EFFECT OF HYDROSTATIC PRESSURE ON THE AGGREGATION REACTION OF POLY-L-VALYL-RIBONUCLEASE

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Polypeptidyl derivatives of proteins (Becker & Stahmann, 1953) have been used frequently to study the effect of modifying groups on the catalytic properties of enzymes (Katchalski et al., 1964). However, in addition to any steric effects of the substrate-enzyme interaction that may be imposed by suitably located polypeptides, one must also consider the effect of the various short-range forces (Kauzmann, 1959) which govern the conformational structure of proteins. By the use of amino acid residues containing alkyl side-chains, the polypeptidyl proteins can serve as effective models for studying non-polar interactions which contribute substantially to the native structure of proteins as well as to protein-protein interactions.

A number of reports (Becker, 1962; Ona, 1960; Krausz, et al. 1963) have indicated that certain polypeptidyl proteins can undergo reversible aggregation reactions at much lower temperatures than their respective native proteins. Although native RNase aggregates only at high temperatures, polyvalyl-RNase (PVRNase) solutions can show a turbidity at temperatures as low as 30°. This low temperature aggregation must be due to the increased apolar interactions afforded by the polyvalyl chains attached to the enzyme, since polyglycyl derivatives are actually more thermally stable than the native enzyme (Becker and Sawada, 1963). This report describes some studies of the thermal aggregation of PVRNase

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at 39° as influenced by salt concentration and hydrostatic pressure.

Methods and Materials. The poly-L-valyl Ribonuclease was prepared from crystalline material (Sigma lot R50B-095) after purification on carboxymethyl cellulose (Taborsky, 1959). The modification reaction was carried out in phosphate buffer at pH 7.6 (Becker & Stahmann, 1953). The average length of the added chains was about 3 based on the addition of 22 moles of valine per mole on 7 sites as determined by dinitrophenylation and analysis. The thermal aggregation studies were conducted in a stainless steel high pressure cell with white sapphire windows and with an optical path length of 1 cm (Morita, 1957). Turbidity was estimated at 320 mμ in a Beckman DU spectrophotometer. The solutions were 2 mg/ml protein in 0.05M phosphate buffer at pH 6.8 and of varying concentrations in sodium chloride as indicated in the results.

Results and Discussion. The thermal aggregation of PVRNase was found to be sensitive to pH, and was maximal between 6 and 7. At pH 6.47 and 49° the time required for the appearance of turbidity as a function of sodium chloride concentration was found as shown on Table I.

Table 1. Effect of salt concentration on turbidity time

NaCl (M)	Time req'd for turbidity (sec)
0.5	75
0.4	75
0.3	120
0.2	135
0.1	160

Alternatively, when solutions of PVRNase with different salt concentrations were warmed in a water bath at the same rate, the turbidity was first observed at 36° with 0.5 M NaCl and at 44° with the 0.1 M salt solution. The presence of higher salt concentrations in the PVRNase solution permitted aggregation to proceed faster and at a lower temperature. Together with the data of Table I, this would suggest that while the low temperature aggregation of PVRNase may be due primarily to attractive apolar interactions, there are also involved repulsive forces which can be diminished by higher ionic strengths.

The effect of hydrostatic pressure on the rate of thermal aggregation is shown in Figure 1. The rate of turbidity increase is highest at 1 atm and decreases markedly at 150 atm and 300 atm. The time scale reflects minutes lapsed after placing the PVRNase solution into the high pressure optical cell at 39°. Hence, the non-zero intercepts for the OD changes determined under 150 and 300 atm are due to the aggregation which takes place in the 7 or 8 minutes required to attain the desired pressures. When the hydrostatic pressure was suddenly released, the rate of turbidity formation abruptly increased to that at 1 atm, showing distinctly the pressure dependence of the aggregation rate.

The diminution of the aggregation rate by applied hydrostatic pressures would suggest that the transition state of the rate limiting step exhibits a positive volume of activation (ΔV^{*}). This volume change for the aggregation reaction can be determined by using the assumed re-

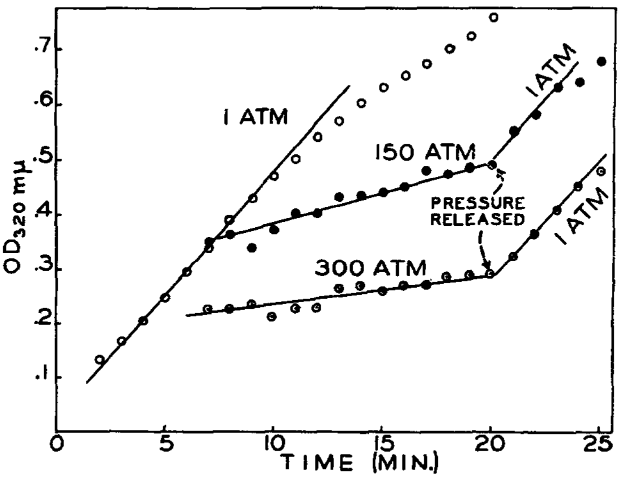

Fig. 1. Time Course of PVRNase Aggregation. Conditions as indicated in text with solutions 0.6M in Sodium cholride.

