From conventional crystallization to better crystals from space: a review on pilot crystallogenesis studies with aspartyl-tRNA synthetases

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Aspartyl-tRNA synthetases were the model proteins in pilot crystallogenesis experiments. They are homodimeric enzymes of M_r~125 kDa that possess as substrates a transfer RNA, ATP and aspartate. They have been isolated from different sources and were crystallized either as free proteins or in association with their ligands. This review discusses their crystallisability with emphasis to crystal quality and structure determination. Crystallization in low diffusivity gelled media or in microgravity environments is highlighted. It has contributed to prepare high-resolution diffracting crystals with better internal order as reflected by their mosaicity. With AspRS from *Thermus thermophilus*, the better crystalline quality of the space-grown crystals within APCF is correlated with higher quality of the derived electron density maps. Usefulness for structural biology of targeted methods aimed to improve the intrinsic physical quality of protein crystals is highlighted.

Keywords: aspartyl-tRNA synthetase, crystal growth, crystal perfection, microgravity

1. Introduction

1.1. Aim and necessity of crystallogenesis studies

In the present post-genomic era of structural biology, the need of efficient high-throughput crystallography increases (Blundell *et al.*, 2002). Despite significant progress, production of crystals is still not entirely under the control of the crystal grower, so that successes in structural biology still primarily rely on advances in the field of crystallogenesis. To overcome the bottleneck, efforts are undertaken either to facilitate high-throughput crystallization (Stevens, 2000) or to produce defect-free crystals that should yield best diffraction and hence highest resolution electron density maps. To reach the latter goal, the mechanisms of crystal formation have to be understood and strategies are needed for producing the desired high-quality crystals.

1.2. Microgravity projects

Elimination of convection and sedimentation in weightlessness attracted the attention of crystal growers who predicted that this environment should favor improvement of crystal quality. After the first trials in the early eighties, showing that protein crystals grew larger in the Space Shuttle (Littke & John, 1984), a number of microgravity projects were sponsored by Space Agencies. Presently a few hundred of proteins have been crystallized in microgravity in over 50 space-missions (Kundrot *et al.*, 2001). These figures reflect a great research effort in a new field, but even if they appear huge they are ridiculously low compared to the many trials conducted on earth.

Microgravity experiments have been based on two strategies. The first consisted in crystallization screening of the largest number of proteins, with the aim of obtaining crystals, possibly of enhanced quality. Here, monitoring growth parameters or running controls on earth (often not feasible because of non-adapted instrumentation) were not the main objectives. In the second strategy, the objective was unraveling the basic processes underlying macromolecular crystal growth. In that case, most investigations were conducted on a few easily available model proteins, with monitoring of as many parameters as possible. Controls were performed in parallel in the same type of crystallization devices and, if possible, using identical protein samples. In both cases assessment of crystal quality by diffraction measurement and electron density map calculation should have been a necessity. However it is only in the past years that evaluation of diffraction quality was carried out on a systematic basis. Structural models derived from space-grown crystals were obtained for a few proteins and their resolution was often better than the best one obtained with earth-grown crystals (DeLucas, 2001). An example is the resolution beyond 0.9Å for pike parvalbumin (Declercq *et al.*, 1999). But, considering the limited number of such structures compared to the many structures solved from conventional crystals, it was concluded by certain scientists that microgravity research is not useful because it had not contributed much to structural biology (comments reported by Reichhardt, 1998). This statement would warrant some justification if the number of solved structures is solely taken into account. It certainly does not hold when considering the contribution microgravity research brought to the understanding of the crystallization process of macromolecules (*e.g.* Carter *et al.*, 1999; Chayen & Helliwell, 1999; Giegé *et al.*, 1995; McPherson, 1998; McPherson, 1997). Microgravity projects were the driving force of most of the bio-crystallogenesis research in the last two decades (De Titta *et al.*, 2001) when prior to this period, the physics and physical chemistry of protein crystallization were not sufficiently explored. Presently, the field is well documented with much knowledge accumulated from studying model proteins, like lysozyme, thaumatin, canavalin and a few others, under the gravitational influence on earth and in space.

