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Pancreatic ribonuclease co-crystallizes with the dinucleotide dCpdG in two distinct forms

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Bovine pancreatic ribonuclease (RNase-A; E.C. 3.1.4.22) is one of the most intensively studied enzymes (Blackburn & Moore, 1982). Its two-step catalytic cleavage of the phosphodiester bond of ribonucleotides is understood in some detail (Richards, Wyckoff, Allewell, Lee & Mitsui 1971; Richards & Wyckoff, 1973; Wodak, Liu & Wyckoff, 1977; Pavlovsky, Borisova, Borisov, Antonov & Karpeisky, 1978).

Recently, however, dinucleotide substrate analogues containing guanine [cytidylyl-2′,5′-guanosine and deoxycytidylyl-3′,5′-deoxyguanosine (dCpdG)], when soaked into RNase-A crystals, were found (Aguilar, Thomas, Mills, Moss & Palmer, 1992; Aguilar, Thomas, Moss, Mills & Palmer, 1992) to bind in a non-productive spatial disposition with the *guanine* moiety occupying the site which had previously been identified (Richards, Wyckoff & Allewell, 1970) as involved in the recognition of *pyrimidine* bases of the substrate. This mode of binding has been called *retro-binding*.

In order to establish whether this unexpected mode of binding is an artefact resulting from the technique of *soaking* the ligand into the crystals, or whether it is indeed a solution phenomenon, we have attempted to co-crystallize the same enzyme-ligand complex, and then to proceed with the detailed crystallographic analysis and explore the binding mode observed in the co-crystals.

In this preliminary report we describe the production of *co-crystals* of RNase-A with dCpdG in *two different forms*, one isomorphous with the $P2_1$ space group used in the earlier structure determinations, and one in space group $C2$ similar to that previously observed with RNase-B (Williams, Greene & McPherson, 1987), the glycosylated form of pancreatic ribonuclease (Brookhaven PDB entry 1RBB).

The $P2_1$ crystals were produced in a manner very similar to that previously used to produce native RNase-A crystals in this form (Carlisle, Palmer, Mazumdar, Gorinsky & Yeates, 1974) but with an approximately fourfold molar excess of the dinucleotide dCpdG present. A mixed solution of 37.5 mg ml^{-1} RNase-A and 5.73 mg ml^{-1} dCpdG in distilled water was prepared, adjusted to pH 5.3, and 200 µl of this was then added to each of several small tubes. These were cooled to 277 K and different volumes of cooled absolute ethanol were added to each tube whilst stirring continuously, to produce a graded array of samples containing 42-47% ethanol by volume. The tubes were left undisturbed at room temperature and after two or three weeks large discrete crystals appeared.

The $C2$ crystals were obtained using the hanging-drop vapour-diffusion method, with PEG 4000 as precipitant. The conditions that produced crystals corresponded to concentrations of RNase-A at 20 mg ml^{-1}, dCpdG at 1.72 mg ml^{-1} (2.1-fold molar excess), and PEG 4000 at 22-24%. When setting up the hanging drops with an automated pipetting system, the pH was adjusted to be between 5 and 6 with citrate/phosphate buffer. Crystals in the form of flat plates appeared after three or four weeks at room temperature. They were fragile, of moderate size (~0.5 mm), and displayed a tendency to twinning.

Following initial characterization with a precession camera (see Fig. 1), data sets were collected on an Enraf-Nonius FAST diffractometer for one sample of each type of crystal using Cu $K\alpha$ radiation at 1.542 Å wavelength passed *via* a graphite (002) monochromator.

