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Characterization of Crystals of an Intact Monoclonal Antibody for Canine Lymphoma

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A monoclonal antibody of the subclass IgG2a specific for canine lymphoma cells has been crystallized by vapor diffusion from polyethylene glycol 8000. The crystals, which occasionally measure nearly a millimeter on edge, have been examined by X-ray diffraction. The crystals are of triclinic space group *P*1 with unit cell parameters of $a = 66.39$ Å, $b = 77.34$ Å, $c = 101.42$ Å, $\alpha = 87.60^\circ$, $\beta = 92.55^\circ$, $\gamma = 97.54^\circ$ and cell volume of $V = 4.84 \times 10^5$ Å³. There is one entire antibody molecule as the asymmetric unit of the crystals. Three-dimensional X-ray diffraction data have been collected to 2.8 Å resolution and a self rotation function calculation shows a pronounced peak indicating at least an approximate non-crystallographic dyad axis.

Keywords: immunoglobulin; X-ray diffraction; protein crystals

Canine lymphoma is the most common hemopoietic tumor in the dog, resembling human non-Hodgkin's lymphoma. It has proven a valuable model for the investigation of anticancer therapeutics (Gillette, 1982; Johnson *et al.*, 1968). Canine lymphoma is conventionally treated with chemotherapy, but it was found that subsequent treatment with a specific monoclonal antibody, Mab 231, raised against the lymphoma, substantially prolonged remission and survival times (Jeglum, 1989). The antibody, which reacts specifically with cells of canine lymphoma (Steplewski *et al.*, 1987), has been shown to exhibit antibody-dependent cellular cytotoxicity as well as complement-dependent cytotoxicity (Rosales *et al.*, 1988).

We undertook the crystallization for X-ray structure analysis of the antibody and report here our success in this attempt and the characterization of the crystals we have obtained. Mab 231 was purified from diluted ascites fluid on a protein A-Sepharose chromatography column following elution with sodium citrate buffer (pH 4.5). The pH was adjusted to 7.0 and the protein dialyzed against phosphate-

buffered saline. It can be stored for long periods of time as the frozen saline solution without loss of activity.

Electrophoresis was carried out by the method of Laemmli (1970) following reduction of the protein with β -mercaptoethanol and boiling. A 5% to 15% polyacrylamide gradient gel with 2% (w/v) sodium dodecyl sulfate was used and gel staining was by Coomassie brilliant blue.

For crystallization the protein, at a concentration of 5 mg/ml was dialyzed at 22°C against distilled water for six to ten hours. At the end of this time most of the antibody had precipitated in the dialysis bag. The precipitate was resuspended in the dialysis bag, the contents centrifuged and the precipitated protein collected.

The precipitate was resuspended in water to a concentration of about 16 mg/ml and gently warmed under the hot water tap in a glass tube until the precipitate was just redissolved and the solution clear to slightly opalescent.

Crystallization samples were then dispensed onto nine-well vapor diffusion plates using 18 μ l droplets with 25 ml reservoirs according to procedures described by McPherson (1990). Each droplet was composed of 8 μ l of the 16 mg/ml protein solution, 2 μ l of a 0.1 M-Tris·HCl buffer, and 8 μ l of the

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reservoir solution. Generally three different buffers were used at pH 7.8, 8.0 and 8.2 yielding three samples at each pH per plate. Plates were equilibrated against reservoirs of 3%, 4% or 5% PEG† 8000 in water.

Crystallization occurred over a six to ten day period of time at 22°C. At 4°C crystals were obtained within 24 hours, but these were not nearly of the same size and quality as those grown at room temperature. The protein could be refrozen in water for storage. So long as the protein is redissolved by gentle heating before dispensing into the depression plates, it can be reproducibly crystallized with little difficulty.

SDS/PAGE of the crystalline antibody demonstrated the presence of intact heavy and light chains consistent with its idiotype classification as a member of the IgG2a subclass (Steplewski *et al.*, 1987) and with crystals composed of entire antibody molecules.

For diffraction analysis the crystals were mounted in quartz capillaries along with a small amount of mother liquor. X-ray diffraction photographs were made using a Buerger precession camera and an Enraf-Nonius generator with a fine focus X-ray tube operated at 45 kV and 32 mA to produce nickel filtered CuK α radiation. For three-dimensional X-ray data collection, a San Diego Area Detector System multiwire detector instrument (Hamlin *et al.*, 1981) was used and the X-rays were generated by a Rigaku RU-200 rotating anode generator operated at 50 kV and 150 mA. A graphite monochromator was used to produce CuK α radiation.

The self-rotation function of the dog lymphoma antibody crystals was calculated using the programs incorporated in the MERLOT molecular replacement system assembled by Fitzgerald (1989).

An extensive search of reciprocal space was pursued to insure that no symmetry other than 1 was present in the diffraction pattern and that the reflections could only be indexed according to a triclinic lattice. The unit cell, of space group *P*1, with angles nearest to 90° was deduced to be that of edge lengths $a = 66.39$ Å, $b = 77.34$ Å, $c = 101.42$ Å (1 Å = 0.1 nm) and cell angles of $\alpha = 87.6^\circ$, $\beta = 92.5^\circ$ and $\gamma = 97.5^\circ$. This cell, inferred from precession photographs, was later confirmed by three-dimensional analysis using the multiwire area detector system.