1.3. A representative model system: the AspRS family

Aminoacyl-tRNA synthetases ensure attachment of amino acids on tRNAs (MW_r~25 kDa) and thus contribute to the correct translation of the genetic code. They are ranked in two classes comprising large monomers (MW_r>100 kDa), homodimers (subunits of ~60 kDa) and $\alpha_2\beta_2$ heterotetramers (>200 kDa). So far, members of each class have been crystallized and models at 2–3Å resolution are available. Structures have modular architectures and have a propensity to undergo conformational changes (Carter, 1993; Martinis *et al.*, 1999).

Dimeric aspartyl-tRNA synthetases (AspRS, E.C.6.1.1.12) from *Saccharomyces cerevisiae* (Amiri *et al.*, 1985) and *Thermus thermophilus* (Poterszman *et al.*, 1993; Becker *et al.*, 2000) were taken as models for pilot crystallogenesis investigations. Other AspRSs originating from *Escherichia coli* (Eriani *et al.*, 1990) and *Pyrococcus kodakaraensis* (Imanaka *et al.*, 1995) were crystallized

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for only structure determination purposes. Crystals of free AspRSs and of complexes with small ligands or with homologous and heterologous tRNAs, often led to X-ray structures (Table 1). In difficult cases, crystallogenesis studies helped either to improve the quality of the crystals or to understand how crystallization failed to produce the better crystals. More recently, crystallization in low diffusivity media (see below) has contributed to obtaining crystals that diffract to high resolution.

This essay discusses what was learned from crystallogenesis studies on AspRSs and highlights data obtained from space-grown crystals. Benefit for a better structural understanding of this family of proteins will be shown and applications for the production of high quality crystals of other proteins discussed.

2. Considerations on methods and techniques

In what follows, particular care was taken to work with well-defined batches of AspRSs and to conduct the required controls. For comparative studies in which the effect of one variable (*e.g.* pH, temperature, microgravity, absence or presence of a gel) was investigated, protocols were always identical except for the parameter analyzed. This holds also for crystallographic analyses done on crystals obtained under different growth conditions (*e.g.* within a gel, in microgravity).

2.1. Importance of purity and homogeneity of protein preparations

So far baker's yeast is the only eukaryote from which an AspRS was crystallized. However, when originating from wild-type yeast cells, the synthetase is partly degraded in its N-terminus. Degradation is seen as a dozen isoforms in isoelectric focusing. The microheterogeneity is due to a statistic cleavage of the first 14 to 33 residues but does not significantly alter the catalytic activity of the protein. Limited trypsinolysis indicates existence of a stable subunit core of MW_r~60 kDa and genetic engineering was the way to get a homogeneous protein. In this case, a bacterial strain carrying a truncated form of the yeast gene was designed to express an active dimer deprived of its 70 first amino acids (Lorber *et al.*, 1987; Sauter *et al.*, 1999; Vincendon, 1990). The biochemical studies on the microheterogeneity of yeast AspRS were among the first to point out the importance of purity for protein crystallization (Giegé *et al.*, 1986). Today, clearly, recombinant proteins have to be produced to obtain well-defined products rather than molecules whose integrity, purity and homogeneity vary from batch to batch. Thus, in case of crystallization drawbacks advanced protein characterization technologies (including mass spectrometry) have to be employed to search for possible microheterogeneities and alternate purification strategies assayed.

Thermostable AspRSs are easier to produce. The two forms co-existing in *T. thermophilus*, a bacterium phylogenetically close to archaea, were overexpressed in *E. coli*. They are easily separable from host proteins by heat treatment followed by centrifugation that removes >90% of them. The bacterial-type AspRS-1 has <29% sequence homology with the archaeal-type AspRS-2.

