

Figure 2. Effect of temperature on undenatured fraction (f) at various pressures for ribonuclease A.

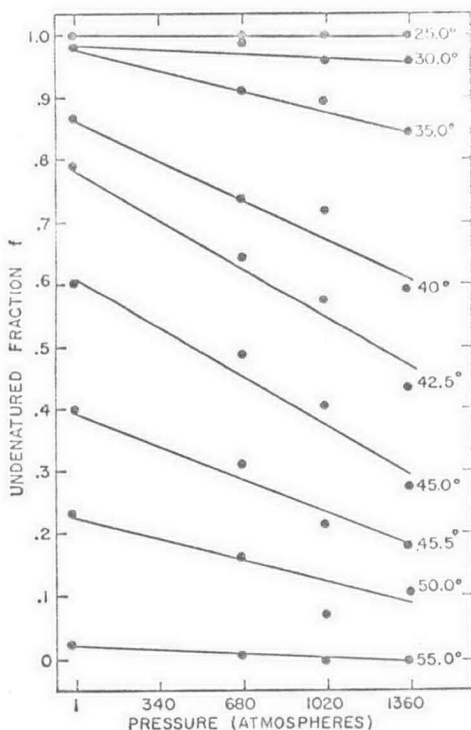


Figure 3. Effect of pressure on undenatured fraction (f) at various temperatures for ribonuclease A.

and 45.0°, respectively, for $\Delta V^\circ/r$. It is also known from direct measurements that the folded configura-

tion (low-temperature form) has greater volume than the unfolded configuration.⁶ The observation (Figure 2) that the amount of folded form decreases with increasing pressure is in agreement with this finding.

The volume changes calculated from the pressure measurements are much lower than those observed by Holcomb and Van Holde,⁶ who found a value of -240 ml./mole at 45.0° by density measurements using twin dilatometers. This immediately suggests a multiple-step process. However, before drawing any more definite conclusions, the possible errors in the values of these two volume changes should be noted.

Although the dilatometric method yields excellent results, a linear extrapolation of the slopes for the high- and low-temperature determinations must necessarily be made to evaluate the volume change occurring for the denaturation process. The determination of the slopes and the necessary extrapolations could affect the evaluation of the volume change by as much as 35%. A similar order of magnitude of error may also exist in the value derived from the pressure measurements since the effect was so small.

Thus, we shall take for the dilatometric volume change -240 ± 100 ml./mole, and for the pressure-calculated value, -30 ± 10 ml./mole. The ratio of these volume changes yields a value for r , the number of independent steps of the mechanism, of somewhere between 3 and 17. The range of values is rather unsatisfactory, but, on the basis of independent equal reaction steps, the number of regions with which the folding to unfolding process occurs is between 3 and 17. Tanford¹⁸ has analyzed data which indicate that three regions seem to unfold independently. Scott and Scheraga have interpreted optical density changes by a two-step mechanism. It seems highly probable that cooperative effects would be present in this transition, and the safest conclusion is one which states that more than one reaction step is involved.

Poly- γ -benzyl-L-glutamate. Reversible thermal transitions as observed for a 4.33 wt. % solution of PBCG at 1, 681, and 1021 atm. are shown in Figure 4 which illustrates the change of observed rotation for the thermal transition at various pressures. Figure 5 shows the effect of temperature on the fraction of folding at the three pressures. The fraction, f , is assumed to be determined by fractional change of the optical rotation upon transition. Open and filled symbols on Figure 4 represent data obtained for increasing and decreasing temperatures, respectively, and illustrate the reversibility of the transition.

Dilatometric measurement was attempted to evaluate the volume change accompanying the transition, but it was found that the change was too small to be