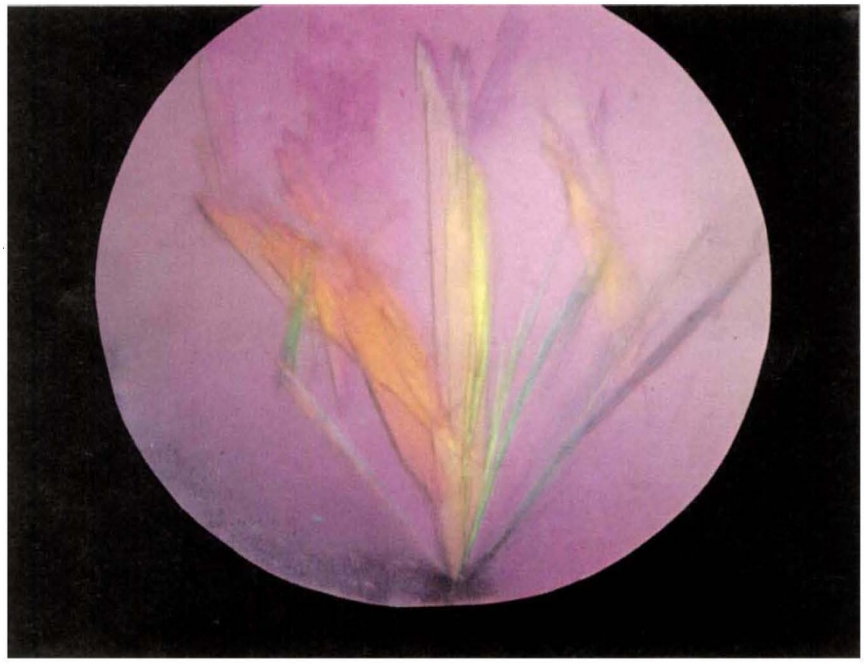
MIAMI BIO/TECHNOLOGY WINTER SYMPOSIUM

MONOCLONAL FINE-TUNING CONTINUES IN *COLI*



Co-crystals of the recombinant F_V fragment of the antibody McPC603, produced in *Escherichia coli*, and its ligand phosphorylcholine.

COURTESY ANDREAS PLÜCKTHUN

MIAMI, Fla.—What if one could produce biologically active, genetically engineered monoclonal antibodies in bacteria? Not only would you have an economic production system, but you would also have a straightforward experimental model for exploring the broader issues of protein engineering, protein folding, and catalysis. There have now been enough reports published to know that it *is* possible to get *Escherichia coli* to make antibody molecules—but how well can the bacterial hosts express these proteins? As reported by Andreas Plückthun (Max Planck Institut fur Biochemie, Martensried, F.R.G.) at January's Miami *Bio/Technology* Winter Symposium, it is possible to harness *E. coli* to express monoclonals in their native state.

The antibody that he has chosen as a model is McPC603, an especially well characterized murine IgA molecule that binds to phosphorylcholine. "We have decided to work on one of the best-studied antibodies there is," explains Plückthun. "The crystal structure of the F_{AB} fragment with the hapten bound was already determined in the early '70s by the lab of David Davies at the NIH (Bethesda, MD)...analogs can be easily synthesized, and we can also obtain some in radioactive form (for a variety of assays)...."

Crystal of the recombinant V_L domain of the antibody McPC603 produced in *E. coli*. It diffracts to more than 2.0 Angstroms.

Plückthun's group has synthesized the genes for the variable (F_V) domains and cloned those for the constant domains. Together, they code for the F_{AB} (antigen-binding) business end of an antibody molecule.

According to Plückthun, "the problem was not getting the genes, but getting a protein out of them." As he explains, "We have tried to directly express the protein, we have made fusions with an insoluble protein, *lacZ*, which then has to be cleaved enzymatically and refolded, we have made fusions to a secreted protein, for instance protein A, which can then be cleaved and reassembled, and we have also taken a C-terminal signal sequence (the *E. coli* protein hemolysin A) and fused it to that. The summary is that all these [methods] can be used to reassemble the protein...by far the most convenient method is the direct expression of both chains in a secreted fashion."

E. coli can co-express and co-secrete both gene products into the periplasmic space—from the same cell. There, the two signal sequences are cleaved off, the disulfide bonds form, the chains fold, and they reassemble to give a completely functional F_V or F_{AB} fragment. Moreover, claims Plückthun, the recombinant fragments can be purified very easily—in a single step via a hapten-affinity column.

"In order for this to work, there's a number of things that have to be taken care of," warns Plückthun. "We need approximately stoichiometric amounts of both chains...both precursor proteins have to be transported to the periplasmic space...we have to get correct processing to get the same N-termini...then the two domains have to fold, we have to form the intramolecular disulfide bonds, and the two chains have to associate."

What about the quality of the proteins that are produced in bacteria? According to Plückthun, the N-termini of both chains are identical to those formed by murine cells, the disulfide bonds are quantitatively there, and the binding constant of the recombinant F_V fragment for the hapten is the same as that for the whole antibody. "And that, of course," he says, "simplifies the problem enormously. We can work with a much smaller molecule and get the same effects."

For the recombinant F_{AB} fragment, however, things are not so straightforward. Although its molar yield from the bacterial expression system is fine, the amount of folded and isolatable material is about 5–10 times less than for the F_V fragment. Plückthun says that the answer to the question is not yet clear, but it is definitely not a question of forming the correct disulfide bonds.

—Jennifer Van Brunt