The molecular mass and trimeric nature of chloramphenicol transacetylase

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Chloramphenicol transacetylase (CAT) is an intracellular enzyme which is the effector of chloramphenicol resistance in a wide range of bacterial species and genera (Shaw, 1983). It was originally believed to be tetrameric, consisting of identical subunits of M_r 20000–24000, as determined by SDS/polyacrylamide-gel electrophoresis (Shaw *et al.*, 1970; Zaidenzaig *et al.*, 1979). A native M_r of approx. 80000 was estimated by both agarose gel filtration (Shaw *et al.*, 1970) and sucrose density gradient centrifugation (Shaw & Brodsky, 1968). The results of hybridization studies *in vivo* were compatible with a tetrameric structure for CAT (Packman & Shaw, 1981), but a recent X-ray crystallographic study (Leslie *et al.*, 1986) has demonstrated that CAT is likely to be a trimer and cannot be a tetramer. To date, known, there has been no direct solution evidence of the trimer form of CAT.

The type III variant of CAT studied by Leslie *et al.* (1986) was isolated and purified as described by Zaidenzaig *et al.* (1979). For the sedimentation equilibrium experiments the protein was dialysed for $\gtrsim 24$ h in a 100 mM-KCl/5 mM-Tris buffer, pH 7.5. Two sedimentation equilibrium experiments were performed. The first was performed in a Beckman Model E Analytical Ultracentrifuge equipped with Rayleigh Interference optics and an RTIC temperature measurement system. The intermediate speed method (Creeth & Harding, 1982) was employed. In this technique the meniscus concentration remains finite and was found by the intercept/slope method (Creeth & Harding, 1982). An exact value for the intial solute loading concentration was not required. A 12 mm cell was employed, the initial loading concentration, c^0 was approx. 2 ml/g, and the value of the partial specific volume used was 0.736 mg/ml evaluated experimentally (Kratky *et al.*, 1973) and also from the amino acid composition. The weight average M_r over the whole solute distribution, $M^0_{r,w}$, was determined by extrapolation of the 'star' average M_r to the cell base (Creeth & Harding, 1982) and found to be 70000 ± 1000. This value will be affected by the small amount of thermodynamic non-ideality present. However, linear extrapolation of the (reciprocal of the) point weight average M_r ($M_{r,w}$) to infinite dilution (Fig. 1) yields an 'ideal' value of 70500 ± 1500). The point $M_{r,w}$ values were obtained from sliding strip fitting procedures to the fringe data (see, for example, Harding, 1984).

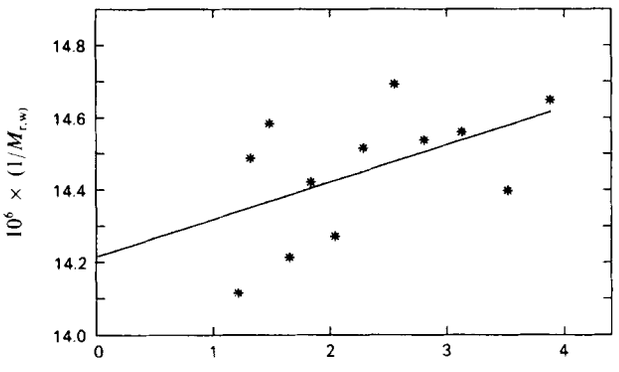

Fig. 1. *Plot of the reciprocal of the apparent point weight average M_r as a function of absolute (fringe) concentration*

A 12 mm path length cell was used with an intial solute loading concentration of ~ 2 mg/ml. Rotor speed, 14288 rev./min; temperature, 20.0°C.

A further low-speed sedimentation equilibrium experiment, performed in an MSE Centriscan ultracentrifuge, using (the less precise) scanning absorption optics yielded a very similar value for the M_r of 72400 ± 1000 (at a solute concentration of 0.3 mg/ml):

The M_r of CAT subunits (from sequence analysis) varies slightly for six microbial variants but is within the range 24000–25000 (W.V. Shaw, unpublished work). The only model therefore consistent with the native and subunit M_r data is a trimer.

Creeth, J. M. & Harding, S. E. (1982) *J. Biochem. Biophys. Methods* **7**, 25–34

Harding, S. E. (1984) *Biochem. J.* **219**, 1061–1064

Kratky, O., Leopold, H. & Stabinger, H. (1973) *Methods Enzymol.* **27**, 98–110

Leslie, A. G. W., Liddell, J. M. & Shaw, W. V. (1986) *J. Mol. Biol.* **188**, 283–285

Packman, L. C. & Shaw, W. V. (1981) *Biochem. J.* **193**, 541–552

Shaw, W. V. (1983) *CRC Crit. Rev. Biochem.* **14**, 1–46

Shaw, W. V. & Brodsky, R. F. (1968) *J. Bacteriol.* **95**, 28–36

Shaw, W. V., Bentley, D. W. & Sands, L. (1970) *J. Bacteriol.* **104**, 1095–1105

Zaidenziag, Y., Fitton, J. E., Packman, L. C. & Shaw, W. V. (1979) *Eur. J. Biochem.* **100**, 609–618

Abbreviation used: CAT, chloramphenicol transacetylase.

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Putative irreversible inhibitors of trypsin-like enzymes: analogues of basic amino acids bearing a carbodi-imide moiety

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The primary specificity of trypsin is determined by the presence of the negatively charged carboxylate of Asp-189 located at the base of the active site which forms a salt-bridge with the protonated basic group of the substrate or inhibitor thus anchoring it inside the catalytic cleft.

Since carbodi-imides ($R^1-N=C=N-R^2$; R^1, R^2 are alkyl or aryl) are both basic and chemically reactive towards carboxyl functions, it should be possible to design active-site-directed irreversible inhibitors for trypsin-like enzymes that are modelled upon reagents incorporating a carbodi-imide moiety. Consequently, we have examined, for the first