CRYSTALLINE CYTOCHROME b_2

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ABBREVIATIONS

ATP	Adenosine triphosphate.
$\underline{b}_2$-DNA	This is the DNA found in the crystals of Type I cytochrome $\underline{b}_2$ (see INTRODUCTION of thesis).
cm.	Centimeters.
CPM	The number of counts or pulses per min. as recorded by a Geiger-Muller, gas-flow or Scintillation counter.
CTP	Cytidine triphosphate.
DNA	Deoxyribonucleic acid.
E	This is the optical density of a solution with a 1.0 cm. light path at the wavelength stated in the subscript.
EDTA	Ethylene diamine tetraacetate.
FMN	Flavin mononucleotide.
g.	Gram.
GM	Geiger-Muller.
GTP	Guanosine triphosphate.
M	Molar.
mCi	Millicurie.
mg.	Milligram.
ml.	Millilitre.
mM.	Millimolar.
PEP	Phosphoenol pyruvate.
RNA	Ribose nucleic acid.
TCA	Trichloroacetic acid.
tris	Tris (hydroxymethyl) amino methane.
UTP	Uridine triphosphate.

SUMMARY

(1) It has been shown that the flavo-haemo-protein of cytochrome $\underline{b}_2$ must undergo modification before it will crystallise under the conditions of the first crystallisation step in the Appleby and Morton procedure.

(2) Air drying or ageing of the yeast is necessary for the release of $\underline{b}_2$-DNA. Preparations of crystalline Type I cytochrome $\underline{b}_2$ from fresh, freeze-dried yeast contained less than the usual amounts of $\underline{b}_2$-DNA.

(3) The protein and DNA of the oxidised Type I cytochrome $\underline{b}_2$ have been shown to be largely dissociated when the enzyme is in solution.

(4) The specificity of the association between the DNA component and the enzyme of crystalline Type I cytochrome $\underline{b}_2$ has been investigated by testing the ability of various nucleic acid preparations to form crystalline complexes with DNA-free (Type II) cytochrome $\underline{b}_2$. It was found that only double-stranded DNA molecules with a molecular weight of roughly 2×10^5 produced the square plate crystals that are characteristic of normal preparations of Type I cytochrome $\underline{b}_2$. High molecular weight DNA and single-stranded DNA, either native or denatured, produced either amorphous

precipitates or various semi-crystalline forms. These effects were independent of the base composition of the samples used. Polyacrylate, a linear, non-cross linked, polyanion produced square plate crystals with Type II cytochrome $\underline{b}_2$.

(5) The ability of $\underline{b}_2$-DNA to anneal extensively with all samples of labelled yeast RNA collected after centrifugation on a sucrose density gradient, even with RNA up to 10 times its own size, has been taken as proof that $\underline{b}_2$-DNA is a breakdown product of higher molecular weight yeast DNA.

(6) The structure of the two crystalline forms of cytochrome $\underline{b}_2$ has been studied by electron microscopy. From the results, the approximate dimensions of a single enzyme molecule of molecular weight 170,000 were determined as 92 x 82 x 26 A^{o}.

(7) Sections of the hexagonal bipyramid crystals (Type II cytochrome $\underline{b}_2$) at right angles to the $\underline{c}$ axis showed a regular hexagonal network with, apparently, one protein molecule forming the side of each hexagon. Sections parallel to the $\underline{c}$ axis showed that the empty, hexagonal tubes of protein ran right through the crystal. The structure deduced from the sections was

in agreement with that observed in negatively stained, sonicated fragments of the same crystal type.

(8) The flat, square plate crystals of Type I cytochrome $\underline{b}_2$ were seen as parallel rows of protein molecules arranged as layers which were stacked on top of each other to form the crystal. It appeared that alternate layers of protein were arranged at right angles to the ones in between. It has not been possible to obtain satisfactory side views of the structure of this crystal type, nor to locate visually the position of the DNA. However, a structure of these nucleoprotein crystals consistent with available data has been proposed.

(9) A yeast protein solution has been prepared that had DNA-dependent RNA polymerase activity that was Actinomycin D sensitive. The enzyme appeared to be producing a hetero-polymer of ribonucleotides and some of its other properties have been briefly described.