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# Crystallographic Data for *Streptomyces avidinii* Streptavidin*

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**Crystallization conditions are reported for *Streptomyces avidinii* streptavidin with and without bound biotin. X-ray examination of the free and bound crystal forms shows the streptavidin-biotin complex crystals to be most suitable for high resolution structure analysis. A complete x-ray data set to 2.6 Å resolution was collected for the streptavidin-biotin crystals using a two-dimensional area detector. Reduction and analysis of the x-ray diffraction pattern show that the complex crystallizes in the tetragonal space group $I4_122$ ($a = b = 98.4$ Å, $c = 125.8$ Å), with half of the streptavidin tetramer in the crystallographic asymmetric unit.**

Streptavidin is tetrameric protein from the yeast strain *Streptomyces avidinii*. Each $M_r$ 15,000 subunit of the tetramer binds one biotin molecule with extremely high affinity ($K_d$ $\sim 10^{-15}$ M), although without apparent formation of streptavidin-biotin covalent bonds (1). Streptavidin was first characterized by Chaiet and Wolf (2), who noted the relatively high tryptophan content of the protein (6/monomer) and total absence of the sulfur-containing amino acids, methionine and cysteine. In subsequent work cloning streptavidin, the protein was found to be homologous with the hen egg white avidin (3). Nevertheless, avian avidin subunits are smaller (125 residues) than streptavidin monomers (159 residues). In addition, avian avidin is naturally glycosylated, while streptavidin is not. Streptavidin is an interesting candidate for x-ray structural studies. There is a basic interest in discovering the structural origins of its high affinity for biotin. The structure determination should also aid in engineering the protein to improve its properties in applications that depend on its specific and high affinity binding properties (4, 5). Here we report crystallization conditions for streptavidin and a streptavidin-biotin complex, together with a crystallographic characterization of the complex crystals that demonstrates their suitability for x-ray structural studies.

Crystallization experiments were carried out with lyophilized preparations of *S. avidinii* streptavidin obtained from Behring Diagnostics and used without further purification. Crystallization reagents included polyethylene glycol 8000, dibasic sodium phosphate, lithium chloride, ammonium sulfate, *d*-biotin (all obtained from Sigma), and citric acid monohydrate (Fisher). Reagents were used as supplied without further purification.

Crystals of streptavidin and streptavidin-biotin complex were both grown by vapor diffusion using the hanging drop technique (6). Biotin-free streptavidin crystals were grown using a 20 mg/ml streptavidin solution in 50 mM MES,[1] pH 6.0. In successful experiments, a 5-μl droplet of the protein solution was mixed with 5 μl of a buffered 30% saturated ammonium sulfate solution on a siliconized coverslip. The protein droplets were equilibrated against 1 ml of similarly buffered ammonium sulfate solution in Linbro plates (Flow Laboratories, McLean, VA) in the usual manner (7). Within 2 weeks at 4 °C, stacks of platelike crystals (typically 0.05 × 0.2 × 0.2 mm) were observed over a wide range of solution buffer conditions including 0.1 M CAPS, pH 10.4, 0.1 M

