Topochemical Catalysis Achieved by Structure-based Ligand Design*

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Recently, a cyclic peptide ligand, cyclo-Ac-[CHPQGPPC]-NH2, that binds to streptavidin with high affinity was discovered by screening phage libraries. From the streptavidin-bound crystal structures of cyclo-Ac-[CHPQGPPC]-NH2 and of a related but more weakly binding linear ligand, FSHPQNT, we designed linear thiol-containing streptavidin binding ligands, FCHPQNT-NH2 and Ac-CHPQNT-NH2, which are dimerized catalytically by the streptavidin crystal lattice of space group I222, as demonstrated by high performance liquid chromatography and mass spectrometry. The catalytic dimerization relies on presentation of the ligand thiois toward one another in the lattice. The streptavidin crystal lattice-mediated catalysis achieved by structure-based design is the first example of catalysis of a chemical reaction by a protein crystal scaffold. The spontaneous and crystal catalyzed rates of disulfide formation were determined by high performance liquid chromatography at pH 3.1, 4.0, 5.0, and 6.0. The ratio of the catalyzed to uncatalyzed rate was maximal at pH 3.1 (kcat/kuncat = 3.8), diminishing to 1.2 at pH 6.0. The crystal structures of the streptavidin-bound dimerized peptide ligands, FCHPQNT-NH2 dimer at 1.95 Å and Ac-CHPQNT-NH2 dimer at 1.80 Å, are described and compared with the structures of streptavidin-bound FSHPQNT monomer and cyclo-Ac-[CHPQGPPC]-NH2 dimer.

Dimerization is a trigger for many important biological processes (1–4). Recently we described a structure-based design strategy for the preparation of ligands that dimerize receptors by topochemistry (5), which involves a chemical reaction mediated by holding reactants in close proximity and in productive orientations on a protein or crystal scaffold. The strategy for preparation by topochemistry of receptor dimerizing molecules is as follows. First, a protein ligand for a receptor monomer is developed by structure-based design or discovered by screening. The three-dimensional structure of the protein-ligand complex is then determined by crystallography. Ligating groups of the appropriate length and orientation are then introduced at sites in the ligand in a region distinct from the binding motif. Dimerization of two such ligands, each bound on a receptor monomer of a native receptor dimer complex, is directed by the receptor monomers, which present the reactive ligating groups of the ligands next to one another. A ligand dimer capable of dimerizing the receptor to produce a native or native-like receptor dimer is thus prepared by topochemistry.

This strategy for preparing a receptor-dimerizing ligand was demonstrated (5) with streptavidin using a high affinity cyclic peptide ligand, cyclo-Ac-[CHPQGPPC]-NH2, with a Kd of 670 nM at pH 7.3, discovered by screening phage libraries against the streptavidin target (36). Streptavidin, a tetrameric protein with a molecular mass of 13,200 Da/subunit produced by Streptomyces avidini, functions as an antibiotic by binding the vitamin biotin with the highest affinity known for a noncovalent protein-ligand interaction, Kd = 10−15 M (6). This remarkable affinity forms the basis for many bioanalytical applications (7–10), as well as a paradigm for studying the structural basis of high affinity protein-ligand interactions (11–13, 37), and for applying structure-based ligand design strategies (14, 15).

In streptavidin, there are four binding sites, one per subunit. In I222 crystals of streptavidin, one crystallographically equivalent pair, site 1 and site 1′, is near a crystallographic 2-fold axis; the other pair, site 2 and site 2′, is not. When bound to I222 crystals of streptavidin, the intramolecular disulfide bonds of neighboring cyclo-Ac-[CHPQGPPC]-NH2 monomers bound at site 1, presented next to one another, undergo disulfide interchange, thus a C2-symmetric peptide dimer adopting the symmetry of the crystal is produced with two intermolecular disulfide bonds passing through the 2-fold axis. A double-headed streptavidin dimerizing agent is thereby prepared by topochemistry. Concomitant with peptide dimerization is the dimerization of the associated bound streptavidin tetramers. We determined the crystal structure of the protein-peptide dimer complex and demonstrated by HPLC† and mass spectrometry that streptavidin crystals of space group I222 mediate the dimerization (5).

