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**Rotation function studies on the subunit structure of supernatant malate dehydrogenase.** By EDWARD J. HILL, DEMETRIUS TSEBNOGLOU and LEONARD J. BANASZAK, *Washington University School of Medicine, Department of Biological Chemistry, St. Louis, Missouri 63130, U.S.A.*

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The rotation function [Rossmann & Blow (1962). *Acta Cryst.* **15**, 24–31] was used to investigate the subunit structure of supernatant malate dehydrogenase. This method indicated that the molecule was a dimer with a twofold molecular axis only  $17^\circ$  from the crystallographic axis. The orientation of this molecular axis has now been confirmed by interpretation of electron-density maps.

The symmetry relating the two subunits of supernatant malate dehydrogenase (s-MDH) has been determined by means of the rotation function (Rossmann & Blow, 1962). This function is a measure of the overlap between a Patterson summation and a rotated version of this summation considered within a limited sphere about the origin. The maximum values of the rotation function correspond to maximum coincidence between these two Patterson summations. This coincidence can be used to identify the relationship between identical or nearly identical units within a crystallographic asymmetric unit (Rossmann & Blow, 1962).

The molecular weight of the asymmetric unit of s-MDH crystals grown from ammonium sulfate in the space group  $P2_12_12$  is about 72,000 Daltons (Banaszak, Tsernoglou & Wade, 1971). The molecular weight of the biologically active s-MDH dimer has been measured by hydrodynamic methods as 73,800 Daltons (Gerding & Wolfe, 1969). Based on this molecular weight and fractionation of the tryptic peptides, the s-MDH molecule is believed to be a dimer of identical or nearly identical subunits (Wade, Glatthaar & Bradshaw, 1972, personal communication). Since the crystallographic asymmetric unit contains one of these dimers, every atom in the first chain can be put into coincidence with the corresponding atom of the second chain by a single rotation plus a translation. If the chains are spatially related by rotations only as would appear to be the case for most polymeric enzymes, the use of the rotation function would describe the symmetry of the s-MDH molecule.

In order to determine this rotation relating the two peptide chains, the rotation function  $R(\kappa, \psi, \phi)$  was calculated using all the 850 independent reflections to  $7.9 \text{ \AA}$  for the stationary Patterson summation.  $\kappa$  is the angle describing the magnitude of the rotation relating the subunits ( $\kappa = 180^\circ$  for a twofold rotation).  $\psi$  and  $\phi$  are the spherical coordinates of the symmetry operation relative to the crystallographic coordinate system. To reduce computing time, only the strongest 10% of the reflections were used to calculate the rotated Patterson summation. The radius of the sphere in which the overlap was considered was  $30 \text{ \AA}$ .

The rotation function was originally calculated over the range of unique rotations varying each of the three angles ( $\kappa, \psi, \phi$ ) by  $15^\circ$  increments. For the space group  $P2_12_12$ , an asymmetric unit of the rotation function lies within the range  $\kappa = 0$  to  $180^\circ$ ,  $\psi = 0$  to  $90^\circ$  and  $\phi = 0$  to  $90^\circ$ . Excluding the exact overlaps for null rotations and crystallographic symmetries the strongest local peak appeared for the rotation  $\kappa = 180^\circ$ ,  $\psi = 15^\circ$  and  $\phi = 45^\circ$ . The value of this peak was 86 while the average background level was 67.6 with a standard deviation of 7.0. To locate the peak more accurately, two of the angles were fixed and the maximum of

the third was determined for each angle at  $4^\circ$  increments. Because  $\psi = 0$  is parallel to the twofold screw axis in the space group  $P2_12_12$ , this angle produces the maximum value of the rotation function. It was difficult to determine