lationship (Hamann, 1963) between rate constants and pressure: $\frac{\partial \ln k}{\partial P} = \frac{-\Delta V^*}{RT}$, where R = molar gas constant and T the absolute temperature. We have chosen k to be equal to $\Delta OD/\Delta time$ (in min.). Since the turbidity (as OD) changes linearly with time (See Fig. 1), we have assumed that our system is undergoing condensation polymerization according to the relationship $\tau = AN_0V_0^2\ (1+kt)$ (Oster, 1947) where τ is the turbidity, A a scattering constant, N_0 the initial monomer concentration, V_0 the volume of the monomer, k the reaction constant, and t the time. This relationship apparently holds in the early phases of the aggregation reaction where the particles formed are small compared to the 320 mμ wave length used for the turbidity measurements.

A plot of the ln $(\Delta OD/\Delta t)$ versus the pressure on the system is shown in Fig. 2. It is seen that the ΔV^* is positive, but that it decreases with increasing salt concentration. Hence, with a smaller ΔV^* barrier at higher ionic strength, the rate of aggregation is increased, thus confirming the observations in Table 1. The low temperature aggregation of PVRNase is apparently made possible by the apolar interactions of the polyvalyl side chains, but the change in the ΔV^* with

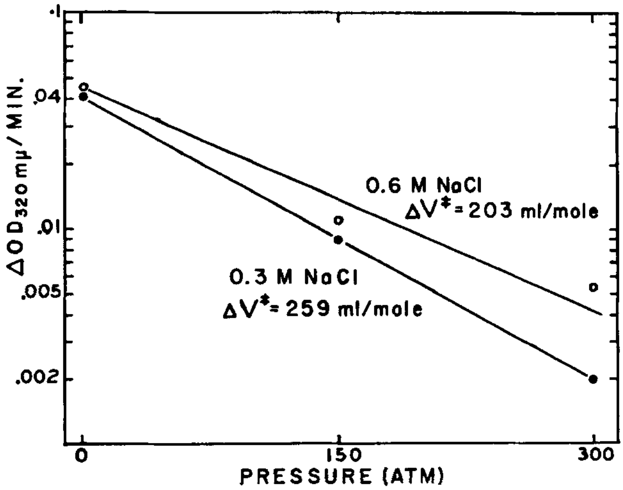

Fig 2. PVRNase Aggregation Rate As A Function of Hydrostatic Pressure. Data obtained from experiments as illustrated in Fig. 1.

salt concentration indicates that the positively charged terminal α-amino groups of the valyl polypeptides exert a sizeable (though opposite) force on the aggregation reaction.

The very large size of the positive ΔV^* can be interpreted as the release of a large number of solvent molecules from the monomer's polyvalyl chains in the rate determining transition state of the aggregation. Kauzmann (1959) has pointed out that the process of transferring hydrocarbons from water solution into nonpolar solvents is accompanied by a volume increase (e.g., +18.1 ml/mole for ethane into hexane). This rather large volume change has been interpreted as due to the release of water molecules "bound" about the alkane molecules. The reaction occurs spontaneously at about room temperature in the direction of forming hydrophobic bonds (i.e., non-covalent alkyl-alkyl interactions with the exculsion of solvating water molecules). While the magnitudes of the ΔV of reactions cannot be directly compared to their ΔV^*, the general size and positivity of the ΔV^* here determined would suggest that the aggregation of PVRNase is due mainly to apolar interactions of the alkyl side chains of the attached polyvalyl residues. Further observations which support this notion are that the aggregation of the polyvalyl derivative occurs at rather moderate temperatures and that such aggregations can be reversed in the cold (0-4°).

The kinetics of the aggregation reaction described here is very similar to the polymerization of tobacco mosaic virus protein subunits reported in summary by Lauffer (1964) who has called the process an entropy driven one involving the release of protein bound water molecules. The PVRNase aggregation system offers a more direct inquiry into the nature of such endothermic processes in that the sites of protein-protein interaction must clearly involve the non-polar residues of the polyvalyl chains.

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