urements for the process were unavailable for comparative purposes. In analogous reasoning the temperature dependence on polypeptide helix coil transition has been used to test helix coil transition theories,[13,14] and from such studies at various chain lengths theoretical parameters for the PBG transition have been established. Recently, direct calorimetric measurements have provided an even more direct evaluation of the theoretical parameters.[15,16]

Analogous information should also be revealed from studies of the effect of pressure on the transition along with a determination of the volume change of the transition. There may be situations where this latter approach is experimentally more feasible than the determination of thermal transitions along with calorimetric measurements. In this paper we report results obtained for two known reversible transitions, ribonuclease and PBG.

Experimental

The ribonuclease A used was Lot RAF 6069, lyophilized and phosphate-free, and was obtained from Worthington Biochemical Corp., Freehold, N. J. The material was used without further purification, and calculations were based on the weight of the vacuum-desiccated material.

The buffer solutions used were 0.01 *M* in potassium acid phthalate and 0.15 *M* in KCl. The solution conforms to that used by Holcomb and Van Holde.[6] In preparation of the ribonuclease solution, the buffer solution was pipetted into a weighed flask containing the ribonuclease; the flask was then tightly stoppered. The pH of the solution was adjusted to 2.80 using a Beckman Zeromatic pH meter. The solution was refrigerated until used. The high pressure optical rotation apparatus has been previously described.[17]

The PBG used was Lot G51. It was obtained from Pilot Chemicals Inc., Watertown, Mass., and was specified to have a molecular weight of 275,000. The material was used without further purification, and calculations were based on the weight of the desiccated material.

The solvent for the PBG was 76 vol. % Eastman practical dichloroacetic acid (DCA) and 24 vol. % Eastman reagent 1,2-dichloroethane (EDC). Both components of the solvent were redistilled prior to use. The solvent mixture was tightly capped and refrigerated until needed.

Theory

The basic reaction process for these polymer transitions can be written in terms of the extreme conditions as

$$\text{folded} \rightleftharpoons \text{unfolded} \qquad (1)$$

where, if the process involves more than one step, there will be a series of intermediate states and equations within eq. 1.

In the case of a complex molecule such as ribonuclease, it is not possible to predict logically the nature of intermediate steps, if any. It becomes convenient either to assume a single-step reaction process of eq. 1 or, as Tanford has done,[18] to suppose that reaction 1 consists of r equivalent and independent steps. This latter situation reduces to the one-step case when $r = 1$. The appropriate result which expresses the pressure dependence upon the fraction of folding f is then given by

$$\left(\frac{\partial f}{\partial P}\right)_T = \frac{f(1-f)\Delta V^\circ}{rRT} \qquad (2)$$

where ΔV° is the standard state volume change per mole of polymer taken under the conditions of infinite dilution, at temperature T and pressure P. A dilatometric determination of the volume change of transition, even at finite concentrations, should be nearly equivalent to ΔV°. With the assumptions contained in eq. 2 it should then be possible to determine $\Delta V^\circ/r$ from pressure measurements, and, when this value is compared to a dilatometric determination of ΔV°, an estimate of r can be made. If a multiple-step mechanism is indicated from the experimental findings, it is quite probable that cooperative effects exist between different portions of the molecule. The assumption of independent steps then becomes invalid. A simple mechanism of successive steps which must follow in a definite order and which are characterized by the same equilibrium constants can be analyzed, but it seems the presentation of such an analysis would be superficial until some more definite facts about the ribonuclease transition are available.

(6) D. N. Holcomb and K. E. Van Holde, *J. Phys. Chem.*, **66**, 1999 (1962).

(7) P. Urnes and P. Doty, *Advan. Protein Chem.*, **16**, 401 (1961).

(8) J. Applequist, *J. Chem. Phys.*, **38**, 934 (1963).

(9) P. Doty and J. T. Yang, *J. Am. Chem. Soc.*, **78**, 498 (1956).

(10) L. Peller, *J. Phys. Chem.*, **63**, 1194 (1959).

(11) R. A. Scott and H. Scheraga, *J. Am. Chem. Soc.*, **85**, 3866 (1963).

(12) S. J. Gill, K. Beck, and M. Downing, *ibid.*, **87**, 901 (1965).

(13) B. H. Zimm, P. Doty, and K. Iso, *Proc. Natl. Acad. Sci., U. S.* **45**, 1601 (1959).

(14) [illegible] and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).

(15) T. Ackermann and H. Ruterjans, *Z. physik. Chem.* (Frankfurt), **41**, 116 (1964).

(16) F. E. Karasz, *Nature*, **202**, 693 (1964).

(17) S. J. Gill and R. L. Glogovsky, *Rev. Sci. Instr.*, **35**, 1281 (1964).

(18) C. Tanford, *J. Am. Chem. Soc.*, **86**, 1050 (1964).