CROSS-LINKING STUDIES ON THE STATE OF ASSOCIATION OF APO A-I AND APO A-II FROM HUMAN HDL

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Received June 2,1976

SUMMARY: The technique of cross-linking with the bifunctional reagent dimethylsuberimidate has been employed in the study of apolipoprotein association. Human apo A-I was found to undergo a concentration dependent self-association, with tetrameric and pentameric forms being the predominant polymeric species at concentrations of apo A-I between 0.5 and 1.1 mg/ml. However, apo A-II showed mainly monomer and dimer forms at concentrations ranging from 0.1-0.7 mg/ml. When these apolipoproteins were mixed, new cross-linked forms of molecular weight 45,000 and 73,500 became prominent. These molecular weights correspond to those of mixed polymers, indicating that these proteins interact in solution.

INTRODUCTION

Apo A-I and apo A-II are the major protein constituents of human high density lipoprotein. Interaction between these proteins may play a role in determining the structure of the HDL[1] particle and its metabolism. Information on the tertiary and quaternary structure of these apolipoproteins is necessary for an understanding of the structure of the HDL particle.

Several studies have been reported recently in which analytical ultracentrifugation was used to study self-association properties of apo A-I (1,2,3) and apo A-II (2,4). Gwynne et al. (1) reported apo A-I to be monomeric up to a concentration of 0.6 mg/ml. Stone and Reynolds (2) found that monomeric apo A-I existed in a slow equilibrium with its dimer in the same concentration range. In a similar study, Vitello and Scanu (3) reported data on apo A-I which fit a monomer-dimer-tetramer-octamer model with a rapid attainment of equilibrium.

[1]The following abbreviations will be used in this paper: HDL, high density lipoproteins: SDS, sodium dodecyl sulfate; DMS, dimethylsuberimidate.

Apo A-II was found to be dimeric (MW = 34,000) at all concentrations investigated by Stone and Reynolds (2), while Scanu and Vitello have fit the self-association of A-II to a monomer-dimer-trimer model (4).

The use of chemical cross-linking methods for study of quaternary structure in proteins has become a widely-used procedure, especially with the imidoester reagents (5). Scanu et al. (6,7) have used this approach for a limited study of apo A-I and apo A-II, and Grow and Fried (8) have used cross-linking to show that A-I and A-II lie in close proximity in human HDL.

We report here the use of the bifunctional reagent dimethylsuberimidate to investigate the self-association properties of apo A-I and apo A-II, as well as the hybridization of these apoproteins when they are incubated with each other.

MATERIALS AND METHODS

Preparation of apolipoproteins: HDL was prepared from fresh human plasma by ultracentrifugal flotation (9). This material was dialyzed, lyophilized, and delipidated with 3:1 ethanol:ether by the procedure of Brown et al. (10). The delipidated HDL was then chromatographed on Sephadex G-200 (2.5 x 190 cm) in 0.2-M Tris HCl (pH 8.2) containing 6-M deionized urea and 0.1-M decyl sulfate (6). Fractions containing purified apo A-I and apo A-II were pooled and dialyzed against 0.15-M NaCl containing 10^{-3}-M EDTA.

Cross-linking: Aliquots of apo A-I and apo A-II were diluted with distilled water to the desired concentration. After 4 hours, one volume of DMS solution (20 mg/ml in 1-M triethanolamine-HCl, pH 8.6) was added to ten volumes of protein solution, and the reaction was allowed to proceed at room temperature. The reaction was stopped at various time intervals by addition of 1-M acetic acid to bring the pH below 7 (11).

SDS gel electrophoresis: Cross-linked samples were analyzed by SDS gel electrophoresis in the buffer system of Shapiro, Vinuela, and Maizel (12) as modified by Maizel (13). Samples were either dried in a vacuum dessicator or used without drying and SDS concentration brought to 1%; the solutions were then heated to 100^{0} for 1-2 min prior to application to the gels. For these studies a 3-27% acrylamide gradient slab gel system was used (14). Gels were scanned at 550 nm with a Clifford Model 445-20 integrating densitometer (Clifford Instruments, Natick, Mass.).

Materials: Dimethylsuberimidate was purchased from Pierce Chemical Co. All other chemicals were of reagent grade.

