PN 1239

Effect of urea on behavior of the protein moiety of human-serum α-lipoproteins in solution

The protein moiety of highly purified human-serum α-lipoproteins (*d* 1.063–1.210 g/ml) has been isolated and its homogeneity established by ultracentrifugal, electrophoretic, end-group and biological analyses. This report presents evidence that the isolated protein may be a monomer which undergoes aggregation in 0.15 M NaCl, phosphate buffer and barbital buffer solutions. The addition of urea or an increase in pH produces a partial dissociation of aggregates to the monomeric form without evidence of denaturation.

High-density lipoproteins were isolated from the plasma of six fasting healthy subjects by differential preparative ultracentrifugation. Further purification was achieved by repeated washing with NaCl–KBr solution (*d* 1.210 g/ml) followed by dialysis against 0.15 M NaCl at 4° for 48 h. The lipoproteins were then extracted with ethanol–ether (3:1, v/v) followed by diethyl ether at −30° by the method of SCANU *et al.*[1]. The resulting freshly prepared solution of lipid-free protein migrates as a single distinct band on agar and starch-gel electrophoresis (Tris–boric acid, pH 8.2) and as a single schlieren-pattern peak during analytical ultracentrifugation (Fig. 1A), in agreement with previously published observations[1,2]. From an extrapolation of data to infinite dilution an $s^{\circ}_{20,w}$ of 4.1 was obtained. Amino acid analyses of the protein confirmed previously reported results[2,3] and revealed that aspartic acid is the only NH_2-terminal amino acid and cysteine or cystine are not present in detectable amounts. The recombination of ^{131}I-labeled protein with whole serum lipids produces a lipoprotein with a biological half-time in the human equivalent to that of ^{131}I-labeled native α-lipoprotein[4].

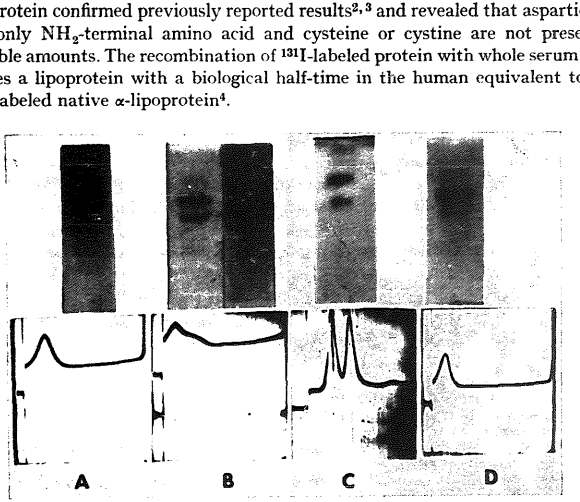

Fig. 1. Starch-gel electrophoresis and sedimentation patterns of α-protein under various conditions. A, α-protein (10 mg/ml) in 0.15 M NaCl + 1 M urea or α-protein (10 mg/ml) in phosphate buffer (pH 10.0); B, α-protein (7 mg/ml) in phosphate buffer (pH 8.0, *I* 0.1) within 48 h; C, α-protein (7 mg/ml) in phosphate buffer (pH 8.0, *I* 0.1) after storage for 2 weeks at 10°; D, Solution B to which urea was added to a final concentration of 1.0 or 4.0 M. All runs at 52640 rev./min, temperature at 24°, time 40, 32, 32 and 32 min, respectively.

The electrophoretic bands and two schlieren-pattern boundaries (Fig. 1B) are demonstrable within 48 h after α-protein is dissolved in 0.15 M NaCl, phosphate buffer (pH 8.0, *I* 0.1) or barbital buffer (pH 8.6, *I* 0.1). An additional electrophoretic band and a third schlieren-pattern peak (Fig. 1C) appear when these solutions are stored for 2 weeks at 10°. Partial dissociation of the aggregates, as evidenced by one major and two minor electrophoretic bands and one schlieren-pattern peak (Fig. 1D), results when the pH of the solution is increased to 11 or when crystalline urea is added to the NaCl, phosphate or barbital-buffer solutions, to a final concentration between 1 and 4 M. Urea concentrations between 1 and 8 M produce no demonstrable alterations in electrophoretic (pH 7.5–9.5) or ultracentrifugal (4.1 S) characteristics of α-protein monomer and no changes in the recombining capacity *in vitro* or *in vivo* of α-protein with serum lipids. Normal ^{131}I-labeled α-protein biological half-time values (3.6 days) are obtained with the α-protein dissolved in 1 M urea or phosphate buffer (pH 8.0). The solubility of α-protein was not altered when a 1 % α-protein solution was diluted from 1.0 M urea to 0.12 M.

The studies of mitochondrial lipoproteins[5] show the formation of aggregates similar to those observed with serum α-protein. It has been postulated that hydrophobic bonding contributes significantly to the interactions involved in mitochondrial structural protein aggregation.

The possibility that the aggregation of α-protein may represent an early phase of denaturation has not been excluded, although no evidence of denaturation has been observed with high concentrations of urea and the aggregation is partly reversed by urea or elevated pH. It is not known whether α-lipoprotein interactions occur *in vivo*, but one may speculate that α-lipoprotein dimerization could explain the two molecular weights reported, *i.e.* 175000 and 375000 (ref. 6). From further studies with urea, bile salts, non-ionic and anionic detergents it may be possible to evaluate the relative contribution of different intermolecular forces in the mechanism of α-protein aggregation and α-lipoprotein formation.

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