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Effects of different post-crystallization soaking conditions on the diffraction of Mtcp1 crystals

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The crystal structure of human Mtcp1 was determined at 2 Å resolution after the X-ray diffraction limit was improved by post-crystallization soaking in 2.0 *M* ammonium sulfate for 1–5 months. The effects of varying the ammonium sulfate concentration and addition of polyethylene glycol to the soaking solution were examined in order to understand the phenomenon and to reduce the soaking time. Soaking the crystal for one week in a solution of 1.5 *M* ammonium sulfate and 2% PEG 3400 gave the desired improvement in diffraction quality. Therefore, different soaking conditions should be explored when crystals show disordered and low-resolution diffraction.

1. Introduction

The MTCP1 (mature T-cell proliferation-1) gene in the human X chromosome was the first candidate gene identifed in the leukemogenesis of mature T cells (Stern, 1993). Aberrant or overexpressed MTCP1 transcripts are found in the rare but recurrent chromosomal translocation t(X;14) in T-cell proliferative diseases (Soulier *et al.*, 1994). MTCP1, TCL1 and TCL1b are members of the novel TCL1 family of proto-oncogenes that are involved in lymphoid proliferation and T-cell malignancies; their physiological function is unknown (Pekarsky *et al.*, 1999; Croce, 1999). Expression of human TCL1 or MTCP1 in transgenic mice causes T-cell leukemia (Virgilio *et al.*, 1998; Gritti *et al.*, 1998). MTCP1 encodes for a protein of 107 amino-acid residues. The crystal structures of human recombinant Mtcp1 and Tcl1 have been determined and they share a unique β-barrel topology (Fu *et al.*, 1998; Hoh *et al.*, 1998). Recently, Tcl1, Mtcp1 and Tcl1b were shown to interact with the Akt protein kinase that has a key role in lymphoid survival (Laine *et al.*, 2000; Pekarsky *et al.*, 2000).

Post-crystallization soaking of Mtcp1 crystals was required to obtain ordered 2.0 Å resolution diffraction data and to solve the structure (Fu *et al.*, 1998, 1999). Freshly grown Mtcp1 crystals show diffraction to only 3.5 Å resolution, with streaks in the diffraction pattern indicating disorder in the lattice. After soaking the crystals in 2 *M* ammonium sulfate for 1–5 months, the diffraction extended to 2.0 Å resolution without streaking. The unit-cell parameters were not significantly changed during the soaking. In contrast, Esnouf *et al.* (1998) observed up to 18% shrinkage in unit-cell volume accompanied by diffraction improved from 3.7 to 2.2 Å resolution on soaking crystals of HIV reverse transcriptase in up to 46% PEG 3400. They concluded that dehydration of the crystals produced the improved diffraction. In order to analyze this phenomenon and in an attempt to reduce the time required to soak Mtcp1 crystals from several months to a more convenient time, the X-ray diffraction was evaluated after soaking in mixtures of PEG 3400 and ammonium sulfate, as well as in different concentrations of ammonium sulfate for times between 1–2 weeks and several months. Varying the soaking conditions accomplished the desired result of yielding high-quality diffraction data in the shorter and more convenient time of one week as opposed to the original 1–5 months. Post-crystallization soaking can be a valuable method for improving the diffraction limit of protein crystals; however, it is necessary to explore a variety of soaking conditions to obtain improved diffraction.

2. Experimental methods

Human recombinant Mtcp1 was expressed and purified as described previously (DuBois *et al.*, 1998). The purified protein was dialyzed into 50 m*M* Tris buffer at pH 7.8 and concentrated to 5.0 mg ml^{-1} for crystallization. Large crystals of Mtcp1 were grown by vapor diffusion at room temperature using 1.3–1.5 *M* ammonium sulfate as precipitant, as described previously (Fu *et al.*, 1998). No crystals or else showers of smaller crystals were grown in ammonium sulfate concentrations outside this range. No crystals grew when PEG was included in the ammonium sulfate precipitant. Cover slips were sealed with vacuum grease. All crystals used in the soaks had dimensions of roughly 0.2 × 0.2 × 0.45 mm. Post-crystallization soaking was performed by transferring the

Table 1
Effect of different soaking conditions on diffraction.