Although I222 streptavidin crystals mediate the disulfide interchange reaction between neighboring bound cyclo-Ac-[CHPQGPPC]-NH2 ligands, they do not catalyze it. The Kd of the product dimer for the two adjacent 2-fold related sites in the I222 streptavidin crystals is ~5 × 10−6 M, on the order of the square of the dimer for a single site, 7.0 × 10−9 M. This high affinity may impede product release and associated catalytic turnover (5). Catalysis of a chemical reaction by a protein crystal lattice has never before been demonstrated.

We reasoned, however, that catalysis could occur for a similar reaction involving a lower affinity, linear peptide whose dimerized product binds weakly enough at the two 2-fold related binding sites in the crystal to allow dissociation and catalytic turnover. Because the measured affinity of the linear peptide FSHPQNT, whose Kd is 64 μM, is several hundred-fold

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†The abbreviations used are: HPLC, high performance liquid chromatography; F0 and Fc, observed and calculated structure factors, respectively.
lower than that of the cyclo-\textit{Ac-[CPhQGPPC]-NH}_2 monomer (36), we wondered whether linear cysteine-containing peptide analogs of \textit{FSpQNT-NH}_2 or of cyclo-\textit{Ac-[CPhQGPPC]-NH}_2 bound to 1222 streptavidin crystals with thiol’s presented toward one another might be catalytically dimerized.

Demonstrated catalysis of a reaction mediated by a protein crystal lattice is a step toward the eventual engineering of such lattices to catalyze diverse chemical reactions. The potential advantages of protein crystals in preparative synthesis, high specific activity, and resistance to inactivation by elevated temperatures and proteolysis have been recognized and demonstrated in the synthesis of peptides with glutaraldehyde cross-linked thermolysin crystals (17).

The design of the thiol-containing ligands, \textit{FChPQNT-NH}_2 and \textit{Ac-CHPQNT-NH}_2, for catalytic dimerization was based on the following observations. In the crystal structure of streptavidin-\textit{FSpPQNT}, the serines of 2-fold related bound peptides are in close proximity; the hydroxyls can be rotated such that the O\textsubscript{Y}-O\textsubscript{Y} distance is 5 Å. Thus we expected that in streptavidin-\textit{FChPQNT-NH}_2, close proximity of 2-fold related cysteines would mediate disulfide formation. Also, because the N-terminal portion of the Ac-\textit{CHPQNT-NH}_2 sequence is identical to that in cyclo-\textit{Ac-[CPhQGPPC]-NH}_2, which dimerizes in the crystal by disulfide interchange, we predicted that streptavidin-bound Ac-\textit{CHPQNT-NH}_2 would also dimerize in the crystal. The structure of the streptavidin-\textit{Ac-CHPQNT-NH}_2 dimer complex in the vicinity of the N-terminal peptide sequence was expected to be similar to that in the streptavidin-cyclo-\textit{Ac-[CPhQGPPC]-NH}_2 dimer complex. The crystal lattice catalyzed dimerization of \textit{FChPQNT-NH}_2 and of Ac-\textit{CHPQNT-NH}_2, described herein, is depicted schematically in Fig. 1.

**EXPERIMENTAL PROCEDURES**

**Peptide Synthesis and Determination of Streptavidin Binding Affinities**—The peptide ligands, \textit{FChPQNT-NH}_2, Ac-\textit{CHPQNT-NH}_2, and \textit{FSpPQNT}, were synthesized with \textit{N-o-Fmoc (9-fluorenylmethoxycarbonyl)-protected} amino acids with acid-labile side chain protecting groups and with acetamidomethyl protected cysteines as described (5, 36).

Streptavidin binding affinities of the peptide monomers were determined by surface plasmon resonance (17, 18) using real-time biopspecific interaction analysis (BIAcore) (19). The BIAcore 2000 system, sensor chip, and coupling reagents were from Pharmacia Biotech Inc. Affinities for each peptide ligand were determined under identical conditions in a competition assay involving a cyclic, disulfide-containing streptavidin binding peptide, cyclo-\textit{Ac-AE-[CPhQGPPC]-EGRK-NH}_2, whose binding affinity has been previously determined (36) by BIAcore, immobilized on the chip surface via the \textit{e-amino group} of the C-terminal lysine residue using standard amine immobilization chemistry (20, 21).