2.2. Crystallization on earth and in space

A large panel of crystallization methods was applied to AspRSs, the most used being vapor diffusion in either hanging or sitting drops inside the classical Linbro plates. For the search of initial crystallization conditions, sparse matrix-type methods were used. Dialysis in cells of large volumes (50–400 µl) was used only under microgravity in the Advanced Protein Crystallization Facility (APCF) (Bosch *et al.*, 1992). Although free-interface diffusion was not used on the ground because convection accelerates mixing of protein and precipitant solutions, it was used under microgravity within the Protein Crystallization facility (PCF) on the European Retrieval Carrier (EURECA). Controls were done under otherwise identical conditions in the laboratory (with the same reactors, solutions, and over the same time span) in parallel with crystallization experiments under microgravity.

2.3. Crystal growth monitoring and structure analysis

A combination of methods was used to accuratly monitor the crystallization proccess and to characterize the outcoming crystals. (i) Nucleation and growth kinetics of *T. thermophilus* AspRS-1 crystals were monitored by video microscopy (Lorber & Giegé, 1996) and growth mechanism visualized by atomic force microscopy (Zhu *et al.*, 2001). (ii) For structure determination, full sets of diffraction data were collected at synchrotron sources (LURE, ESRF and Hasylab-DESY). (iii) Bragg reflection profiles and topographies were recorded to monitor defects in the crystalline lattice using a quasi-plane wave on beamline D25 at LURE. For details see Lorber *et al.* (1999) and Ng *et al.* (2002).

3. Achievements and limits of conventional crystallization

The first AspRS crystallized was that from *S. cerevisiae*, followed by the enzymes from *E. coli*, *T. thermophilus* (first form), *P. kodaka raensis* and *T. thermophilus* (second form). Crystallizations were also conducted on AspRSs complexed with ligands. Ammonium sulfate was the crystallizing agent in most cases; however it could be replaced by sodium formate (Poterszman *et al.*, 1993) or ethylene glycol (Schmitt *et al.*, 1998). The pH for crystallization was around neutrality, except for AspRS-2 from *T. thermophilus* that crystallizes at pH 9.5 (Charron *et al.*, 2001a), the most basic pH ever observed for the crystallization of a synthetase. Crystals of yeast and *E. coli* AspRSs are obtained at 4°C while those of the *T. thermophilus* enzymes grow best at room temperature. Surprisingly, ammonium sulfate at high concentration does not disrupt the interaction between AspRS and tRNA and was found to be the best crystallization agent for complexes as first found with the AspRS/$tRNA^{Asp}$ complex from yeast (Giegé *et al.*, 1980; Lorber *et al.*, 1983) (Table 1). Today, crystals of most RNA/protein complexes are obtained in the presence of this salt (reviewed in Dock-Bregeon *et al.*, 1999). Although yeast AspRS was soon crystallized (Dietrich *et al.*, 1980), crystals suitable for a structural analysis were only obtained recently (Sauter *et al.*, 1999). The reason was uncontrolled proteolysis during purification generating microheterogeneity (see above). Overproduction of a deletion mutant (AspRS-70) gave a homogeneous protein that opened the way to better crystals. In the presence of ammonium sulfate, this mutant crystallizes as tetragonal dipyramides as does the original protein isolated from yeast.

But as opposed to the latter, their diffraction is isotropic and extends beyond the previous limit (2.3 *vs* ~4Å). Despite numerous attempts, the protein with the entire sequence overproduced in *E. coli* could not be crystallized yet. As examples, Figure 1 displays AspRS crystals from yeast and *T. thermophilus*, either as free enzymes (panels a,b,e,f,h) or complexed with $tRNA^{Asp}$ (panels c,d). None of these crystals were found after blind screenings, but were the result of rational or semi-rational searches of crystallization conditions.