(*a*) Ammonium sulfate (AS) soaks.

AS (*M*)	Time (weeks)	Resolution (Å)	$a = b$ (Å)	Δa (Å)	c (Å)	Δc (Å)	Volume (Å^3)	ΔV (Å^3)
2.0†	4–20	2.0	62.7	0.5	86.0	−1.0	292787	−0.1
1.5‡	0	3.5	62.4	0	86.9	0	293026	0
1.3	12	2.4	62.4	0	86.9	0	293026	0
1.5	16	2.6	61.3	−1.8	88.3	1.6	287342	−1.9
2.0	16	2.6	61.5	−1.5	87.7	0.9	287255	−2.0
2.5	16	3.9	61.5	−1.5	88.0	1.3	288238	−1.7
3.5	1	3.4	61.1	−2.1	87.5	0.7	282892	−3.5
3.5	2	3.6	61.1	−2.1	87.1	0.2	281590	−3.9

(*b*) Ammonium sulfate (AS) and PEG 3400 soaks.

AS (*M*)	PEG [%(*w*/*v*)]	Time (weeks)	Resolution (Å)	$a = b$ (Å)	Δa (Å)	c (Å)	Δc (Å)	Volume (Å^3)	ΔV (Å^3)
1.5	2	1	2.2	62.9	0.8	85.7	−1.4	293630	0.2
1.8	2	1	2.5	62.2	−0.3	87.5	0.7	293161	0
1.8	5	1	2.5	62.8	0.6	84.2	−3.2	287574	−1.9
2.0	2	1	2.6	62.4	0	87.0	0.1	293364	0.1
2.0	5	8	2.1	62.8	0.6	85.8	−1.2	293047	0
2.0	5	9	2.0	63.0	1.0	54.8	−2.4	291471	−0.5
2.3	2	1	2.5	62.8	0.6	85.5	−1.6	292014	−0.3
2.5	2	1	2.5	62.6	0.3	86.6	−0.3	298890	0.3
2.5	2	9	2.5	62.8	0.6	85.9	−1.2	293380	0.1
2.5	2.5	10	2.0	62.8	0.6	85.4	−1.7	291672	−0.5
2.5	5	9	2.7	62.8	0.6	86.2	−0.8	294413	0.5

† Values taken from previous study (Fu *et al.*, 1999). ‡ Control crystals that were not soaked.

crystals to a fresh drop with the test solution, which consisted of ammonium sulfate alone or combined with different molecular-weight PEGs. Protein crystals dissolved when subjected to PEG 8000 and PEG 10 000. However, PEG 3400 did not appear to dissolve the crystals. Therefore, different mixtures of ammonium sulfate and PEG 3400 were evaluated. Crystals were soaked for varying times from one week to several months before testing the diffraction. Each experiment was run in duplicate and the unit-cell parameters were averaged. X-ray diffraction data were collected on an R-AXIS II imaging-plate detector mounted on an RU-200 Rigaku rotating-anode X-ray generator. Three frames were collected for each crystal using 1.5° oscillations and 30 min exposure at 0, 90 and 180°. X-ray diffraction data were reduced with the *HKL* package (Otwinowski & Minor, 1997) to obtain the resolution of the diffraction and the unit-cell parameters of the crystal. All crystals were processed in the space group *P*6222.

3. Results and discussion

The diffraction of Mtcp1 crystals was evaluated after soaking in different conditions in which the concentrations of ammonium sulfate and PEG 3400 and the soaking time were varied. This was performed to better understand the observed improvement in crystal quality and to reduce the time needed to obtain such results. The soaking conditions, the effects on the crystals and diffraction resolution are listed in Table 1. The results of these new studies were compared with the original observation that soaking the Mtcp1 crystals in 2 *M* ammonium sulfate for several months produced an improvement from 3.5 to 2.0 Å resolution in the diffraction pattern.