Immobilization on the sensor chip surface was done at a flow rate of 5 μl/min, 25 °C. Between injections of reagents, the sensor chip was continuously washed with running buffer composed of 10 mM HEPES, pH 7.3, 150 mM NaCl, 3.4 mM EDTA, and 0.005% surfactant P20. The sensor surface was activated by a 2-min pulse of a mixture containing 0.20 M N-hydroxysuccinimide and 0.20 M N-ethyl-N-\textit{[3-diethylamino]propyl}-carbodiimide. The cyclo-\textit{Ac-AE-[CPhQGPPC]-EGRK-NH}_2 peptide solution at a concentration of 0.1 mW in 0.1 M sodium borate, pH 8.5, was then injected for 2 min followed by a 7.0-min injection of 1.0 M ethanolamine HCl to inactivate any remaining N-hydroxysuccinimide ester groups. In order to wash out any noncovalently bound peptide, the immobilization condensation with a 2-min pulse of 6.0 M guanidine HCl, pH 2.1.

The competition assay involved preincubation of streptavidin at a concentration of 167 nM with increasing concentrations (from 0 to 7.5 mM) of each of the different peptide ligands. Each streptavidin-peptide ligand solution was injected over the immobilized peptide surface, as well as over a blank surface identical except lacking the immobilized peptide, in the running buffer, at a flow rate of 10.0 μl/min for 4.0 min. After subtracting the nonspecific response for the blank surface from the specific signal of the immobilized peptide surface, the resulting streptavidin binding response was plotted as a function of the competing peptide concentration to produce the inhibition curves shown in Fig. 2.

In order to regenerate the peptide surface before assay of each peptide concentration, 6.0 M guanidine HCl, pH 2.1, was injected to dissociate remaining bound peptide ligand.

Because the concentration of the cyclo-\textit{Ac-AE-[CPhQGPPC]-EGRK-NH}_2 peptide immobilized on the sensor chip is high (~30 μM), the IC\textsubscript{50} values for all the peptide ligands in this study rely on determining the relationship between the apparent association constant \( K_a \) and the true association constant \( K_a^* \). Calculation of the true \( K_a^* \) values of the peptide ligands in this study and the true \( K_a^* \) values determined previously (36), for cyclo-\textit{Ac-AE-[CPhQGPPC]-EGRK-NH}_2 and applying the appropriate correction factor to the \( IC_{50} \) values for all the peptide ligands in this study. Thus, for this purpose, the \( IC_{50} \) for competition of the unbound cyclo-\textit{Ac-AE-[CPhQGPPC]-EGRK-NH}_2 peptide ligand in solution with sensor-bound cyclo-\textit{Ac-AE-[CPhQGPPC]-EGRK-NH}_2 was determined in this study. Similar protocols for determining true \( K_a^* \) values from the results of competition assays involving high concentrations of ligands immobilized on the sensor chip have been described (22, 23).

**High Performance Liquid Chromatography and Mass Spectrometry**—The crystal-catalyzed dimerization of FChPQNT-NH\textsubscript{2} and of Ac-CHPQNT-NH\textsubscript{2}, which dimerizes in the crystal by disulfide interchange, was investigated by HPLC and by HPLC coupled to mass spectrometry by procedures previously described for the crystal-induced dimerization of cyclo-\textit{Ac-[CPhQGPPC]-NH}_2 (5). A Hewlett-Packard 1090 analytical HPLC instrument containing a C18 reverse phase column was used with a linear gradient from 0.05% trifluoroacetic acid in water to 0.05% trifluoroacetic acid in 50% water/50% acetonitrile over a 35-min time period. The fraction dimerized was calculated by dividing the integrated area of the dimer peak by the sum of the integrated areas of the monomer and dimer peaks. HPLC with a Michrom BioResources Ultrafast Microprotein Analyzer coupled to electrospray ionization mass spectrometry with a Finnigan MAT SSQ710 instrument was also performed on selected samples.
fuge tube. After centrifugation of the washed crystals, the supernatant was removed, and fresh washing solution was added. After vortexing, the crystals were left soaking for several more hours. The washing procedure was done a total of three times before reusing the crystals. Because the same batch of crystals of a particular form was used for each pH study, the amount and surface area of the crystals should be the same, and differences in rates should reflect differences in pH only. At pH 5.0, two determinations of the crystal catalyzed rate of disulfide formation were performed, one with recycled crystals and one with fresh crystals. No large differences were observed between these two determinations.