Resolution of bacterial AspRS crystals is comprised in the medium range (1.9–3.2Å) with best resolution for the free enzyme from *P. kodakaraensis* (Schmitt *et al.*, 1998) and the lowest for the *E. coli* enzyme complexed to cognate $tRNA^{Asp}$ (Eiler *et al.*, 1992). For yeast AspRS, best resolution of the free enzyme is ~2Å (Sauter *et al.*, 2001); it is only 7Å for the cubic form of the complex with

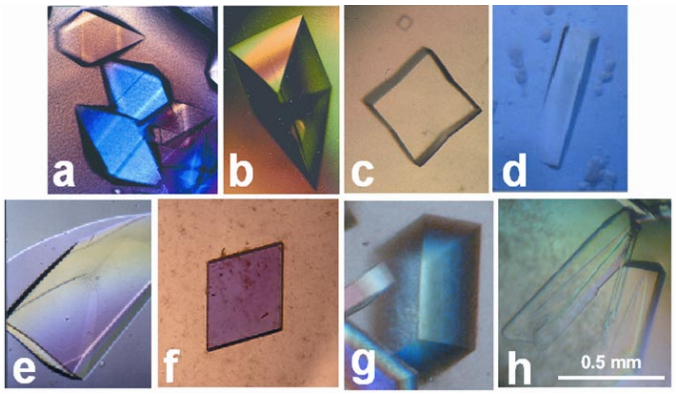

Figure 1
Crystals of free and ligand-complexed AspRSs. (Top row) (a,b) Tetragonal dipyramides and trigonal prisms of yeast AspRS-70, and (c,d) cubic and orthorhombic crystals of the yeast AspRS-tRNA^{Asp} complex. (Bottom row) (e,f) orthorhombic and monoclinic crystals of AspRS-1 from *T. thermophilus*. (g) Crystals of the latter prepared on the space station are twice as thick as controls prepared in parallel on earth. (h) AspRS-2 crystals prepared by macroseeding.

tRNA^{Asp} (Lorber *et al.*, 1983) but becomes 3.0Å for its orthorhombic form (Ruff *et al.*, 1988). Crystals contain rather high solvent contents reaching up to 78%. Interestingly, highest resolution (1.9Å) is accompanied by lowest solvent content (57%) (Schmitt *et al.*, 1998) and lowest resolution (7Å) by highest solvent content (78%) (Lorber *et al.*, 1983). This holds also when comparing the tetragonal and trigonal crystals of yeast AspRS-70, where highest resolution is correlated with lowest solvent content (Sauter *et al.*, 2001).

4. Controlling crystallization and applications for better crystals

4.1. Analysis of undersaturated solutions and use of phase diagrams

The homogeneity of the sample is a key parameter and dynamic light scattering (DLS) has shown that monodispersity of protein solutions favors crystallization (Mikol *et al.*, 1990). This feature applies for yeast AspRS that remains monodisperse under conditions yielding crystals (*i.e.* when ammonium sulfate is the crystallizing agent), but aggregates in crystallization unfriendly solutions (*i.e.* in PEG solutions) (Mikol *et al.*, 1991). Having defined the best prenucleation environment, a phase diagram can be used to define conditions producing good crystals. Indeed, at low supersaturation where nucleation is limited, few large tetragonal dipyramides of yeast AspRS diffracting to high-resolution could be obtained (Fig. 1a). Best crystals grew outside the dead zone of the phase diagram, where the amount of free protein in the soluble phase is limiting and impurity incorporation favored (Sauter *et al.*, 1999). A phase diagram was also useful to find a second crystal form of thermostable AspRS-1 that grows in the presence of PEG.

4.2. Nucleation and growth mechanisms

AspRS-1 crystals from *T. thermophilus* show an original growth mechanism. They are obtained from an initial precipitate when the crystallizing agent is ammonium sulfate or sodium formate. Inside the precipitate a few crystals nucleate and grow from micrometer to submillimeter size within a month. Gradually, the smallest crystals dissolve while the largest continue to grow. Once the later is alone, it promotes the dissolution of the precipitate and a clear halo appears around it until all soluble protein is dissolved. Growth ceases after all precipitated protein has disappeared (Ng *et al.*, 1996). This process was discovered for salts one century ago and is known as Ostwald ripening. It is the preferred growth mechanism of *T. thermophilus* AspRS-1 crystals in salt solution and was also observed for smaller proteins and for a spherical virus (Ng *et al.*, 1996).