3.1. Effects of soaking in ammonium sulfate

Freshly grown Mtcp1 crystals routinely showed diffraction to about 3.5 Å resolution, with streaky spots indicative of lattice disorder (Fig. 1*a*). After soaking for several months in 2*M* ammonium sulfate, the diffraction improved to 2 Å resolution (Fig. 1*b*), without significant changes in the unit-cell volume (Fu *et al.*, 1999). In order to investigate the possible causes of this effect, several soaking experiments were performed at different concentrations of ammonium sulfate (Table 1*a*). If the effect of the long soak is primarily dehydration, then higher ammonium sulfate concentrations should be required for the effect to occur. On the other hand, if the resolution improvement occurs with lower salt concentrations, then the cause is not simply a consequence of dehydration.

Crystals of Mtcp1 were soaked in 1.3 *M* ammonium sulfate, a concentration close to the conditions for crystal growth. After 12 weeks of soaking crystals in 1.3 *M* ammonium sulfate, the diffraction limit was increased to 2.4 Å resolution without streaking and with no change in unit-cell parameters. This result suggests that the diffraction improved primarily because of a time-dependent increase in lattice order. This could have been accomplished by the increased sulfate-ion concentration in the lattice improving the stabilization of charged amino-acid side chains at the protein surface. It could also be the consequence of a chemical change in either the protein or solvent owing to oxidation or amidation. Soaking in 1.5–2.0 *M* ammonium sulfate for 16 weeks did not result in any further improvement in the diffraction. Further increases in ammonium sulfate concentration from 2.5 to 3.5 *M* resulted in a 3–4% decrease in the volume of the unit cell, mostly because of a decreased length of the *a* and *b* axes. Moreover, the diffraction was not improved in the shorter time frame of about one week. Therefore, dehydration alone does not improve the Mtcp1 diffraction, in contrast to the effect reported for HIV reverse transcriptase crystals, where unit-cell volume shrinkages of up to 18% were seen and diffraction improved from 3.7 to 2.2 Å resolution. The solvent content of HIV reverse transcriptase crystals decreased from 56% before soaking to 47% after soaking (Esnouf *et al.*, 1998). However, the Mtcp1 protein molecules are relatively tightly packed in the crystal lattice with a solvent content of 36.5%, which may preclude substantial shrinkage.

Soaking the crystals in ammonium sulfate alone did not result in diffraction to 2.0 Å resolution as reported previously (Fu *et al.*, 1999). However, crystals of larger size were used for the original study (0.25 × 0.25 × 0.5 mm), compared with 0.2 × 0.2 × 0.45 mm in this study.

3.2. Effects of soaking in mixtures of ammonium sulfate and PEG 3400

Diffraction data were also improved by soaking the crystals in various mixtures of ammonium sulfate and PEG 3400 (Table 1*b*; Fig. 1*c*). The crystals cracked when transferred directly into 10% PEG 3400. The highest resolution diffraction of 2.0 Å was obtained by soaking for 9–10 weeks in either 2.5 *M* ammonium sulfate, 2.5% PEG 3400 or 2.0 *M* ammonium sulfate, 5% PEG 3400. Since the soaking time of about ten weeks was undesirably long, several tests were performed using shorter soaks of about one week in mixtures of ammonium sulfate and PEG 3400. One week of soaking in 1.8–