Crystallography of Complexes of Streptavidin with FCHPQNT-NH₂ and Ac-CHPQNT-NH₂ Disulfide-bonded Dimers—Crystals of the streptavidin peptide dimer complexes were prepared by soaking large diamond plate apo-streptavidin crystals grown from ammonium sulfate solution at pH 4.0 (24) for several days in synthetic mother liquor composed of 50% saturated ammonium sulfate, 50% 1.0M Tris, pH 7.0—Crystalsofthestrep-

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\[ \text{FIG. 2. Inhibition curves, calculated by BIACore, for the competition of streptavidin between cyclo-Ac-AE(\text{CHPQGPCC})EGRK-NH₂ immobilized on the sensor chip and Ac-FSPQNT-NH₂, FCHPQNT-NH₂, or CHPQNT-NH₂, dimer in the solution flowing over the sensor chip.} \]

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**RESULTS**

Design of Binding Conditions Appropriate for Product Release and Hence Catalytic Turnover—Fig. 2 shows the inhibition curves for the competition of streptavidin between a high affinity cyclic peptide, cyclo-Ac-AE(\text{CHPQGPCC})EGRK-NH₂ (36), immobilized on the sensor chip and peptide ligands incubated at concentrations from 0 to 5 mM with streptavidin at a concentration of 167 nM in 100 mM HEPES, pH 7.3, in the solution flowing over the chip. The K_d for the FCHPQNT-NH₂ monomer is 10 μM; for the Ac-CHPQNT-NH₂ monomer and dimer produced by incubation in K₃Fe(CN)₆, the K_d values are 17 and 71 μM, respectively. For FSPQNT the K_d, 46 μM is comparable to that determined by microcalorimetry, 125 μM (28).

The affinity for streptavidin of HPQ-containing peptides decrease as the pH decreases from neutral to acidic pH (28). Thus an expected decrease in affinity at low pH for the thiol-containing peptide ligands is expected to facilitate dissociation and permit catalytic turnover at low pH.

HPLC and Mass Spectrometry Show That I222 Streptavidin Crystals Catalyze Disulfide Formation—Freshly dissolved FChPQNT-NH₂ is predominantly monomeric (Fig. 3A), and treatment of the peptide with K₃Fe(CN)₆ to promote disulfide formation results in complete dimerization, yielding a peak of greater retention time (Fig. 3B). Incubation at pH 4.0 of the FCHPQNT-NH₂ monomer for 3 days in the presence of crushed I222 streptavidin crystals induces an increase in the dimer peak along with a concomitant decrease in the monomer peak (Fig. 3C). Incubation of the peptide monomer alone (Fig. 3D) or in the presence of noncrystalline streptavidin (Fig. 3E) under

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\[^2\text{Because the program X-PLOR does not allow a bond between symmetry-related atoms, the van der Waals radii of the 2-fold related sulfurs in the disulfides were decreased to zero. In such refinements there is no constraint on the S-S bond length or any other disulfide parameters involving atoms from both monomers (such as } \chi_3, \text{ the dihedral about the S-S bond).} \]

\[^3\text{B. A. Katz, unpublished observations.} \]

\[^4\text{B. A. Katz and R. T. Cass, unpublished observations.} \]
The same conditions yield significantly less dimer. Thus the I222 streptavidin crystals accelerate disulfide formation. In Fig. 3F peptide monomer at a molar ratio of at least 21-fold with respect to crystalline streptavidin subunits was 87% dimerized after 6 days, showing that catalytic turnover occurs in I222 crystals, which act as a heterogeneous catalyst for the disulfide formation reaction. A similar catalytic effect was observed for Ac-CHPQNT-NH\textsubscript{2} (data not shown).

Table I

|                  | FCHPQNT-NH\textsubscript{2} dimer | Ac-CHPQNT-NH\textsubscript{2} dimer |
|------------------|----------------------------------|----------------------------------|
| No. atoms (including disorder)\textsuperscript{a} | 2143                             | 2106                             |
| No. discretely disordered groups\textsuperscript{b} | 7                                | 6                                |
| No. waters (including disorder) \textsuperscript{c} | 191                               | 157                              |
| No. side chains with refined occs\textsuperscript{a} | 3                                | 3                                |
| Diffraction Statistics |                                 |                                   |
| Resolution (Å)    | 50-1.74                          | 50-1.74                          |
| No. observations | 46268                            | 60759                            |
| No. unique observations | 20222                              | 19758                            |
| Redundancy        | 2.3                              | 3.1                              |
| R\textsubscript{merge} (%) | 8.9                                | 7.4                              |
| Refinement Statistics |                                 |                                   |
| Refinement resolution | 7.5-1.95                           | 7.5-1.80                          |
| No. merged reflections | 13414                              | 17106                            |
| \[F_{\text{w}}/F_{\text{o}}\] cut-off | 1.5                              | 1.0                              |
| R\textsubscript{free} (%) | 19.1                               | 18.9                             |
| Overall completeness (%) | 72.2                              | 74.0                             |
| At highest resolution (%) | 46.3                              | 43.0                             |
| RMS Deviations\textsuperscript{d} |                                 |                                   |
| Bond lengths (Å)  | 0.019                            | 0.018                            |
| Bond angles (°)   | 3.7                               | 3.5                              |
| Torsion angles (°) | 27.6                             | 27.6                             |

