Small-Angle X-Ray Scattering of Pig Thyroglobulin in Solution

By HARRY BRUMBERGER, JEFFREY L. LIPTON, GARY DORFMAN and EILEEN NAKANO
Department of Chemistry, Syracuse University, Syracuse, N. Y. 13210, U.S.A.

(Received 20 May 1974)

Small-angle X-ray scattering measurements on native pig thyroglobulin in phosphate buffer, pH 6.9, yield a radius of gyration of 6.4 nm (64 Å), a particle volume of approx. $1.5 \times 10^3 \text{ nm}^3$ ($1.5 \times 10^6 \text{ Å}^3$), an axial ratio of 2.2:1 (assuming an ellipsoidal shape), and a solvation of 0.63 g of solvent/g of protein.

The shape and solvation of the thyroid protein thyroglobulin have been the subject of a number of investigations; axial ratios ranging from about 9:1 (Lundgren & Williams, 1939) to 2:1 (Bloth & Bergquist, 1968) have been reported, on the basis of various experimental methods. A preliminary report of some of the results presented here was given at the 3rd International Conference on Small-Angle Scattering (Brumberger *et al.*, 1974).

In an attempt to determine these parameters by a different and possibly more unambiguous method, we have measured the small-angle X-ray scattering of native pig thyroglobulin (Mann Research Laboratories, New York, N.Y. 10006, U.S.A., lots U-2586 and U-4196) in phosphate buffer (0.010 M- NaH_2PO_4 , H_2O –0.012 M- Na_2HPO_4 –0.005 M- NaCl –0.003 M- NaN_3 , pH 6.9) at concentrations from approx. 3 to approx. 30 mg/ml. The commercial preparation was chromatographed in a buffer on a column (2.6 cm $\times$ 40 cm) of Sephadex G-200 gel, the 'native' fraction collected and rechromatographed on Sepharose 6B; $s_{20,w}^0 = 20.1 \text{ S}$, measured by sedimentation velocity (Lipton, 1972). X-ray experiments were performed with a Kratky camera (Kratky, 1967), nickel-filtered $\text{CuK}\alpha$ radiation, proportional counter and pulse-height discriminator; the sample was kept at $4.0 \pm 0.5^\circ\text{C}$ in most experiments for which parameters are reported here. The 'infinite-slit' geometry of the camera was used (Kratky *et al.*, 1960). The smoothed difference curves (solution-scattering minus solvent-scattering, measured in the same cell) were corrected for collimation effects (Kratky *et al.*, 1960; Heine & Roppert, 1962; Kent & Brumberger, 1964).

The average radius of gyration, R_g , of the protein molecule in solution was determined from the Guinier approximation to the small-angle portion of the scattering curves and found to be $6.4 \pm 0.1 \text{ nm}$ (Guinier *et al.*, 1955; Kratky & Pilz, 1972) independent of concentration (Fig. 1). The volume v of the particle can be calculated from the relationship:

$$v = \frac{I(0)(\lambda)^3}{4\pi Q} \quad (1)$$

(Kratky & Pilz, 1972) where $I(0)$ is the extrapolated zero-angle intensity from the linear portion of the

Guinier plot, l the distance from sample to detector, λ the X-ray wavelength (0.154 nm), and Q the 'invariant':

$$Q = \int_0^\infty I(m)m^2 dm \quad (2)$$

with $m = l \cdot 2\theta$ (2θ = scattering angle). The most reliable value for v from our data appears to be

Fig. 1. Logarithmic (Guinier) plots of relative intensities I versus m^2

Protein concentrations: ●, 7.98 and 31 mg/ml; ○, 2.82 and 7.80 mg/ml; ○, 31 mg/ml. The runs grouped together under each symbol represent three sets taken with different camera geometries (slit widths, angular resolutions etc.); relative intensities are directly comparable only within each set.

approx. $1.5 \times 10^3 \text{ nm}^3$. The shape anisotropy factor f , defined as the ratio of the experimental radius of gyration to that of a sphere of equal volume (Kratky, 1971; Alexander, 1969) is 1.16. Assuming a prolate ellipsoid of revolution (Bloth & Bergquist, 1968; Edelhoch & Rall, 1964), f can be expressed in terms of the axial ratio $p = a/b$ (a is the semiaxis of rotation):

$$f^2 = \frac{p^2 + 2}{3p^{2/3}} \quad (3)$$

This yields $p = 2.2$, in excellent agreement with recent electron-microscopic observations (Bloth & Bergquist, 1968).

The degree of homogeneous solvation can also be found (see, e.g. Durchschlag *et al.*, 1971); if x is the solvation (g of solvent/g of protein) and q the swelling ratio, i.e.:

$$q = \frac{vN}{\bar{v}M} \quad (4)$$

(N is Avogadro's number, M the molecular weight and $\bar{v}$ the partial specific volume of the protein), then:

$$x = d_2 \bar{v}(q - 1) \quad (5)$$

where d_2 is the solvent density. If we accept the values of 670000 and 0.723 reported in the literature for M and $\bar{v}_{20}$ respectively (Tarutani & Ui, 1969; Derrien *et al.*, 1949), we find $x = 0.63$.

Alexander, L. E. (1969) *X-Ray Diffraction Methods in Polymer Science*, chapter 5, Wiley-Interscience, New York

- Bloth, B. & Bergquist, R. (1968) *J. Exp. Med.* **128**, 1129–1136
- Brumberger, H., Lipton, J. L., Dorfman, G. & Nakano, E. (1974) *Int. Conf. Small-Angle X-Ray and Neutron Scattering 3rd, Grenoble, 1973*, abstracted in *J. Appl. Cryst.* **7**, 181
- Derrien, Y., Michel, R., Pedersen, K. & Roche, J. (1949) *Biochim. Biophys. Acta* **3**, 436–441
- Durchschlag, H., Puchwein, G., Kratky, O., Schuster, I. & Kirschner, K. (1971) *Eur. J. Biochem.* **19**, 9–22
- Edelhoch, H. & Rall, J. E. (1964) in *The Thyroid Gland* (Pitt-Rivers, R. & Trotter, W. R., eds.), vol. 1, pp. 113–130, Butterworths, London
- Guinier, A., Fournet, G., Walker, C. B. & Yudowitch, K. L. (1955) *Small-Angle Scattering of X-Rays*, John Wiley and Sons, New York
- Heine, S. & Roppert, J. (1962) *Acta Phys. Austr.* **15**, 148–166
- Kent, P. & Brumberger, H. (1964) *Acta Phys. Austr.* **17**, 263–265
- Kratky, O. (1967) in *Small-Angle X-Ray Scattering* (Brumberger, H., ed.), pp. 63–120, Gordon and Breach, New York
- Kratky, O. (1971) in *Proc. Eur. Biophys. Congr. 1st* (Broda, E., Locker, A. & Springer-Lederer, H., eds.), S-III, 373–396, Verlag Wiener Medizinische Akademie, Vienna
- Kratky, O. & Pilz, I. (1972) *Quart. Rev. Biophys.* **5**, 481–537
- Kratky, O., Porod, G. & Skala, Z. (1960) *Acta Phys. Austr.* **13**, 76–128
- Lipton, J. L. (1972) Ph.D. Thesis, Syracuse University
- Lundgren, H. P. & Williams, J. W. (1939) *J. Phys. Chem.* **43**, 989–1002
- Tarutani, O. & Ui, N. (1969) *Biochim. Biophys. Acta* **181**, 116–135