The volume of the unit cell is $V = 4.84 \times 10^5$ Å³ and if one assumes that the asymmetric unit of the crystal is one entire antibody molecule of 150,000 daltons, then the volume to mass ratio for the crystal is $V_m = 3.22$ Å³/dalton. This is near the center of the range for most crystalline proteins (Matthews, 1968) while any other assumption would lead to entirely unreasonable values.

Three-dimensional data collection, repeated in

entirety for several crystals to assure accuracy, demonstrated that the X-ray diffraction pattern was strong to 3.5 Å but declined rapidly and was essentially unobservable beyond about 2.8 Å. Between 3.5 Å and 2.8 Å, the data were weak but a substantial number of statistically significant reflections were nonetheless present to the resolution limit. From a total of 268,000 observations, independent reflections greater than three times the estimated error were recorded. To achieve a high degree of redundancy the data were recorded from seven different crystals and merged. The observations from all crystals were combined with a standard residual of $R = 0.08$.

The crystals, which can be grown very large, greater than 0.8 mm on all edges, are mechanically stable for periods of up to several months in their mother liquor and show a stability in the X-ray beam comparable to most other protein crystals. This allowed collection of data over two to three day periods without unacceptable levels of deterioration due to X-ray damage.

Using the program package MERLOT, a three-dimensional rotation function using the algorithm of Crowther (1972) was computed using X-ray data from 14 to 5 Å resolution. A pronounced peak corresponding to a non-crystallographic dyad axis was observed well above the general level of the function. No other symmetry feature was suggested by the rotation function results.

The monoclonal antibody to canine lymphoma is one of only a few intact immunoglobulin molecules that have been crystallized in a form suitable for X-ray diffraction analysis. As such it may provide additional insight to the architectural variability within this class of proteins and, hopefully, some clues to its mechanism of action.

Although the quality of the crystals and their reproducibility is very good, the diffraction pattern is somewhat limited in extent and is only marginally useful beyond 3.0 Å resolution. This lack of extent suggests that some disorder may be present in the crystals and that portions of the protein may not be resolved in the final analysis. This was the case, for example, with another intact immunoglobulin, κ 01 (Colman *et al.*, 1976), for which it was found that the Fc portion of the immunoglobulin could not be clearly seen in the electron density map. Nevertheless, valuable structural information was obtained for the remainder of the protein molecule.

The rotation function results suggest the presence of a dyad axis within the asymmetric unit. This probably represents the relationship between the two Fab portions of the structure. We cannot, however, at this stage of the analysis, say whether the observed dyad axis is exact or only approximate.

The crystals of the canine lymphoma antibody are entirely suitable for a full three-dimensional X-ray diffraction analysis. We are proceeding toward this end using both molecular replacement and conventional multiple isomorphous replacement techniques.

† Abbreviations used: PEG, polyethylene glycol; SDS/PAGE, sodium dodecyl sulphate/polyacrylamide gel electrophoresis.

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References

- Colman, P. M., Deisenhofer, J., Huber, R. & Palm, W. (1976). Structure of the human antibody molecule kol (immunoglobulin G1): an electron density map at 5 Å resolution. *J. Mol. Biol.* **100**, 257-282.
- Crowther, R. A. (1972). In *The Molecular Replacement Method* (Rossman, M. G., ed.), Gordon & Breach, New York.
- Fitzgerald, P. M. D. (1989). *Merlot: an Integrated Package of Computer Programs for the Determination of Crystal Structures by Molecular Replacement*, Merck, Sharp and Dohme Laboratories, Rahway, NJ.
- Gillette, E. L. (1982). In *Design of Models for Testing Cancer Therapeutic Agents* (Fidler, I. J. & White, R. J. eds), Van Nostrand and Reinhold, New York.
- Hamlin, R., Cork, C., Howard, A., Nielsen, C., Vernon, W., Matthews, D. & Xuong, Ng. H. (1981). Characteristics of a flat multiwire area detector for protein crystallography. *J. Appl. Crystallogr.* **14**, 85-89.
- Jeglum, K. A. (1989). Chemotherapy of canine lymphoma, pp. 922-925, *Proc. Seventh ACVIM Forum*, San Diego, CA.
- Johnson, R. E., Cameron, T. P. & Kinard, R. (1968). Canine lymphoma as a potential model for experimental therapeutics. *Cancer Res.* **28**, 2562.
- Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)*, **227**, 680-685.
- Matthews, B. W. (1968). Solvent content of protein crystals. *J. Biol. Chem.* **33**, 491-497.
- McPherson, A. (1990). Current approaches of Macromolecular Crystallization. *Eur. J. Biochem.* **189**, 1-23.
- Rosales, C., Jeglum, K. A., Obrocka, M. & Steplewski, Z. (1988). Cytolytic activity of murine anti-dog lymphoma monoclonal antibodies with canine effector cells and complement. *Cell Immunol.* **115**, 420.
- Steplewski, Z., Jeglum, K. A., Rosales, C. & Weintraub, N. (1987). Canine lymphoma-associated antigens defined by murine monoclonal antibodies. *Cancer Immunol. Immunother.* **24**, 197-208.

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