AspRS-1 from *T. thermophilus* can also be grown from PEG solutions, without or within agarose gels, and comparable growth rates in both conditions (Zhu *et al.*, 2001). At low supersaturation, these crystals grew by a screw-dislocation spiral mechanism. High-resolution AFM images showed the two-dimensional arrangement of individual molecules and confirmed that each layer of the spiral had the height of one synthetase dimer (Zhu *et al.*, 2001).

5. Crystallization in low diffusivity media

5.1. Crystallization under microgravity

Table 2 lists the microgravity experiments done with yeast and *T. thermophilus* AspRSs. The first trials were done in the PCF instrument that flew in EURECA. Design of this free-interface crystallization experiment was both naive and too ambitious. The aim was to find better crystals of the yeast AspRS/tRNA^{Asp} complex. Results were essentially negative, and *a posteriori* the reasons for failure are well understood: no feasibility assays and test-case experiments, unadapted crystallization set-up, and too long duration of the mission. However this mission provided information on the equilibration process between the protein chamber and the reservoir containing the crystallization agent (Fig. 2): unexpectidly, the diffusion of the precipitant proved to be irregular as shown by the parabolic shape of the diffusion zone. Thus uncontrolled perturbations occurred in the rather large crystallization vessels of PCF. Fluid flow and protein crystal movements were observed at several instances during microgravity crystal growth (Boggon *et al.*, 1998; Lorber *et al.*, 2000). At least two lessons were learned from this trial. First, the free-interface crystallization technique was not the best choice for controlled microgravity experiments.

Crystallization by dialysis was a better option and PCF was not the versatile instrument required for that purpose. Second, feasibility and scenario of a space experiment need to be tested, before real applications. After a successful pilot experiment with lysozyme, it turned out that APCF was the versatile instrument required for crystallizations by either vapor diffusion or dialysis methods (Riès-Kautt *et al.*, 1997). It was used three times with *T. thermophilus* AspRS (Table 2) and it could be concluded that the dialysis method is the best adapted for crystal production under microgravity; vapor diffusion gave crystals as well, but their observation and recovery was delicate. Interestingly, as for experiments on earth (Fig. 1e), the synthetase seems to crystallize from a precipitate by Ostwald ripening (Ng *et al.*, 2002) yielding very few and large crystals (Fig. 1g).

5.2. Crystallization in gels

Hydrogels such as silica and agarose have been rediscovered recently in the field of protein crystal growth (Robert & Lefaucheux, 1988; Robert *et al.*, 1999). They allow growth of high quality crystals that may yield structures near to atomic resolution as shown for thaumatin (Sauter *et al.*, 2002). They may also enhance the crystal behavior during cryocooling as shown for *T. thermophilus* AspRS-1 crystals prepared in agarose gel (Zhu *et al.*, 2001). Another advantage of the gel is the quiescence of the solution due to the absence (or strong reduction of convection currents). In this medium, matter transport is limited by diffusion. Thus, gels mimic at least in part what happens under reduced gravity. An interesting practical aspect is that crystals are immobile at the position where

Table 1 Crystallization and crystallography of AspRSs.

