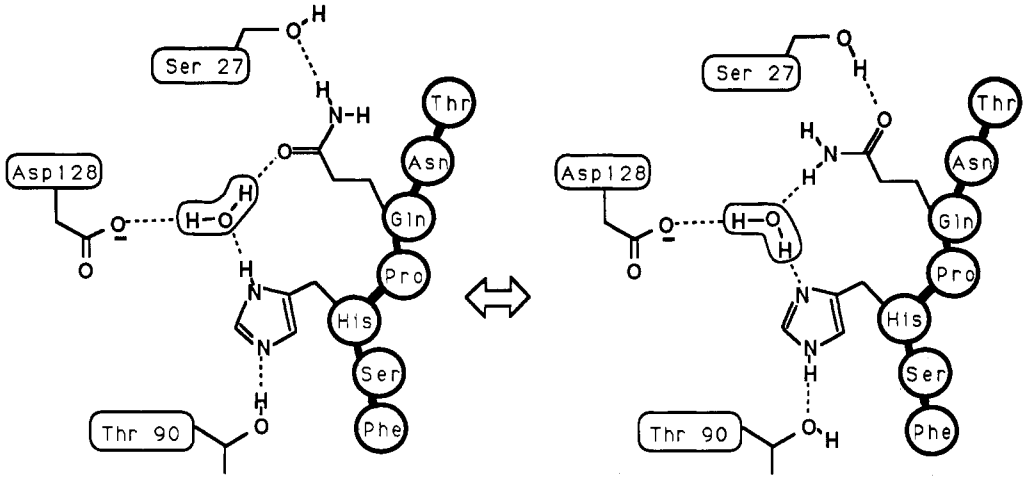

FIGURE 3: Schematic illustration of alternate streptavidin–His-Pro-Gln interactions at neutral pH. Circles show the peptide sequence.

Table I: Thermodynamic Parameters for Streptavidin–Ligand Interactions[a]

ligand	K_a (M^{-1})	$\Delta G°$ (kcal/mol)	$\Delta H°$ (kcal/mol)	n	$T\Delta S°$ (kcal/mol)
FSHPQNT	7944 (±224)	−5.32 (±0.01)	−19.34 (±0.48)	1.06 (±0.03)	−14.0
HDHPQNL	3542 (±146)	−4.84 (±0.03)	−19.00 (±0.64)	0.96 (±0.02)	−14.2
d-biotin[b]	2.5×10^{13}		−32.0		

[a] Experiments were performed in 50 mM potassium phosphate, pH 7.62, buffer at 25 °C. The data were analyzed by assuming four noninteracting sites on the streptavidin tetramer. The fitting parameters of K_a, $\Delta H°$, and n were obtained through a nonlinear least-squares fit of the reaction heat observed for each ith step of a titration experiment for a simple association reaction (see text). Data are reported as the mean for two separate titrations with ±1 standard deviation in parentheses. [b] Weber et al., 1992.

observed changes in binding affinity may reflect the differences in interactions made by the terminal residues. Nearly a 1000-fold reduction in binding of the tetrapeptide His-Pro-Gln-Tyr is also consistent with the idea that extended interactions occur between longer peptides and streptavidin.

The small overall free energies of binding indicate that the favorable enthalpies are largely offset by unfavorable entropy effects (Table I). Although the release of a number of water molecules bound at the biotin-binding site in apostreptavidin entropically favors peptide binding, the ordering of the His-Pro-Gln sequence, and the accompanying restriction in motion of the remaining peptide residues, even to the extent observed in the crystal structure, is likely to account for some of the unfavorable reaction entropy.

Both peptides bind streptavidin with nearly a 1000-fold higher affinity at pH 7 than at pH 5, suggesting that the uncharged form of the histidine is preferred in the binding site. The preference for a singly protonated imidazole may reflect increased entropy in the hydrogen-bonding network that stabilizes the peptide–streptavidin complex. In its singly protonated state at neutral pH, the histidine imidazole can participate in hydrogen-bonding networks with its proton on either the δ- or ϵ-nitrogen (Figure 3). The ability to form what are probably nearly energetically equivalent hydrogen-bonding networks provides a type of resonance stabilization to the peptide–streptavidin complex that includes a favorable entropic contribution due to reorientations of the bound water molecule, the hydroxyl groups of Thr90 and Ser27, and the peptide glutamine side chain. In contrast, the doubly protonated histidine imidazole found at low pH can form a single hydrogen-bonding network in which the relative orientations of the sequestered water molecule and the Thr90 and Ser27 hydroxyls are fixed.