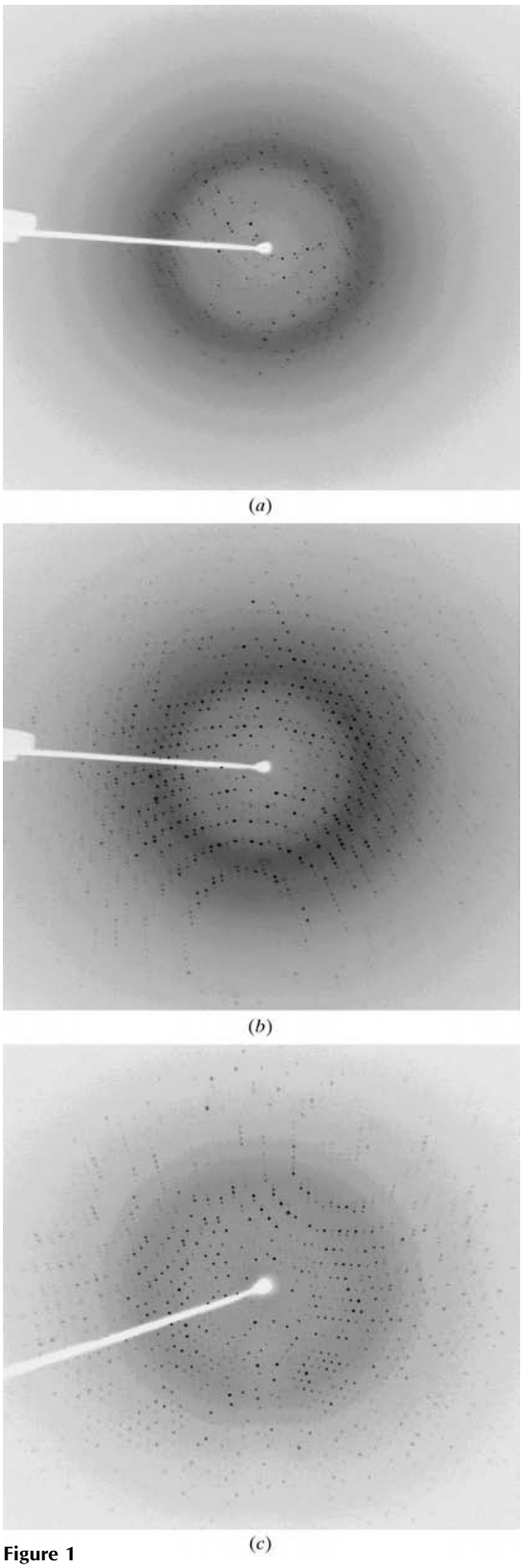

Figure 1
Diffraction patterns of Mtcp1 crystals after various post-crystallization conditions. (*a*) Mtcp1 crystals without post-crystallization soak yielded reflections with disorder and diffraction to 3.5 Å resolution. (*b*) Crystal after post-crystallization soaking in 2 *M* ammonium sulfate for several months gives improved diffraction resolution to 2.0 Å. (*c*) Crystal after one week soak in 1.5 *M* ammonium sulfate and 2% PEG 3400 showed diffraction to 2.2 Å resolution without disorder.

2.5 *M* ammonium sulfate with 2% PEG 3400 resulted in a diffraction limit of 2.5 Å resolution, a substantial improvement over the 3.5 Å resolution for freshly grown crystals. The best result for the purpose of time reduction was obtained by soaking for one week in 1.5 *M* ammonium sulfate with 2% PEG 3400; the crystals showed diffraction to 2.2 Å resolution. A post-crystallization soak for one week is clearly more convenient than the original time frame of 1–5 months. Therefore, varying the soaking conditions with mixtures of ammonium sulfate and PEG 3400 was required to obtain a more optimal soak time as well as the improvement in ordered diffraction from these crystals.

4. Conclusions

Comparison between experimental soaking conditions and the observed improvements in the crystal quality of Mtcp1 shows that several mechanisms can be involved. The effects observed did not require dehydration because they occur in nearly the initial growth conditions. Indeed, dehydration and shrinkage of the unit cell by using higher salt concentrations alone did not improve crystal quality, in contrast to the results for crystals of HIV reverse transcriptase. However, using low concentrations of PEG did improve the speed and ultimate magnitude of the improvements in diffraction. While PEG could be acting a dehydrating agent, little change was seen in the unit cell, suggesting a more complex mechanism. This is important for the general problem of improving resolution in protein crystals, because it shows that careful control of soaking and aging conditions is required to find the best conditions for diffraction. In the case of Mtcp1 crystals, a time-dependent increase in lattice order was observed as well as a more rapid improvement after careful selection of conditions. The mechanism for improving the lattice order could be improved stabilization of charged amino-acid side chains at the protein surface owing to the increased sulfate-ion concentration; alternatively, small rearrangements of the protein surface could arise from a chemical change in either the protein or solvent owing to oxidation or amidation that is enhanced by the presence of ammonium sulfate and PEG. It is possible that entirely different conditions could have the same result. Therefore, increases in resolution and improvement in data quality are dependent on varied soaking conditions for different proteins and such conditions should be investigated to obtain optimal diffraction.

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