Organism Ligand(s)	crystallization method, T(K), precipitant, buffer, additives, pH, ions in structure	Space group, cell parameters(Å), (mol./A.U.), resolution, R-value, %solvent, PDB code	References
Archaea			
P. kodakaraensis			
ATP (none or ade.)	V.D.-h.d., 297K, Tris-HCl, ethylene glycol, 2-mercaptoethanol, KCl, pH7.5, Mn^{2+}	$P2_12_12$, a=124.8, b=125.0, c=87.2, (1dimer), 1.9Å, R=0.168, 57%, **1B8A**	Schmitt *et al.* (1998)
Eubacteria			
E. coli			
none	V.D.-s.d., 277K, Am.Sulf., isopropanol, Bis-Tris propane, NaCl, pH7.0, Mg^{2+}	C2, a=117.7, b=162.0, c=131.6, β=110.4°, (3 monomers), 2.7Å, R=0.198, 59%, **1EQR**	Boeglin *et al.* (1996) Rees *et al.* (2000)
***E.coli* tRNA^{Asp}**	**V.D.-h.d., 277K, Am.Sulf.,** Bis-Tris propane, pH6.5, SO_4^{2-}	**$C222_1$, a=102.7, b=128.1, c=231.7, (1 monomer+1tRNA),** 3.2Å, 71%	**Eiler *et al.* (1992)**
***E.coli* tRNA^{Asp}+ade.**	**V.D.-h.d., 277K, Am.Sulf.,** Bis-Tris propane, glycerol, pH6.8, SO_4^{2-}	**$P4_32_12$, a=b=101.2, c=231.8, (1 monomer/tRNA/ade.),** 2.4Å, R=0.208, 63%, **1COA**	**Eiler *et al.* (1999)**
yeast tRNA^{Asp}+ade.	V.D.-macroseeding, 277K, Am.Sulf., Bis-Tris propane, pH6.7, SO_4^{2-}	$P2_1$, a=75.8, b=222.8, c=80.8, β=111.8°, (1dim/2tRNA/2ade.), 2.6Å, R=0.204, 65%, **1IL2**	Moulinier *et al.* (2001)
T. thermophilus (**1**)			
none	V.D.-s.d., 288K, sodium formate,	$P2_12_12_1$, a=61.4, b=156.1, c=177.3, (1dimer),	Poterszman *et al.* (1993)
	Tris-HCl, pH7.5	2.2Å, 62% idem but 2.0 Å	Delarue *et al.* (1994) Ng *et al.* (2002)
none	V.D.-h.d.+agarose, 293K, PEG8000, Tris-HCl, pH7.8	$P2_1$, a=85.1, b=113.3, c=90.2, β=104.3°, (1dimer), 2.65Å, 62%	Zhu *et al.* (2001) Charron *et al.* (2001b)
aspartyl-ade.	soaking, 288K, Am.Sulf.,	$P2_12_12_1$, a=60.1, b=155.5, c=171.1, (1dimer),	Poterszman *et al.* (1994)
	Tris-HCl, pH7.5, SO_4^{2-}	2.4Å, R=0.194, 60 %, **1G51**	
E.coli tRNA^{Asp}	V.D.-h.d., 290K, sodium citrate, Na-HEPES, pH7.5	$P6_3$, a=b=251.5, c=88.7, (1dimer/2tRNA), 3Å, R=0.248, 73%, **1EFW**	Briand *et al.* (2000)
T. thermophilus (**2**)			
none	V.D.-h.d., 293K, PEG8000, CHES, NaCl, pH9.5	$P2_12_12_1$, a=57.3, b=121.9, c=166.9, (1dimer), 2.5Å, 58%,	Charron *et al.* (2001a)
Eukaryotes			
S. cerevisiae			
none	V.D.-s.d., 277K, Am.Sulf., MES-KOH, pH6.7	$P4_12_12$, a=b=92, c=185, (1 monomer), 3.5Å, 59-64%, partially proteolyzed	Dietrich *et al.* (1980)
none	V.D.-s.d., 277K, Am.Sulf., pH5.6	$P4_12_12$, a=b=90.2, c=184.9, (1 monomer), 2.3Å, R=0.202, 64%, **1EOV**, AspRS-70 mutant	Sauter *et al.* (2000)
yeast tRNA^{Asp}	V.D.-s.d., 277K, Am.Sulf., Tris-HCl, pH 7.8-8.5	I432, a=b=c=354, (1dimer/2tRNA), 7Å, 78%	Giegé *et al.* (1980) Lorber *et al.* (1983)
yeast tRNA^{Asp} (yeast tRNA^{Asp}+ATP)	V.D.-h.d., 277K, Am.Sulf., Tris-maleate, pH7.5	$P2_12_12$, a=210.2, b=146.2, c=86.1, (1dimer/2tRNA), 3Å, R=0.225, 69%, **1ASY**, **1ASZ**	Ruff *et al.* (1991) Cavarelli *et al.* (1994)

Abbreviations : mol./A.U., molecule(s) per asymmetric unit ; V.D., vapor diffusion ; h.d., hanging drop ; s.d., sitting drop ; ade., adenylate; Am. Sulf., ammonium sulfate.

they nucleate. Consequently they do not sediment and have well-developed faces and optimal volume.