The greater affinity for the uncharged form of the peptide histidine is somewhat surprising given that the biotin-binding site contains an aspartic acid side chain. Although formation of an electrostatic interaction between a positively charged

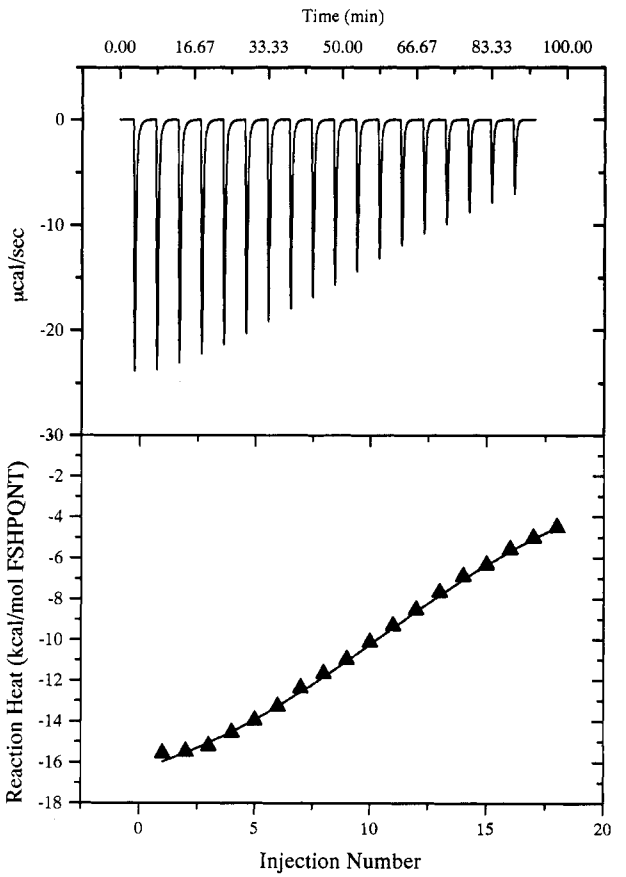

FIGURE 4: Upper panel: measured heats of reaction for titrating streptavidin with the peptide Phe-Ser-His-Pro-Gln-Asn-Thr at neutral pH. Lower panel: fitted binding curve for data shown in the upper panel (see text).

histidine and aspartic acid side chains may be anticipated, the free energy of transfer to the binding site might be less favorable for the positively charged histidine, which would be expected

to be better solvated as a charged rather than an uncharged species. In this context, it should also be pointed out that both biotin and azobenzoic acid dyes bind streptavidin as negatively charged molecules (Weber et al., 1988, 1992).

DISCUSSION

Purified heptapeptides whose sequences were obtained by random screening methods (Devlin et al., 1990; Lam et al., 1991) bind streptavidin with 0.1–1.0 affinities (Table I). A major factor in stabilizing the peptide–streptavidin complex is formation of a hydrogen-bonded network that includes side chains of the His-Pro-Gln sequence conserved among screened peptide sequences (Devlin et al., 1990; Lam et al., 1991), streptavidin Asp128, Thr90, and Ser27 side chains, and an immobilized water molecule. Electron density maps and binding data indicate that residues on either side of the His-Pro-Gln sequence interact with the streptavidin surface. This suggests that more stringent screening assays might reveal a longer concensus sequence. Indeed, the ability to initially detect such weakly binding peptides (Devlin et al., 1990; Lam et al., 1991) may only be possible given that the tetrameric streptavidin can simultaneously bind several of the multiple peptides displayed on the phage surface (Devlin et al., 1990) or synthetic bead (Lam et al., 1991).

These results have implications for drug discovery using peptide epitope strategies. Although the consistent recurrence of His-Pro-Gln-containing sequences among the *several million* peptides screened argues convincingly for an optimal binding sequence, the peptide utilizes only a subset of the possible interaction sites of the binding pocket. This reflects in part the limited chemical functionality of the peptide, which lacks an analogue of the polarizable ureido group important for tight binding of biotin. This general limitation might be overcome by using synthetic strategies that incorporate combinations of modified or nonpeptide fragments for initial epitope selection. It seems possible that computer modeling from the crystal structure of the peptide complex could produce nonpeptide ligands that incorporated features of biotin, since analogous hydrogen-bonding networks are observed in the crystal structures of both ligand complexes. However, the stereochemical features of biotin that produce a tight fit in the binding pocket are not easily derived from the bound peptide structure, since there is little detailed correspondence between the ligand structures (Figure 2) and the peptide ligand itself prevents ordering of a surface loop which buries biotin. Drug design directly from the peptide sequence is much more difficult, since the peptide assumes a distinctive conformation that depends on a bridging water molecule whose existence would be unknown in the absence of the crystal structure.

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Registry No. His-Asp-His-Pro-Gln-Asn-Leu, 143063-73-4; Phe-Ser-His-Pro-Gln-Asn-Thr, 143063-74-5; streptavidin, 9013-20-1; biotin, 58-85-5.