\textsuperscript{a} Restricted, isotropic temperature factors were refined for both structures. Bulk solvent contributions were included for both structures.

\textsuperscript{b} Two major conformations were found for these residues.

\textsuperscript{c} These waters were disordered between two positions less than 2.5 Å from one another.

\textsuperscript{d} Also includes ligand groups. Density for all side chain atoms or for terminal atoms in these groups was weak or absent, and temperature factors were high. Discretely disordered groups are not included in this category. Occupancies for poorly defined groups of atoms were refined. Occupancies for complete residues (main chain plus side chain atoms) for disordered loops and N termini were also refined.

\textsuperscript{e} Data with R\textsubscript{free} > 50% were rejected along with data with values > 3.5 \( \sigma \) from the mean for each bunch of symmetry equivalents.

\textsuperscript{f} R\textsubscript{merge} = \[\sum_{i} \sum_{h} \{ |F_{i}(h) - \langle F_{i}(h) \rangle\} / \sum_{i} \sum_{h} \langle F_{i}(h) \rangle\], where \( \langle F_{i}(h) \rangle \) is the \( h \)-th observation of the intensity of reflection \( h \).

\textsuperscript{g} R\textsubscript{merge} = \[\sum_{i} \{ |F_{i}(h) - \langle F_{i}(h) \rangle| / \langle |F_{i}(h)\rangle\} \] (for reflections from 7.5 Å to the highest resolution).

\textsuperscript{h} Cross-validation R-factor using 10% of the data withheld from the refinement (25).

\textsuperscript{i} Root mean square deviations from ideal bond lengths, bond angles, and torsion angles.

5 To establish the crystal form of these microcrystals in the incubation samples, they were used to seed eight streptavidin crystallization drops. Six of the eight drops seeded with these microcrystals yielded showers of small diamond plates before ten unseeded drops had crystallized (all 10 unseeded drops eventually yielded large diamond plates). Two drops seeded with diamond plates that were used in the incubations behaved similarly to the other seed drops.
seen that no catalysis will occur above pH \(-6.25\).

The Disulfide-bonded Ligands Dimerize Streptavidin—Fig. 6 shows the \(|2F_o - F_c|\), \(\alpha_c\) map for the refined crystal structure of streptavidin-Ac-CHPQNT-NH\(_2\) dimer, pH 7.0. The numerous, intricate hydrogen bonding and van der Waals interactions that mediate the binding of HPQ-containing peptide ligands to streptavidin have been described (37) and are shown in Fig. 6 and briefly summarized in the legend. The intermolecular disulfide, defined by weak density, is in a position similar to that for the N-terminal disulfide in streptavidin-cyclo-Ac-[CHPQGPPC]-NH\(_2\) dimer. The \(S_i-S_i\) distance is 2.06 Å, and the \(\chi_3\) dihedral is 71°, which are close to the optimal values for a right-handed disulfide (29).

Fig. 7 shows the \(|2F_o - F_c|\), \(\alpha_c\) map superimposed on the refined structure of streptavidin-FCHPQNT-NH\(_2\) dimer, pH 7.0. The stacking of the N-terminal peptide Phe residues against one another may help stabilize the protein-ligand dimer complex. There are potential NH \(\cdots\) aromatic \(\pi\) interactions (30), shown in cyan, between the C-terminal amide NHs and the Phe aromatic rings.