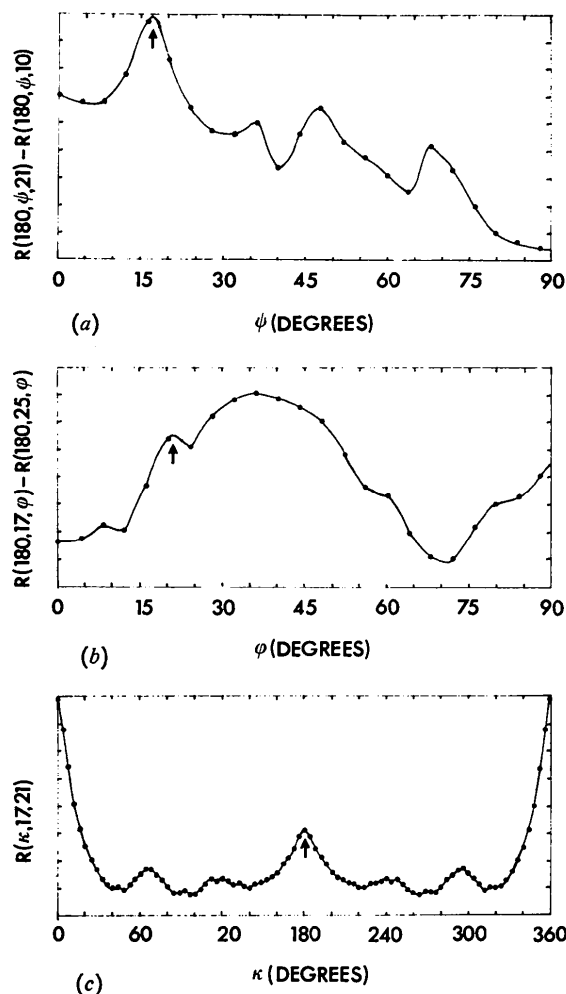


Fig. 1. The rotation function  $R(\kappa, \psi, \phi)$  (in arbitrary units) calculated to  $7.9 \text{ \AA}$  resolution; (a) shows the  $\psi$  dependence; (b) shows the  $\phi$  dependence; (c) shows the  $\kappa$  dependence. The arrows mark the orientation of the molecular dyad which was obtained from the  $\alpha$ -carbon model derived from an electron density map at  $3.0 \text{ \AA}$  resolution. The relative scale along the ordinate along with the standard deviations and error peak levels are discussed in the text.

the precise  $\psi$  position since  $\psi = 15^\circ$  was near this large crystallographic peak and hence it represented only a small plateau on the fall-off from this peak. To illustrate this plateau, Fig. 1(a) shows the difference between the fall-off along  $\varphi = 21^\circ$  (the final  $\varphi$  value) and an arbitrary direction ( $\varphi = 10^\circ$ ). From this Figure, the  $\psi$  value of the local twofold axis was determined to be  $17^\circ$ . Fig. 1(b) shows the  $\varphi$  dependence of the rotation function. There is a narrow peak at  $21^\circ$  and a broad unexplained peak between  $25^\circ$  and  $50^\circ$ . Fig. 1(c) shows the  $\kappa$  dependence of the rotation function. The magnitude of the rotation function for the null rotation ( $\kappa = 180^\circ$ ) is 81. The average background of the function was 67.9 with a standard deviation of 6.8.

It should be emphasized that these calculations were made before any phases were known and were based only on the native X-ray intensities to 7.9 Å resolution. However, knowledge of the spatial relationship between the two subunits has been helpful in interpreting a 3.0 Å resolution electron-density map of s-MDH (Hill, Tsernoglou, Webb & Banaszak, 1972).

After this map was interpreted, a three-dimensional least-squares fit of the  $\alpha$ -carbon positions in the two subunits to a twofold symmetry axis (molecular dyad) indicated that the 'best' directions for the rotation function angles are  $\kappa = 180^\circ$ ,  $\psi = 17^\circ$ ,  $\varphi = 21^\circ$ . The orientation of the molecular dyad obtained from the molecular model is marked by ar-

rows in Fig. 1(a), (b) and (c) to permit comparison with the results from the rotation function. Although the results in  $\varphi$  were made ambiguous by the broad peak, the values for  $\kappa$  and  $\psi$  are certainly consistent with these results.

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**Crystallographic data for carbobenzoxy-L-alanyl- $\beta$ -alanine.** By A. DEL PRA, *Institute of Organic Chemistry, 35100 Padova, Italy*

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Crystals of carbobenzoxy-L-alanyl- $\beta$ -alanine have been examined by X-ray methods. They crystallize in the orthorhombic system, space group  $P2_12_12_1$ , with  $a = 17.02$ ,  $b = 15.60$ ,  $c = 5.91$  Å and  $Z = 4$ .

Carbobenzoxy-L-alanyl- $\beta$ -alanine,  $C_6H_5-CH_2-O-CO-NH-CH(CH_3)-CO-NH-(CH_2)_2-COOH$ , is the monomer used for obtaining the sequential polypeptide poly-L-alanyl- $\beta$ -alanine, which is a good model for the study, both in the solid state and in solution, of the conformational properties of co-polyamides.

The compound was prepared by basic hydrolysis of carbobenzoxy-L-alanyl- $\beta$ -alanine ethyl ester (Scatturin and co-workers, 1973, in preparation). Single crystals were grown from ethanol solution as colourless prisms, with rectangular cross section, elongated along [001]. The crystal symmetry and unit-cell dimensions were determined from Weissenberg and precession photographs of zero and upper layers. The unit cell chosen proved to be reduced (Buerger, 1957).

The crystal density was measured by flotation in KBr solution. The physical and crystallographic data are reported in Table 1.

No further work is contemplated.

I thank Dr A. Scatturin for the samples of carbobenzoxy-L-alanyl- $\beta$ -alanine.

Table 1. *Crystallographic data*

M.W.	294
m.p.	132°C
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
$a$	$17.02 \pm 2$ Å
$b$	$15.60 \pm 2$
$c$	$5.91 \pm 1$
$V$	$1569.2$ Å <sup>3</sup>
$D_x$	$1.24$ g cm <sup>-3</sup>
$D_m$	1.23
$Z$	4
$F(000)$	624
$\mu$ (Mo $K\alpha$ )	$8.06$ cm <sup>-1</sup>
$\mu$ (Mo $K\alpha$ )	1.02

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