Crystallographic evidence for the structural isomorphism of deer and beef catalase

D. J. Burkey and A. McPherson[1]

Department of Biological Chemistry, The Milton S. Hershey Medical Center, The Pennsylvania State University, Hershey (PA 17033, USA), 23 November 1976

Summary. 2 crystal forms of deer liver catalase have been grown and analyzed by X-ray diffraction. Both are of space group $P2_12_12_1$ with cell dimensions I: a = 88.5 Å, b = 140.5 Å and c = 235 Å; II: a = 124 Å, b = 128 Å and c = 154 Å. The first of these 2 forms appears to be virtually isomorphous with crystals of beef liver catalase.

Catalase (E.C. 1.11.1.6) is a hemeprotein of 2.5×10^5 daltons which catalyzes the reaction $2H_2O_2=2H_2O+O_2$. The enzyme is composed of 4 identical subunits[2], each containing a ferroprotoporphyrin IX group with the iron atom as Fe^{+3}. Sumner in 1943 developed a simple procedure for the purification and crystallization of beef liver catalase[3] and this has since been applied to obtain the enzyme from a wide variety of organisms[4–8]. Although crystals of beef liver catalase have been extensively studied by both X-ray diffraction and electron microscopy[9], structural studies of the protein from other species have received little attention. In the course of our studies on the enzyme from a wide variety of sources, we have obtained crystals entirely suitable for high resolution X-ray diffraction analysis of catalase from deer liver. The crystals have been grown in 2 distinct modifications, and one of these is virtually isomorphous with a common form of beef liver catalase crystal. This occurs despite very different ambient crystallization conditions.

Methods. Deer liver was obtained from an American White Tail deer accidentally killed by an automobile. Beef liver was obtained from the local butcher. Both catalases were isolated according to preparatory techniques developed by Sumner[3], except that the final product was precipitated with 45% ammonium sulfate followed by extensive dialysis at room temperature with 0.01 M sodium cacodylate, pH 6.2.

Large rectangular prisms of beef liver catalase were grown using the vapor diffusion method[10] in about 3 days from sample depression solutions containing 20 µl of 1% beef liver catalase and 20 µl of 0.1 M sodium cacodylate, pH 6.4. The vapor reservoirs contained 8% ethanol. When the crystals reached a desired size, the depressions were flooded with 0.1 ml of 25% 2-methyl 2–4 pentanediol to stabilize them during exposure to X-ray.

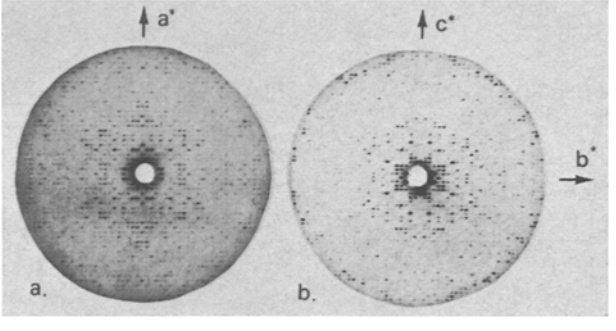
Deer liver catalase crystals of 2 different crystal forms were also grown using the vapor diffusion technique. Large rectangular prisms identical in morphology to those of beef liver catalase were crystallized from a solution comprised of 20 µl of 1% deer catalase, 20 µl of 6% polyethylene glycol 4000 and 10 µl 1.0 M sodium cacodylate pH 6.28 in the sample depression. The vapor reservoir contained 6% polyethylene glycol 4000. The second deer liver catalase crystal form, flat plates, grew out of the precipitate formed in the sample depresseion when 10 µl of 1% deer liver catalase and 10 µl of 4% polyethylene glycol 1000 were present. The reservoir contained 4% PEG 1000. For X-ray analysis, the crystals were sealed in quartz capillaries by conventional methods and diffraction photographs recorded on a Supper precession camera using nickel filtered CuK_α radiation generated by an Elliot rotating anode source operated at 40 kV and 40 mA. The photographic diffraction records were measured and converted to integrated intensities by an Optronics P1000 high-speed rotating drum microdensitometer. All calculations and data reduction were performed on-line with a Digital PDP-11/40 minicomputer using software (Scan 11) written by Paul Bethge and modified by John I. H. Patterson.

Crystallographic results. Precession photographs of the $\varnothing$kl zone of the beef liver catalase crystals possess mm symmetry with the $\varnothing k\varnothing$ and $\varnothing\varnothing l$ present only when $k=2n$ and $l=2n$. The $h\varnothing l$ zone also demonstrates mm symmetry with $h\varnothing\varnothing$ present only when $h=2n$. Upper levels of both zones show mm symmetry with no systematic absences relative to the zero level. The space group of these crystals is, therefore, $P2_12_12_1$ with a = 88 Å, b = 141 Å and c = 234 Å. Beef liver crystals with the same lattice parameters and symmetry were previously reported grown from sodium citrate at pH 8.2[9].