Since movements and g-jitters occur during space missions, as already suspected during the EURECA mission and demonstrated in further flights (Boggon *et al.*, 1998; Lorber *et al.*, 2000), it was decided to combine the benefits of both microgravity and gel in one unique experiment. The concept was successfully tested with thaumatin (Lorber & Giegé, 2001) and was applied with more confidence for AspRS crystallization during the last 2001 mission.

6. Crystal analyses

6.1. Crystal quality and novel structural information

Mosaicity measurements and X-ray topography have been used to characterize the internal order of macromolecular crystals. The of *T. thermophilus* is among the largest proteins investigated so far by both of these methods (Lorber *et al.*, 1999). The analysis of crystals grown in formate and diffracting X-rays to 2Å resolution, indicated a very low mosaicity with a full width at half maximum of Bragg reflection profiles in the 14–27 arcsec range. Topographs revealed several growth sectors characterized by differences in contrast, that have each a mosaicity of ~10 arcsec.

On the other hand, crystallogenesis studies on AspRSs yielded at least three bodies of novel structural information. First, concerning yeast AspRS, the fact to have at disposal two structures solved at similar resolution of the free and tRNA complexed synthetase, allowed to discover structural changes within the protein structure correlated with functional states (Sauter *et al.*, 2000). Second, concerning the two AspRSs from *T. thermophilus*, resolution of their structure allows comparison of two structures fulfilling the same function within the same organism (Charron *et al.*, 2001a). Third, for *T. thermophilus* AspRS-1 the better crystals obtained in space

Table 2 AspRS crystallization experiments under microgravity.

Mission duration, date	Protein crystallized, instrument and technique	Protein chamber volume (µl)	Results gained in space *vs.* on earth	References
EURECA 1 year, 08-1992/93	Yeast AspRS/tRNA^{Asp} complex, PCF, F.I.D.	368	Salt diffusion kinetics	this paper, Fig. 2
IML-2 10 days, 07-1994	*Thermus* AspRS-1 APCF, V.D.-h.d.	67	Larger crystals	Ng *et al.*, 1997
LMS 14 days, 06-1996	*Thermus* AspRS-1 in salt solution APCF, dialysis	67	Larger crystals, better diffraction with higher signal/noise ratio and lower mosaicity	Ng *et al.*, 2002
ISS-3 4 months, 08-2001	*Thermus* AspRS-1 in PEG solution trapped in gel, APCF, dialysis	67	Thicker crystals, (analysis in progress)	this paper

Abbreviations: F.I.D., free interface diffusion; V.D.-h.d., vapor diffusion with hanging drop.

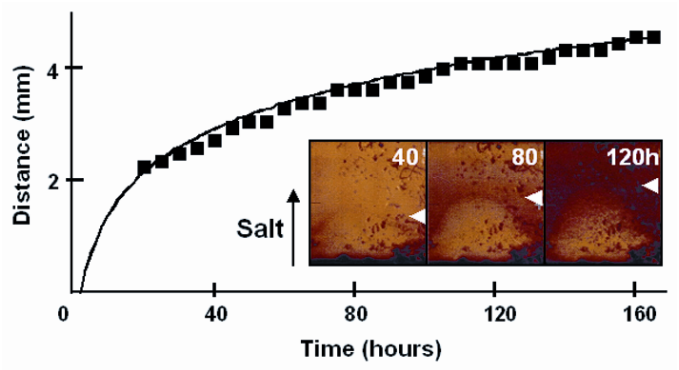

Figure 2
Salt equilibration kinetics by free-interface diffusion in PCF. Plot of the displacement of the ammonium sulfate front inside the AspRS/tRNA^{Asp} solution as observed by the formation of a precipitate. Experimental values can be fitted with a log function. (Inset) White arrows indicate the displacement of the precipitate after 40, 80 and 120 hours of equilibration.

(see below) give insight among others into the hydration shell of the synthetase (Ng *et al.*, 2002).

