02.1–6 THE CRYSTALLOGRAPHIC STUDIES OF RIBONUCLEASE T_1. By Hirofumi Ohishi, Takashi Amisaki, Shigetoshi Sugio and Ken-ichi Tomita, Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565, Japan.

The structure of ribonuclease T_1 determined as a complex with a highly specific inhibitor, 2'-guanylic acid revealed not only the folding manner of the enzyme but also some interesting features of protein-nucleotide interactions. (U.Heinemann & W.Saenger,(1982) Nature, 299, 27) To understand a specific base recognition mechanism in detail, we independently performed the X-ray analyses of three dimensional structures of RNase T_1-2'-GMP and RNase T_1-3'-GMP complexes.

Crystal data of both complexes are shown in Table 1. Reflection data were collected up to 1.9Å resolution for 2'-GMP complex and 2.1Å for 3'-GMP complex.

The structure of 2'-GMP complex was solved by the multiple isomorphous replacement method using $La(NO_3)_3$ and $UO_2(CH_3COO)_2$ derivatives and the mean figure of merit was 0.78 for 3319 reflections at 2.4Å. The electron density map was of exellent quality so that tracing of polypeptide chain could be straightforward. The protein model was constructed in a "Richards' box" with Kendrew-wire model (2cm/Å) with reference to the amino acid sequence reported previously (K.Takahashi (1965) J. Biol.Chem., 240, 4117). The structure is now refining by using Hendrickson-Konnert least-squares procedure. According to our present model of RNase T_1-2'-GMP complex the inhibitor molecule seems to interact with the protein as shown in Table 2.

The structure determination of 3'-GMP complex is in progress by using molecular replacement methods and/or difference Fourier synthesis with 2'-GMP complex structure.

Table 1. The crystal data of RNase T1-2'-GMP and RNase T1-3'-GMP

Complex	RNase T1-2'-GMP	RNase T1-3'-GMP
Crystal system	orthorhombic	orthorhombic
Space group	$P2_12_12_1$	$P2_12_12_1$
a (Å)	46.65(1)	47.58(1)
b	50.26(1)	50.92(1)
c	40.60(1)	40.32(1)

Table 2. The list of possible interactions between the guanine base of 2'-GMP and RNase T1

Hydrogen bonds

Guanine base	RNase T1	
N1	side chain C=O	Glu 46
N2	backbone C=O	Asn 98
O6	backbone N-H	Asn 44
N7	backbone N-H	Asn 43
or	side chain N-H	Asn 43

Stacking

base	to	phenolic group Tyr 45
base	to	phenolic group Tyr 42

02.1–7 THE REFINED STRUCTURE AT 1.85Å RESOLUTION OF CALOTROPIN D1, A SULFHYDRYL PROTEASE EXHIBITING UNUSUAL PROPERTIES. By R. Hilgenfeld, G.P. Pal, U. Heinemann* and W. Saenger, Institut für Kristallographie, Freie Universität Berlin, Takustr. 6, D-1000 Berlin 33; *Institute of Molecular Biology, University of California, 405 Hilgard Ave., Los Angeles, CA 90024.

Calotropin D1 is a sulfhydryl protease of molecular weight 23,400 which can be isolated from the latex of the madar plant, Calotropis gigantea. The enzyme shows close similarity to other plant cysteinyl proteases such as papain, ficin, and actinidin. However, calotropin completely fails to hydrolyze small synthetic amide and ester substrates such as benzoyl-D,L-arginine p-nitroanilide and carbobenzoxy glycine p-nitrophenyl ester which papain readily acts upon. This unique catalytic behavior of calotropin must be accounted for by structural differences between the active sites of calotropin and papain. We have therefore undertaken an X-Ray crystallographic study of calotropin D1. At 3.2Å resolution, this did not unambiguously reveal such differences (Heinemann et al., J.Mol.Biol. (1982) 161, 591). The current refinement of calotropin D1 at high resolution (up to 1.85Å), however, resulted in detection of some minor but important unique features of the active site architecture. A description of these is given, together with a first account on the amino acid sequence of calotropin D1 as revealed from the electron density map.

02.1–8 CYTOCHROME c_4: FURTHER EXAMPLES OF THE CYTOCHROME FOLD. By A.M. Damas[1], J.E. Grieve[2], R.O. Gould[4], M.M. Harding[1,3], J. Helliwell[4], M.Z. Papiz[4], and L. Sawyer[2].

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The structure of the dihaem, bacterial cytochrome c_4 has been determined by a combination of SIR and wavelength-optimised anomalous dispersion (WOAD) techniques at a nominal resolution of 2.3Å. (A further discussion of WOAD can be found in Papiz et al., Abstracts, this meeting). The resulting electron density map, based on phasing information from rotation camera data for:

(i) a single UO_2^{++} derivative measured with λ=1.760Å
(ii) the native protein at λ=1.380Å and λ=1.739Å and
(iii) diffractometer data to 5Å resolution at λ=1.542Å

for native with $Pt(NO_2)_4^{=}$ and UO_2^{++} derivatives (J.Mol.Biol. 153, 831, 1981), shows that the polypeptide adopts the "cytochrome fold" (Dickerson, Sci. Am., March 1980) typical of high potential low spin cytochrome c. Further, this motif appears twice in the same molecule which raises questions about the evolution and role of the protein in the electron transport mechanism of denitrifying bacteria. A comparison of the two halves with each other and with other cytochromes c will be presented together with a discussion of possible interaction between the two haems.