Photographs of the $\varnothing$kl and $h\varnothing l$ zones of the prismatic crystals of deer liver catalase proved the space group to be $P2_12_12_1$ with a = 88 Å, b = 140 Å and c = 235 Å. In addition, the intensity distributions of the $\varnothing$kl photographs from the beef and deer liver catalase crystals were strikingly similar although not identical. The 2 photographs were densitometered, the intensities corrected for Lorentz and polarization effects and scaled to one another using a coefficient of the form $A\exp(-B\sin\theta/\lambda)$. Following this fitting, a residual, R, was calculated using the expression

$$R=\sum_{k=0}^{n}\sum_{l=0}^{n}\frac{(|F_{0kl}(\text{beef})-F_{0kl}(\text{deer})|)}{|F_{0kl}(\text{beef})|}.$$

For all data to a resolution of 4.2 Å, R was found to be 0.22. The flat plate form of deer liver catalase was examined on the precession camera and also shown to have space group symmetry $P2_12_12_1$ but with lattice parameters a = 124 Å, b = 128 Å and c = 154 Å. The $hk\varnothing$ and $\varnothing$kl zones of this crystal are shown in the figure, a and b, respectively. Assumption of 1 molecule of 250,000 per asymmetric unit yields a volume to mass ratio, V_m, of



a Precession photo of $hk\varnothing$ level of the second crystal form of deer liver catalase. *b* Precession photo of the $\varnothing$kl of the second crystal form of deer liver catalase where a = 124 Å, b = 128 Å and c = 154 Å.

2.44 A^3/dalton. This is near the center of the range of values described by Matthews for other crystalline proteins[11] and is close to those of the beef liver and first deer liver crystal forms. Thus in all of the crystals described here, the asymmetric unit consists of 4 entire protein subunits.

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Discussion. The results indicate that the catalase molecules from deer and beef liver are essentially isomorphous. Considering the differences in amino acid composition that must be expected for the 2 species and the surprisingly low residual observed to a rather high resolution, it seems fair to conclude that the overall conformation of these 2 catalases must be almost identical. The differences observed in the diffraction pattern are roughly equivalent to those that might be expected, for example, by the introduction of a single heavy atom into each subunit of the tetramer and would easily be accounted for by the amino acid side chain differences.

Thus in spite of the substantial difference in the animal species involved, it can be stated with considerable certainty that the 3dimensional structure of the catalase molecule has been essentially conserved. One may speculate, therefore, that this hemeprotein, like hemoglobin and myoglobin, very likely exhibits a structural constancy among widely different species. A similar kind of result was obtained by North et al.[12] who showed the close identity of seal and sperm whale myoglobin by direct examination of their respective diffraction patterns.

Beef liver catalase crystals are presently under investigation in 2 laboratories, and will likely result in the 3dimensional structure of that molecule within the next 2 years. Since the deer liver form yields data to high resolution which can probably be phased directly from a beef liver catalase structure, the deer liver catalase molecular structure should follow soon thereafter.

Lysergylpeptides in the course of peptide ergot alkaloid formation

A. Baumert, D. Gröger and W. Maier

Institut für Biochemie der Pflanzen der Akademie der Wissenschaften der DDR, Weinberg, DDR-401 Halle/Saale (German Democratic Republic), 2 December 1976

Summary. Radioactive d-lysergyl-Val-Leu-OMe, d-lysergyl-Val-Val-OMe and d-lysergyl-Val-Val-Pro-OMe were synthetized according to the dicyclohexylcarbodiimide/1-hydroxybenzotriazole procedure. These compounds are not used by intact mycelium of Claviceps as immediate precursors for cyclolalkaloid biosynthesis.

The mechanism of peptide alkaloid formation in Claviceps, especially of the cyclol moiety, is still obscure. Feeding of lysergic acid and appropriate amino acids led to a specific incorporation into ergot alkaloids[1]. Furthermore neither d-lysergyl-L-alanine nor d-lysergyl-L-valine were incorporated as intact units into ergotamine or ergotoxine respectively[2–4]. Also other potential intermediates like dipeptides, diketopiperazines and tripeptides were split prior to incorporation by the ergot fungi[5–7]. For example, Val-Leu-Pro (sequence of ergokryptine) and Val-Val-Pro are no free intermediates in ergotoxine biosynthesis[6,7]. A plausible scheme for cyclol type ergot alkaloid formation was recently developed[7,8]. Apparently the peptide chain formation takes places in a concerted fashion on a multienzyme complex. As a key intermediate, an enzyme-bound lysergyltripeptide was proposed. Concomitantly with releasing this lysergyl derivative from the enzyme, an acylated diketopiperazine could be formed. We tested now whether a lysergyltripeptide (as –OMe derivative) is converted in vivo by the fungus into the corresponding peptide alkaloid. Also 2 appropriate lysergyldipeptides were fed which, after coupling with the 'starter'-molecule proline, might in turn be transformed into alkaloids. Previous experiments[4] revealed that ergot fungi demethylate methyl esters of lysergyl amino acids quite easily.

Material and methods. L-Valyl-L-valin-(U-^{14}C)methyl ester, L-valyl-L-leucine-(1-^{14}C)methyl ester were synthetized by the mixed anhydride method with isobutyl chloroformate[9] and triethylamine. L-Valyl-L-valyl-(U-^{14}C)-L-proline

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