TH-AM-D1 WHY DO SOME PROTEINS CRYSTALLIZE FROM 2-METHYL-2,4-PENTANEDIOL? Serge N. Timasheff and Eugene P. Pittz*, Graduate Department of Biochemistry, Brandeis University, Waltham, Mass. 02154.

The crystallization of ribonuclease A from 55% 2-methyl-2,4-pentanediol (MPD) is a puzzle, since MPD is expected to be a good denaturant. In order to elucidate this question, the thermodynamic interactions between ribonuclease A and solvent components were investigated in water-MPD mixtures of various compositions at pH 5.8. The results show that, at all solvent compositions, addition of protein increases the chemical potential of MPD, with a resultant preferential exclusion of MPD from contact with protein. From the interaction parameters obtained, it can be shown that addition of MPD to an aqueous solution of ribonuclease A to a final solvent composition of 55% MPD should result in phase separation, if total protein concentration is higher than 9 mg/ml. The preferential exclusion of MPD from contact with protein is attributed to unfavorable interactions with charged groups on the surface of the protein. (Supported by NIH grants GM 14603, CA 16707, and NSF grant BMS 72-02572).

TH-AM-D2 CRYSTALLOGRAPHIC STUDIES OF PROTEIN FOLDING. W. Traub*, A. Yonath*, A. Podjarny*, A. Sielecki*, B. Honig* and J. Moult* (Introd. by D.A. Torchia) Structural Chemistry, Weizmann Institute, Rehovot, Israel.

Denaturants have been extensively used to study protein unfolding and refolding in solution, but have provided relatively little information about the conformational changes involved. The use of cross-linked triclinic lysozyme crystals has enabled us to investigate some of these changes in detail by means of X-ray structure analysis. Soaking of cross-linked crystals in denaturant solutions of increasing concentrations caused corresponding increases in crystal volume and decreases in minimum observable X-ray spacings. These changes proved partly reversible on diluting the solutions. The effects of various denaturants were studied by following crystal volume and minimum X-ray spacing as a function of concentration, as well as by means of detailed structure analyses at several intermediate stages in the denaturation and renaturation cycles. It appears that at least two different denaturation mechanisms are involved, with detergent-like reagents disrupting the hydrophobic interactions joining the two wings of the lysozyme molecule and hydrophilic denaturants interacting primarily with polar groups on the molecular surface. These studies have also indicated that denaturant-mediated conformational changes involve relative movements of fixed domains in the protein structure.

TH-AM-D3 FIBRINOPEPTIDE B AND AGGREGATION OF FIBRINOGEN. John R. Shainoff, Research Div., Cleveland Clinic, Cleveland, OH 44106

It is now possible to remove fibrinopeptide B without fibrinopeptide A from rabbit, bovine, and human fibrinogen. Copperhead venom procoagulant enzyme, previously shown to remove B faster than A, has been found to release little A at temperatures below 14°. At low temperatures, tight aggregation of the derivative lacking B blocked release of A by the enzyme. Transient release of A occurred, but stopped as removal of B approached completion. Overall losses of A amounted to 8% and 2% from human and rabbit fibrinogen, respectively. Resultant clots with A intact dissolved on warming to 37°, whereafter release of A resumed with secondary coagulation ensuing. When dissolved at 37° with PMSF-inactivated enzyme, the fibrin remained highly soluble (>12 mg/ml). Ultracentrifugation showed constant levels of 8S monomer together with 16S aggregates in amounts accounting for the total protein at concentrations down to 0.16 mg/ml, and monomer alone at lower concentrations. From changes in saturation level of monomer at lower temperatures, the enthalpy of aggregation appeared to be of the order of -20 kcal/mole, approximately half that known to be associated with aggregation of fibrin lacking both fibrinopeptides. Other experiments indicate that removal of the two peptides unmasks separate aggregation sites, each of which place the monomers in an alignment necessary for crosslinking by factor XIIIa. The formation of 16S aggregates through release of peptide B contrasts with the well known formation of 24S aggregates on removing either A alone or both A and B. To explain this difference, it is suggested that removing A promotes aggregation of monomers in a double stranded array while B masks sites for strengthening binding within the strands. With peptide A intact, aggregation may be limited to alignment of monomers in a single stranded array.