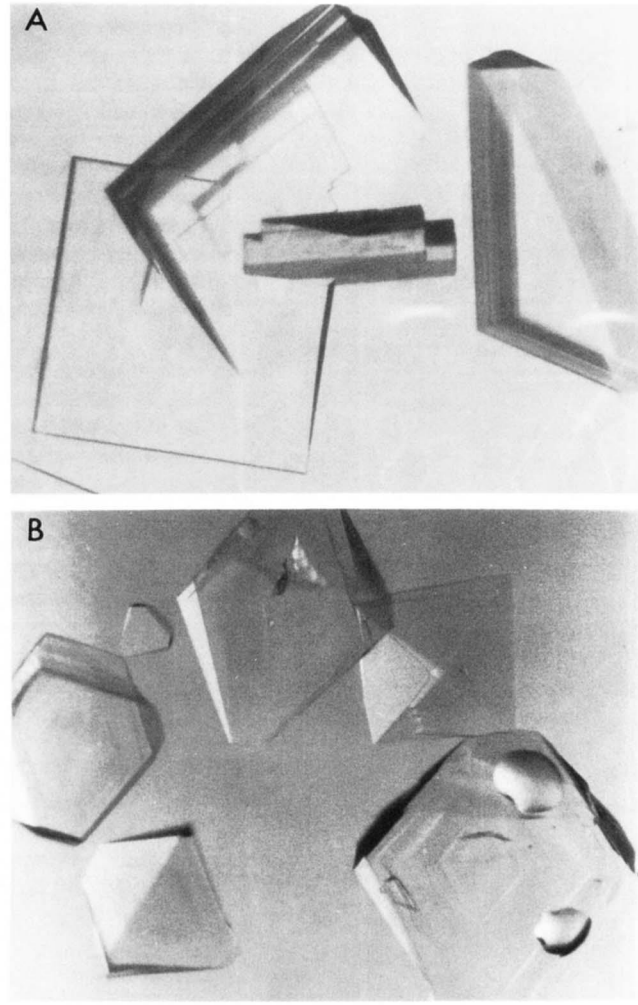


FIG. 1. **Photomicrographs of streptavidin crystals (*part A*) and crystals of a streptavidin-biotin complex (*part B*).** Both pictures are the same relative scale (1:10).

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[1] The abbreviations used are: MES, 2-(*N*-morpholino)ethanesulfonic acid; CAPS, 3-[cyclohexylamino]-1-propanesulfonic acid; CHES, 2-(*N*-cyclohexylamino)ethanesulfonic acid.