RESULTS

Figure 1 shows a study of the concentration dependence of apo A-I self-association. It can be seen that as the concentration of apo A-I was increased from .06 to 1.1 mg/ml, there was a shift in the cross-linked forms

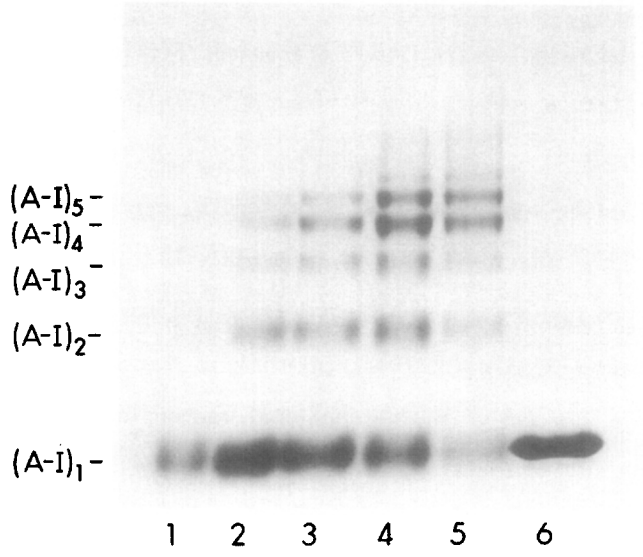

Figure 1: Concentration dependence of apo A-I self-association. Equal amounts of apo A-I were diluted with water 4 hours before initiation of cross-linking with DMS (1.8 mg/ml) and the cross-linking reaction was terminated after 90 minutes. The following concentrations of A-I present in the reaction mixture were: (1) 0.06 mg/ml; (2) 0.19 mg/ml; (3) 0.26 mg/ml; (4) 0.48 mg/ml; (5) 1.1 mg/ml. Position 6 contained apo A-I that was not cross-linked.

observed: monomeric (mol. wt. 28,000) and dimeric (mol. wt. 56,000) species predominated at low concentrations, and tetrameric (mol. wt. 112,000) and pentameric (mol. wt. 140,000) species predominated at higher concentrations. The initial identification of the cross-linked species of A-I from their molecular weights was achieved by the simultaneous electrophoresis of cross-linked polymers of aldolase as molecular weight markers (5). Identical observations were made on 6% acrylamide cylindrical SDS gels.

In order to verify that the cross-linked species observed were not derived from random cross-linking of monomers, and that the amount of DMS was not limiting, several experiments were performed. Figure 2 shows the results of a time course experiment in which aliquots of apo A-I were incubated with DMS for various lengths of time, with additions of freshly prepared DMS re-

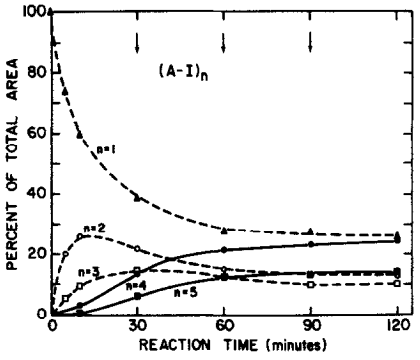

Figure 2: Formation of cross-linked polymers of apo A-I as a function of the time of reaction. Forty μl aliquots of apo A-I were reacted with 4 μl of DMS (final concentration, 1.8 mg/ml) and the reaction was terminated at various times. At 30, 60, and 90 minutes, 4 μl aliquots of freshly-prepared DMS (20 mg/ml) were added (indicated by arrows). Final apo A-I concentrations were: 0,5,10,30 min - 0.56 mg/ml; 60 min - 0.52 mg/ml; 90 min - 0.48 mg/ml; 120 min - 0.44 mg/ml. Samples were electrophoresed on SDS slab gels and scanned. The area of each oligomeric peak was divided by the sum of the areas of all peaks (including $n > 5$) to determine the relative amounts of the various polymers.

agent at the times indicated by the arrows. The proportion of apo A-I monomer can be seen to decrease with time, as do the amounts of dimer and trimer, after their initial formation. Only the tetramer and pentamer can be seen to continually rise before reaching a plateau. Polymeric forms higher than pentamers together amounted to less than 15% of the total area. The fact that the proportions of the various polymers remain constant after 60 minutes suggests that the cross-linking is mostly intramolecular. We have found that cross-linking of carbonic anhydrase at comparable or higher concentrations yields only a monomeric species of 29,000 molecular weight. Likewise, cross-linking of apo A-I in the presence of 0.1% SDS, which is known to produce disaggregation, also yields only the 28,000 molecular weight monomer. These findings suggest that the cross-linking of A-I is not random, but occurs within various polymeric species.

