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FOR THE RECORD

Extending the diffraction limit of protein crystals: The example of glutamine synthetase from *Salmonella typhimurium* in the presence of its cofactor ATP

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Glutamine synthetase (GS) plays a central role in cellular nitrogen metabolism in bacteria, catalyzing the ATP-dependent condensation of ammonia with glutamate to yield glutamine in the presence of divalent cations. GS is regulated by product feedback, by oxidative modification, and by adenylylation (Rhee et al., 1989). The 3.5-Å structure of completely unadenylylated GS was determined by Almassy et al. (1986) and refined by Yamashita et al. (1989). To improve the atomic model, and our understanding of the regulation and reaction mechanism, it has been necessary to grow GS crystals that diffract to higher resolution. We find that the cofactor ATP affects the size, growth rate, and diffraction quality of GS crystals.

Two crystal forms grown in different concentrations of ATP or the reaction products ADP and Pi are found to have distinct morphologies, stabilities, space groups, and resolution limits. Form XVI crystals, grown at high ATP concentration (greater than 500 μM), grow to greater than 1 mm, belong to space group C222, and have unit-cell constants $a = 235.1$ Å, $b = 138.7$ Å, and $c = 205.6$ Å. They diffract to no better than 3.2 Å resolution and have a short lifetime in the X-ray beam. These crystals tend to redissolve.

Form XIV crystals are grown at low ATP concentrations (less than 200 μM). They are routinely $0.8 \times 0.6 \times 0.2$ mm and belong to space group C2, with unit-cell dimensions $a = 235.5$ Å, $b = 134.5$ Å, $c = 200.1$ Å, and $\beta = 102.8°$. They diffract to 2.4 Å resolution, are suitable for data collection, and are stable for at least 6 months. Both Form XIV and XVI crystals have been observed earlier (Heidner et al., 1978; Janson et al., 1984), and Form XIV crystals have been used to determine the GS structure at 3.5 Å resolution. However, before we determined the importance of ATP, crystals grew irregularly, were rarely large enough for X-ray diffraction studies, and had limited resolution. The present study reveals that the ATP concentration is a crucial variable in obtaining large, better diffracting crystals.

The two differences in the growth conditions between crystal Forms XIV and XVI are the concentrations of ATP and of spermine tetrahydrochloride. The ATP:GS monomer ratio is 0.5 in the best Form XIV crystals, and it is equal to or greater than 1 in Form XVI crystals.

Two lines of evidence suggest that high ATP concentration makes GS more soluble, accounting for the redissolving of Form XVI crystals. The first is that increased concentrations of positively charged spermine tetrahydrochloride stabilize the growth of GS containing high ATP. This is presumably because of charge compensation. The other line of evidence is that addition of 2 mM ATP to crystallization drops dissolves Form XIV crystals. High ATP may change the packing of GS molecules, either because of an additional ATP binding site on the GS monomer, or because of the added negative charges of ATP. Whether ATP actually alters the packing of GS molecules we will learn only when we determine the structure of Form XVI crystals.

It may be significant that the best crystals are obtained under the condition in which the ATP concentration is 6 times the GS dodecamer concentration. This suggests that half of the active sites are occupied by ATP in the best quality crystals. This could imply that glutamine synthetase shares some characteristics of a half-of-the-sites reactive protein (Fersht, 1977). In fact, the studies on the inactivation of rat liver GS by [^{35}S]- or [methyl-^{14}C]L-methionine-*S*-sulfoximine indicated that about four molecules of methionine sulfoximine phosphate were bound per molecule of totally inactivated GS, which has eight subunits. This result might imply that rat liver GS nor-

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mally uses only four subunits for catalysis; the remaining four subunits may function in regulation (Tate & Meister, 1971). However, "half-of-the-sites" behavior was not observed in the inactivation of sheep brain GS or *Escherichia coli* GS (Ronzio et al., 1969; Weisbrod & Meister, 1973). Also, studies by equilibrium dialysis (Ginsburg, 1969) show that *E. coli* dodecamer GS binds 12 molecules of two feedback inhibitors, AMP and tryptophan. Nevertheless, the question "Does dodecamer *S. typhimurium* GS have some half-of-the-sites character?" may deserve further investigation.

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