Large Shifts in Protein and Peptide Coordinates Accompany Dimerization—Overlaid in Fig. 8 are the refined structures of the complexes of streptavidin with FCHPQNT-NH\(_2\) (37) and with the disulfide-bonded dimers of FCHPQNT-NH\(_2\), Ac-CHPQNT-NH\(_2\), and cyclo-Ac-[CHPQGPPC]-NH\(_2\) (5). Global shifts in protein and peptide ligand coordinates occur due to changes in crystal packing that often involve significant contraction or expansion in the crystal lattice (5). For each pair of structures compared the root mean square deviations between corresponding atoms of core residues range from 0.25 to 0.63 Å (Table II). The root mean square deviations for residues at the binding site exhibiting the most structural plasticity range from 0.21 to 0.87 Å (Table II). Disulfide formation and dimerization causes large differences in the positions of the stacking peptide Phe residues, shown in green balls and sticks, in the streptavidin-bound FCHPQNT-NH\(_2\) dimer structure compared with their more separated positions, shown with pink balls and sticks, in the streptavidin-bound FSHPQNT monomer structure.

**DISCUSSION**

Structural Constraints Involved in Crystal Lattice Mediation of Disulfide Formation between Streptavidin-bound Ligands—In I222 streptavidin crystals, the thiolate of bound neighboring 2-fold related FCHPQNT ligands are estimated to be separated by \(-5.0\) Å, based on the structure of streptavidin-FSHPQNT (37), and the thiolate of neighboring Ac-CHPQNT-NH\(_2\) ligands are estimated to be separated by \(-3.6\) Å, based on the structure of streptavidin-cyclo-Ac-[CHPQGPPC]-NH\(_2\) monomer (37). Thus in I222 streptavidin crystals, disulfide formation can be catalyzed when the ligand thiolate are positioned 3.6-5.0 Å from one another in appropriate orientations.
In I4122 streptavidin crystals (37), bound ligands are also presented next to one another (Fig. 9). In the I4122 crystal structure of streptavidin-cyclo-Ac-[CHPQFC]-NH2, the 2-fold related N-terminal sulfurs are separated by 10.8 Å (Fig. 9) and can be brought to a separation of 6.4 Å by breaking the disulfide bond and rotating about the Cβ-Sγ bond. No enhancement of disulfide formation was observed upon incubation of FCH-PQNT-NH2 or Ac-CHPQNT-NH2 in apostreptavidin crystals of space group I4 122. Thus, a separation of ~6.4 Å between neighboring bound thiol may be too large to allow catalysis of disulfide formation. The lack of catalysis by the tetragonal crystals could also reflect nonoptimal orientations of the bound ligand thiol.

The streptavidin I222 crystal lattice mediates formation by disulfide interchange of a “head to head” disulfide bonded cyclo-Ac-[CHPQGPPC]-NH2 peptide dimer from the corresponding bound monomers (5). Fig. 8 shows the relationship of the neighboring streptavidin-bound cyclo-Ac-[CHPQGPPC]-NH2 monomer units (in white) of the peptide dimer in the I222 lattice. The relationship of the neighboring streptavidin-bound cyclo-Ac-[CHPQGPPC]-NH2 monomers in the I4, 22 lattice is different; the Sγ atom of the N-terminal Cys is closer to the Sγ atom of the C-terminal Cys of the neighboring ligand than to the N-terminal Sγ of the neighboring ligand (Fig. 9). The N- and C-terminal Sγ atoms can be brought to a separation of 4.8 Å by breaking the disulfide bond and rotating about the Cβ-Sγ bond (Fig. 9).

This relationship suggested that for some ligands that contain a disulfide or 2 thios, “head to tail” dimerization could be promoted or catalyzed by the I4, 22 lattice. Whereas in both space groups neighboring bound ligands are related by 2-fold symmetry axes, the 2-fold axis relating the neighboring streptavidin-bound cyclo-Ac-[CHPQGPPC]-NH2 ligands in I222 crystals is along the x-direction of Fig. 8, appropriate for head to head dimerization, whereas in I4, 22 crystals it is along...
the z-direction of Fig. 9, appropriate for “head-to-tail” dimerization. Recently we showed that streptavidin crystals of space group 14,22 promote head to tail dimerization of cyclo-Ac-[CHPQGPPC]-NH$_2$.$^3$

Engineering Protein Crystal Lattices as Biocatalysts for Chemical Reactions—An industrially and technologically significant field of chemistry involves heterogeneous catalysis by small molecule crystals or crystal surfaces (31, 32). The catalysis of disulfide formation between thiols of neighboring ligands in streptavidin is the first example of catalysis mediated by a protein crystal lattice. Key features of the design of the ligands that are catalytically dimerized are weaker binding of
linear peptide ligands compared with their cyclic counterparts and weaker binding at low pH, which allows product dissociation and catalytic turnover.