The $P2_1$ crystals provided the better quality data set, diffracting to 1.7 Å (θ_{max} = 27°, $-18 < h < 18$, $0 < k$

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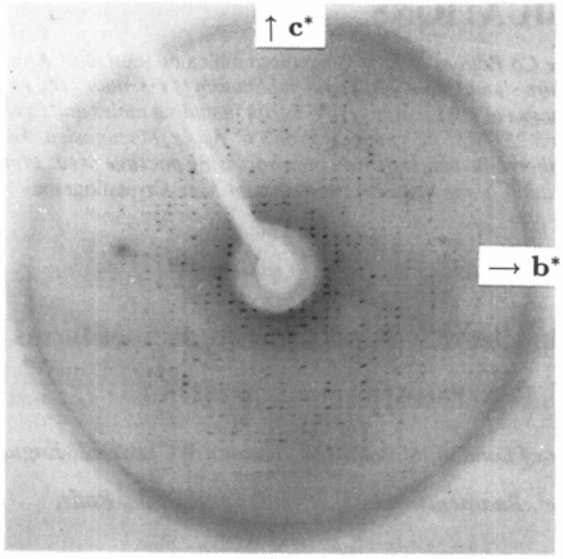
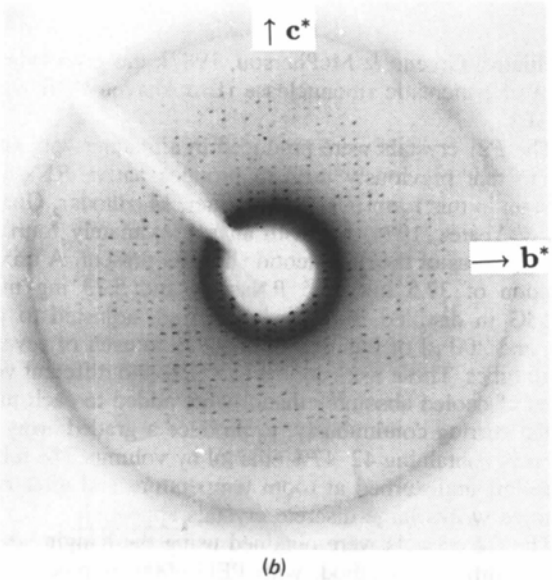


(*a*)



(*b*)

Fig. 1. Precession photographs of a *C*2 space group co-crystal of RNase-A with dCpdG showing (*a*) the 0*kl* zone and (*b*) the 1*kl* upper level.

< 22, 0 < *l* < 31), yielding 8400 independent reflections with $I \geq 3\sigma(I)$ (45% completeness) from a total of 36 135 measurements; the current *R* factor is 0.19. The cell parameters were *a* = 30.47(7), *b* = 38.72(7), *c* = 53.68(8) Å, β = 106.32(9)°; these values are virtually unchanged from the native $P2_1$ form.

The *C*2 crystals, on the other hand, diffracted to only 2.5 Å, and the quality of the reflections was such that the diffractometer software was unable to refine the *C*2 cell, the data being collected instead in an equivalent primitive *P*1 cell. The *C*2 parameters were *a* = 33.14(5), *b* = 52.86(8), *c* = 101.4(2) Å, β = 90.10(7)°, with *Z* = 8 molecules per unit cell, *i.e.* two per asymmetric unit. 15 835 reflections were collected, and merged to give 7692 independent reflections of which 5892 had $[I \geq 3\sigma(I)]$ representing 49% completeness to 2.5 Å (θ_{max} = 18°, -13 < *h* < 13, 0 < *k* < 21, 0 < *l* < 40). The current *R* factor is 0.27.

An initial examination of electron density maps, prior to completion of the refinement, unequivocally shows that in the $P2_1$ crystals the dinucleotide dCpdG is bound to the RNase molecule in the same or a very similar disposition as in the earlier studies which revealed the *retro-binding* phenomenon. The same also seems to have occurred in at least one of the sites of the two protein molecules in the *C*2 asymmetric unit, suggesting that this mode of binding to RNase-A is not just an artefact of soaking, but indeed may be a solution phenomenon for small nucleotides containing guanine.

A fuller report of the refined crystallographic complexes will be submitted for publication on completion of the refinement.

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