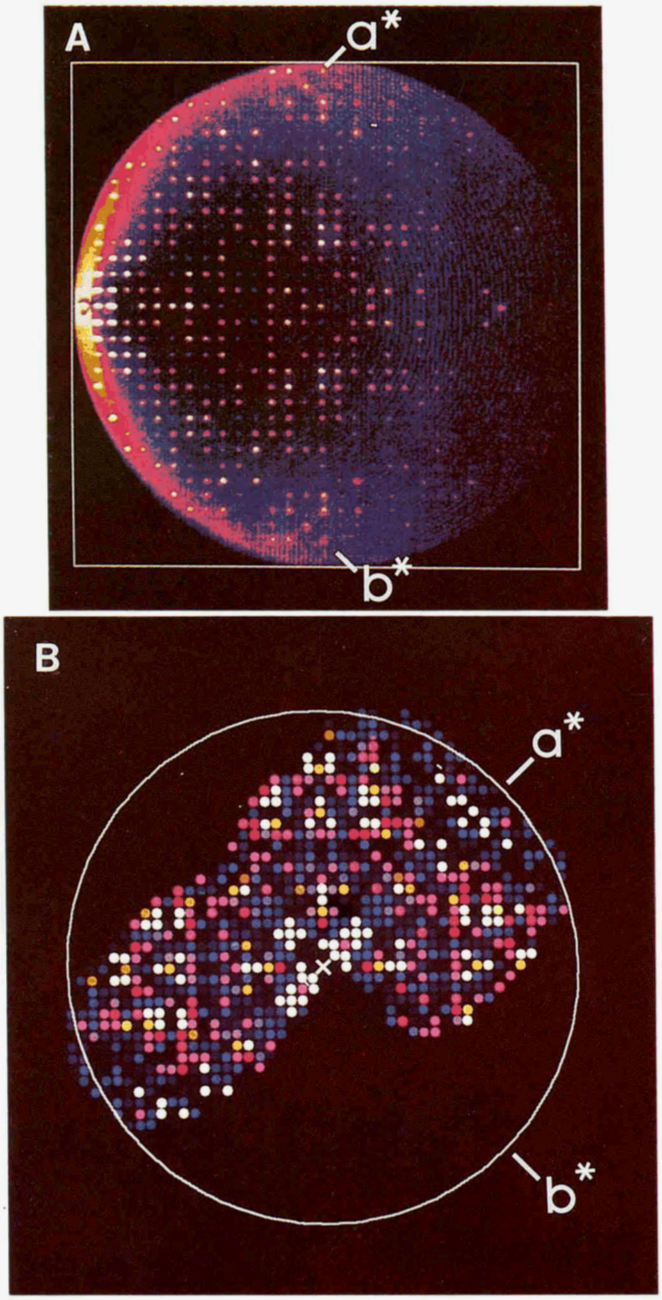


FIG. 2. *A*, pseudo-precession photograph of (*hk*0) zone of a streptavidin-biotin complex crystal. The image was generated by summing annuli that contain the zero-level data in a sequence of consecutive data frames from the two-dimensional area detector. The main beam is on the left of the image and the $a^*$ and $b^*$ axes are inclined 45° to the horizontal. The right edge of the frame corresponds to 2.2Å resolution. *B*, pattern of Lorentz and polarization-corrected diffraction data in the (*hk*0) zone. The zone is oriented as in *part A* with the $a^*$ and $b^*$ axes inclined 45° to the horizontal. The corrected intensities were obtained from integrating frames corresponding to a continuous 274° scan about the oscillation camera spindle axis. Regions missing from the pattern were not collected for this combination of crystal orientation and spindle rotation angle.

CHES, pH 9.1, and Sorensen's citrate at pH 4.0 and pH 2.2 (8). Subsequent experiments at 30 °C produced larger (0.1 × 1.0 × 1.0 mm), apparently single crystals (Fig. 1*A*) of the protein. X-ray diffraction experiments showed that although the crystals diffracted to about 3 Å resolution, they were highly mosaic and would prove difficult subjects for x-ray structure analysis.

Crystallization experiments with the streptavidin-biotin complex were carried out by preincubating the protein (33 mg/ml) for 8 h at 4 °C with a 4-fold molar excess of *d*-biotin. Streptavidin-biotin samples were microcentrifuged at 12,000 × *g* for 2 min to separate the complex from undissolved biotin. Hanging drop crystallization trials following the protocol outlined above were carried out using 10-μl drops of protein-precipitant solution equilibrated against 1 ml of precipitant. Experiments were set up at 4 °C with the aid of a programmable pipetting device (9). The streptavidin-biotin complex formed crystals with a tetragonal bipyramidal habit, up to 0.5 × 1.0 × 1.0 mm in size (Fig. 1*B*), over a range of conditions and times from days to weeks. Optimal crystal growth occurred when the complex solution in the drop was mixed with and equilibrated against 9–13% (w/v) solutions of polyethylene glycol 8000 in pH 6.0–8.0 citric acid-sodium phosphate buffer (9), 1 M LiCl. However, crystals of apparently similar quality grew when alternative buffers useful in the pH 6–8 range were used, or 2 M NaCl or KCl was substituted for 1 M LiCl.

Streptavidin-biotin complex crystals were characterized by collection of a complete set of x-ray diffraction data. The data were recorded with a Nicolet/Xentronics imaging proportional counter using monochromatized CuK$\alpha$ radiation generated by an Elliott GX-21 x-ray generator. The raw data consisted of 1657 0.25° oscillation frames collected for two independent orientations of the same crystal. Data collection required 4 days during which no crystal radiation damage was observed. Raw detector data were indexed and integrated using the XENGEN software (10). The XENGEN program was able to index the diffraction pattern consistent with a tetragonal unit cell of $a = b = 98.4$ Å, $c = 125.8$ Å. Subsequent examination of the indexed and integrated intensity distribution (Fig. 2, *A* and *B*) determined the space group as $I4_122$. The volume of the asymmetric unit is $7.62 \times 10^{-4}$ Å$^3$. Assuming a typical Matthews coefficient of 2.5 Å$^3$/dalton (11), the result is consistent with a crystallographic asymmetric unit containing half the native streptavidin tetramer. Therefore, the streptavidin tetramer contains a molecular diad that, in this crystal, lies concident with one of the unit cell 2-fold symmetry axes.

Unambiguous determination of the space group allowed averaging of symmetry-related intensities to provide a final parent data set that could be analyzed statistically. Although reflections were observed to 2.2 Å resolution, intensities fell off rapidly beyond 2.6 Å resolution, where the data on average were only 1$\sigma$ above background. The truncated native data set to 2.6 Å resolution contained 84,062 observations of 9,194 crystallographically independent reflections. The average $R_{\text{merge}}$, ($\langle \Delta I \rangle / \langle I \rangle$), for symmetry-related reflections was 0.057. Efforts to obtain isomorphous heavy atom derivatives are underway.

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