The I222 streptavidin lattice at the ligand binding sites resembles an enzyme in several features: binding of the adjacent reactive ligands is in productive orientations for the ensuing reaction; binding involves H-bonds, ordered water molecules, and hydrophobic interactions at several subsites away from the ligand. Best viewed by zooming in.

**Table II**

Comparison of structures of complexes of streptavidin with FSHPQNT, with FCHPQNT-NH$_2$ dimer, with Ac-CHPQNT-NH$_2$ dimer, and with cyclo-Ac-[CHPQGPPC]-NH$_2$ dimer

|                  | FSHPQNT | FCHPQNT-NH$_2$ dimer | Ac-CHPQNT-NH$_2$ dimer | Cyclo-Ac-[CHPQGPPC]-NH$_2$ dimer |
|------------------|---------|----------------------|------------------------|----------------------------------|
| FSHPQNT          |         |                      |                        |                                  |
| FCHPQNT-NH$_2$ dimer | 0.402   | 0.253                | 0.355                  |                                  |
| Ac-CHPQNT-NH$_2$ dimer | 0.457   | 0.366                | 0.631                  |                                  |
| Cyclo-Ac-[CHPQGPPC]-NH$_2$ dimer | 0.208   | 0.358                | 0.448                  |                                  |

**Fig. 9.** Structure of I4122 streptavidin-cyclo-Ac-[CHPQFC]-NH$_2$ in the vicinity of the 2-fold related ligand binding sites.

**Fig. 10.** Cavity in I222 streptavidin crystals looking down the 2-fold axis where catalytic dimerization of FCHPQNT-NH$_2$ and of Ac-CHPQNT-NH$_2$ occurs. Residues from one subunit are labeled in white; residues from the crystallographically inequivalent subunit are labeled in yellow.
from the “active site”; the effective concentrations of the reactants are greatly raised; and the crystal packing interactions must marginally stabilize the transition state to yield the most rate enhancements observed. Also, just as participation of more than one subunit in enzyme catalysis is a common theme of biochemistry, topochemical catalysis within a protein crystal lattice also relies on adjacent protein molecules acting in concert.

Structure-based enzyme engineering (33, 34) bridges the disciplines of chemistry, structural biology, and biotechnology. The design of protein crystal lattices capable of catalyzing targeted reactions is a challenge with the potential to enlarge the scope of engineering artificial enzyme systems. Such catalyzed reactions may include ligation reactions like asymmetric synthesis of peptides, peptidomimetics, or organic molecules.

There are some features of protein crystal lattices that could be exploited in the design of biocatalysts. The solvent channels contain space for insertion by mutagenesis of engineered loops or segments bearing catalytic residues onto the surface of the protein comprising the crystal. The size and site of insertion must preserve the ability of the protein to crystallize in the intended lattice. A catalytic active site engineered at a crystal packing interface could allow access of relatively large substrates to and from the active site for catalytic turnover. For example, the cavity or channel between the 2-fold related neighboring ligand sites in I222 streptavidin crystals is about 18.9 × 12.6 Å (Fig. 10). Finally, the ability of some protein crystal lattices, like the I222 streptavidin lattice, to expand and contract in response to the binding of different ligands allows some tolerance to accommodate substrates that may not fit precisely in the existing lattice. This variability in crystal packing within a crystal lattice resembles structural plasticity within the protein itself, which also increases the repertoire of potential ligands.

An advantage of protein crystal catalysts is stability toward autolysis and exogenous proteolysis (16). Furthermore, the intermolecular protein-protein interactions within the crystal lattice may significantly enhance stability against heat and other denaturants by preventing unfolding, aggregation, or dissociation of the proteins (35). In addition, stability toward organic solvents to increase the scope of possible catalyzed reactions can be attained by covalent cross-linking of neighboring proteins in the lattice (16). Cross-linking in the presence of tightly bound ligands at or near the engineered active site following removal of such ligands would leave the active site intact (cross-link-free) in an engineered crystal cross-linked elsewhere. Such stable protein crystal lattices engineered to catalyze targeted reactions may well have industrial and biomedical applications in the future.

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