## Structural Role of the Metal in Crystalline Complexes of Ribonuclease

PREVIOUS investigations[1,2] of the various crystalline modifications of bovine pancreatic ribonuclease (hereinafter abbreviated as RNase) have shown that the growth of several of these depends on the presence of a metal compound in the crystallization medium. To be reported here are some observations throwing light on the structural role of the metal atoms in one of these modifications, namely, RNase I (space group $P2_12_12_1$).

This modification is normally obtained by crystallization in the presence of nickel[1], and appears occasionally in crystallization samples containing copper[2], but cannot be grown in the absence of a metal. This fact in itself would suggest that the metal plays a part in maintaining the particular arrangement of the protein molecules.

The structural role of the metal is further corroborated by the observation that crystals of this form become exceedingly fragile on soaking in a nickel-free medium, especially if exposed to repeated changes of the medium. The medium used for nickel-depletion was either 75 volume per cent 2-methyl-2,4-pentanediol (hereinafter abbreviated as MPD), or a nickel-free buffer in this solvent of $pH$ about 6 (as measured by the glass electrode). The fragility of the nickel-depleted crystals rendered them quite difficult to mount for X-ray diffraction investigations. When the depleted crystals were put in a medium containing about $10^{-4}$ M nickel chloride (in 75 volume per cent MPD), they regained their hardness. The loss and uptake of nickel here could be followed qualitatively by exposure of the crystals to hydrogen sulphide or by immersion in a solution of dimethylglyoxime in 75 volume per cent MPD.

This behaviour would indicate that the nickel incorporated in the crystal is in mobile equilibrium with the surrounding medium, while at the same time it participates in stabilizing the contacts between protein molecules. Such a nickel ion might be complexed by two groups contributed by each of two neighbouring molecules. These groups might be carboxyl or imidazole, either of which would be subject to a competitive equilibrium between $Ni^{++}$ and $H^+$. Such an equilibrium is indicated by the limited $pH$ stability range of form I ($pH$ 5·6–7·8)[1], and may also be involved in the depletion of the nickel in the crystals. The pale green colour of the crystals would suggest carboxyl (nitrogenous complexes of nickel are usually blue). Presumably, the intermolecular contacts in the nickel-depleted crystals involve hydrogen bonds between the groups which had held the nickel previously. It is of interest to note that uncomplexed carboxyl groups should be predominantly unionized in these crystals, since carboxylic acids (for example, acetic acid) show nominal (glass-electrode) $pK_a$ values of about 7 in 75 volume per cent MPD.

More striking results were obtained on treatment of RNase I crystals with the platinum complex $K_2[Pt(C_2O_4)_2]$. Now, these crystals normally show perfect cleavage on (100) to give fragments showing unimpaired diffraction patterns; indeed, cleavage provides a useful means of obtaining specimens of a desired size for diffraction investigations. However, on soaking the crystals in a 2·5 × $10^{-4}$ M solution of $K_2[Pt(C_2O_4)_2]$ in 75 volume per cent MPD for some weeks, they became yellow and much harder. The cleavage on (100) was abolished, so that the crystals showed only irregular fracture when pressed very firmly with a glass point. The diffraction intensities of the treated crystals were altered, but unfortunately so were the lattice parameters (original: $a = 43·96$ Å, $b = 75·58$ Å, $c = 37·51$ Å; final: $a = 45·10$ Å, $b = 75·90$ Å, $c = 33·96$ Å). Thus, we cannot regard this case as a good isomorphous substitution.

On the other hand, if we soak the RNase I crystals under the same conditions in a solution of the dithiooxalate analogue, $K_2[Pt(C_2S_2O_2)_2]$, they are strongly stained, but the cleavage and even the diffraction pattern are unaltered, thus indicating diffuse labelling of the protein molecules with the heavy-atom dye. In view of the close structural analogy between $[Pt(C_2O_4)_2]^=$ and $[Pt(C_2S_2O_2)_2]^=$, their differing behaviour towards RNase I should perhaps be ascribed to the lability of the oxalate groups in the former complex. Thus, the oxalate groups could be shed in an exchange of platinum for the nickel ions stabilizing the crystal, so that the platinum takes over the function of linking molecule to molecule. The dithio-oxalate, in which platinum is bound more stably to sulphur, is presumably incapable of undergoing such an exchange reaction.

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[1] King, M. V., Magdoff, B. S., Adelman, M. B., and Harker, D., *Acta Cryst.*, **9**, 460 (1956).
[2] King, M. V., Bello, J., Pignataro, E. H., and Harker, D., *Acta Cryst.*, **15**, 144 (1962).

## CERAMICS

### High-strength Calcium Silicate Hydrate

LIGHT-WEIGHT calcium silicate hydrate (unit weight about 70 lb./ft.$^3$) is made by curing cast mixtures of hydrated lime and silica for a number of hours in saturated steam at high temperatures. In previous work by Taylor and Moorehead[1], it was shown that the maximum compressive strength for cast specimens depends on the lime–silica ratio and the specific surface area of silica flour used. A maximum compressive strength of 3,850 lb./in.$^2$ at one day was achieved at a unit weight of 64 lb./ft.$^3$ (strength/weight ratio 60) for specimens cured for 10 h in saturated steam at 186° C.

Subsequent work has been aimed at producing calcium silicate hydrate of high strength with as low as possible unit weight. To achieve this mixtures of lime and silica (0·5 : 1·0 by weight) were proportioned for maximum strength, mixed in a mortar with 0·067 parts by weight of water until they were uniformly damp and then introduced into a specially designed pressure mould to produce 1-in. cubes. The pressure was applied in two stages to overcome friction between the plunger and the mould. Shortly after the specimens were demoulded they were cured for 7 h in saturated steam at a temperature of 185° C. The relationship between moulding pressure and resulting mean compressive strength (S.D. ± 2,584 lb./in.$^2$) and mean unit weight (S.D. ± 2·0 lb./ft.$^3$) from three samples, one day after pressing, is given in Fig. 1. An increase in moulding pressure from 0 to 15,000 lb./in.$^2$ resulted in an increase in mean compressive strength from 3,500 lb./in.$^2$ to 30,000 lb./in.$^2$ for an increase in mean unit weight from 70 lb./ft.$^3$ to 123 lb./ft.$^3$. Beyond 15,000 lb./in.$^2$ moulding pressure the compressive strength remained unchanged but the unit weight slowly increased. The strength/weight ratio of about 240 for specimens pressure-moulded at 20,000 lb./in.$^2$ is to be compared with the 60 quoted here for ordinary cast calcium silicate hydrate, and 20 for good quality dense concrete at an age of one month (neglecting any size and shape factor). Specimens moulded at a pressure as low as 3,000 lb./in.$^2$ still had a compressive strength of 20,500 lb./in.$^2$, a unit weight of 102 lb./ft.$^3$,