6.2. Crystal packing aspects

Crystallogenesis studies helped to understand why certain AspRS crystals were of poor quality. This is the case of the cubic crystals of the yeast AspRS/tRNA^{Asp} complex, whose diffraction limit was never better than 6Å (Lorber *et al.*, 1983) while that of the orthorhombic crystals reached 2.4Å (Ruff *et al.*, 1988). Examination of the packing of the complex pointed to the rather mobile dihydrouridine loop of tRNA^{Asp} that probably does not form a tight intermolecular contact inside the cubic crystals (Giegé *et al.*, 1994). Another example is native yeast AspRS whose entire polypeptide chain could never be crystallized despite many efforts put on purification. Modeling of its N-terminal domain, showed that it perturbs one packing contact in the tetragonal crystal lattice (Sauter *et al.*, 2001).

A comparison of the packing of the two crystal forms of AspRS-1 from *T. thermophilus* grown in salt or PEG solutions supports a correlation between molecular surface area involved in contacts and crystal perfection. Indeed, the larger the contact area, the better the diffraction properties of the crystals (Charron *et al.*, 2001b). Interestingly, this thermophilic AspRS mainly develops hydrophobic Van der Waals contacts in both orthorhombic and monoclinic lattices, despite the overall-accessible surface of the protein is more hydrophilic than average (Charron *et al.*, 2001b). This contrasts with what observed in yeast tetragonal AspRS crystals, where packing interactions are made predominantly by H-bonds and a few Van der Waals contacts (Sauter *et al.*, 2001). Whether these features are characteristics of the thermophilic and mesophilic nature of the two proteins is yet not known.

6.3. Better 3D structure from space-grown crystals

When compared to crystallization on earth, microgravity has repeatedly produced smaller numbers of crystals with augmented volume (*e.g.* DeLucas, 2001; McPherson, 1996). A rigorous comparison of the crystallographic properties of *T. thermophilus* AspRS-1 crystals prepared in parallel on earth and in space within the APCF has indicated that this large multidomain protein behaves like the small monomeric lysozyme (Dong *et al.*, 1999) or phospholipase (Dong *et al.*, 2000). Even when the diffraction limit was the same, the plots of the intensity of Bragg reflections over background for space-grown crystals were shifted toward higher values compared to those of earth control crystals. Topographs revealed an up to 5-fold reduction in mosaic spread, meaning that the reflections were more intense and sharper (Lorber *et al.*, 1999). This accelerated spot indexing and yielded more detailed electron density maps in which more atoms could be observed. In any region where the map derived from earth-grown crystals was of low quality and not interpretable, the map from space crystals was clear, well resolved and allowed an unambiguous model building. This was extremely useful for structure model building. Finally, a higher number of hydrogen-bonded water molecules was visible that is probably responsible for an enhanced stability of the protein in the crystals (Ng *et al.*, 2002).

7. Conclusions and perspectives

Methodological and technical advances in crystallographic analyses, including high-brilliancy X-ray synchrotron sources and fast computing, have dramatically accelerated structure determination of biomacromolecules (Blundell *et al.*, 2002; Rossmann & Arnold, 2001). However, the limiting factor is the same as two decades ago and preparation of high-quality crystals remains the corner stone of structural biology. In many instances, molecular flexibility and adaptability, together with structure processing, are prerequisites for protein function. As a consequence, macromolecules often present isoforms and adopt alternate conformations. Altogether this leads to structural heterogeneity that often hampers crystallization. As shown here with yeast AspRS, engineering a compact protein core is

therefore a good strategy to obtain crystals suitable for structure determination. Improving crystal quality may also arise by preventing growth defects. Stabilizing the protein structure by ligands or other additives, thereby minimizing crystal poisoning by undesired protein conformers, can do this. Likewise, more regular growth and thus crystals with less packing defects can be obtained when crystallizing under proper supersaturation conditions either in free solution or in gelled media (or in microgravity). Better crystal quality may also arise by stabilizing the protein structure by additional water-mediated hydrogen bonds, as seen with crystals from AspRS-1 from *T. thermophilus* grown under microgravity. By combining these different approaches, crystals diffracting to higher resolution can be expected.

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