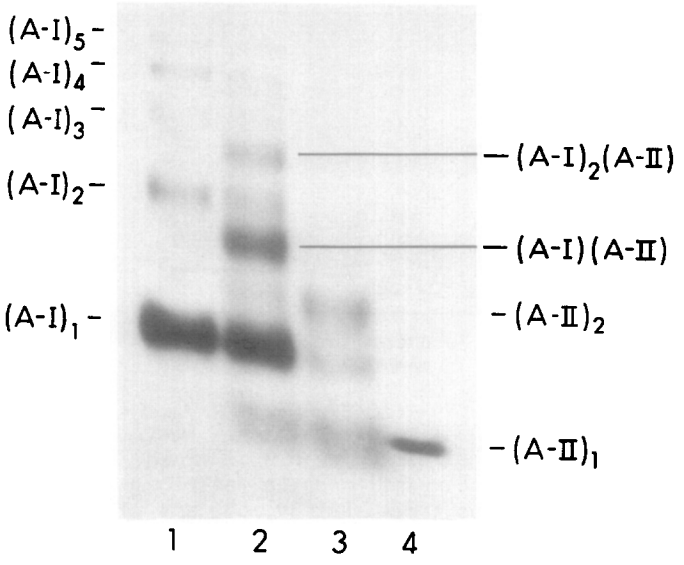

Figure 3: Cross-linking of a mixture of apo A-I and apo A-II. Apolipoprotein samples were cross-linked with DMS for 90 min at a concentration of 1.8 mg/ml, and applied to the gels as follows: position 1, apo A-I (0.16 mg/ml); position 2, apo A-I (0.16 mg/ml)/apo A-II (0.28 mg/ml) mixture; position 3, apo A-II (0.28 mg/ml). Position 4 contains a sample of apo A-II which was not cross-linked.

Because of conflicting data in the literature on the speed with which equilibrium between self-associated forms is attained (2,3), it was necessary to determine whether cross-linking patterns would be affected by the length of incubation of diluted apolipoproteins prior to the cross-linking reaction. To answer this question, samples of apo A-I were diluted ten-fold and the reaction initiated at 1 minute, 1 hour, and 4 hours after dilution. Each of these samples were found to give equivalent results, and the rate of equilibrium would, therefore, appear to be faster than the rate of cross-linking.

Apo A-II was also cross-linked with DMS and was found to exist as monomers (MW = 17,000) and dimers over the concentration range of 0.1-0.7 mg/ml. A sample of cross-linked apo A-II at a concentration of 0.28 mg/ml is shown in Figure 3 (position 3). These results agree with the reports of Stone and

Reynolds (2) and Vitello and Scanu (4). The absence of polymeric forms higher than dimers suggests that the tetramers and pentamers observed with apo A-I are not the result of interparticle cross-linking.

In addition to the study of the self-association of these apoproteins, the hybridization of the two forms was also investigated. Figure 3 shows the results of cross-linking a mixture of apo A-I (0.16 mg/ml) and apo A-II (0.28 mg/ml) following a 4 hour incubation at room temperature. Two prominent bands are observed on this gel which are not observed upon cross-linking of either apo A-I or apo A-II individually. Using the oligomers of apo A-I and apo A-II as molecular weight markers, the hybrid bands were found to have molecular weights of 45,000 and 73,500 (Fig. 4). We conclude that the 45,000 molecular weight band is the (A-I)(A-II) mixed dimer, and have tentatively identified the 73,500 molecular weight component as having a composition of $(A\text{-}I)_2(A\text{-}II)$.

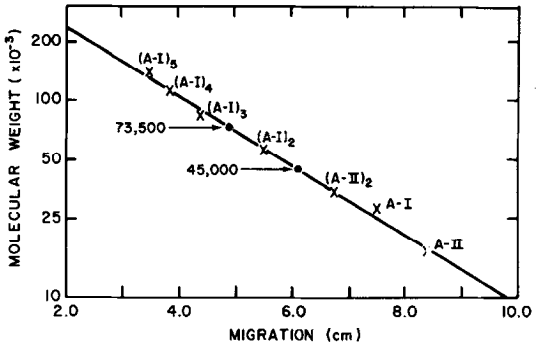

Figure 4: Determination of molecular weights of apo A-I - apo A-II hybrid species. The rates of migration for bands observed in electrophoresed samples of mixtures of apo A-I and apo A-II were measured and are plotted in the figure, with identification based on adjacent samples of cross-linked apo A-I and apo A-II alone. Bands which did not correspond to the self-associated forms are identified by molecular weight values. The points shown in the figure are from a single gel, but the molecular weights indicated are the average of three experiments. The standard deviation was 0.6% for the 45,000 molecular weight band and 1.3% for the 73,500 component.

DISCUSSION

We have employed the technique of chemical cross-linking with dimethylsuberimidate to study the state of association of the apolipoproteins of HDL. Results with apo A-II confirmed the findings of others that this protein is in equilibrium with a dimeric form over a wide range of concentrations. However, apo A-I exhibited a greater concentration dependence of association, with tetramers and pentamers predominating at higher concentrations. These data support the findings of Vitello and Scanu (3). However, the largest species we found in significant amounts was pentameric, while these authors found their data best fit a monomer-dimer-tetramer-octamer model. Time course studies and other cross-linking data rule out interparticle cross-linking as a source of the tetrameric and pentameric species observed.

We have also found that apo A-I and apo A-II exist in a state of association in mixtures. This is in agreement with the findings of Reynolds and Simon (15), who found a boundary by analytical ultracentrifugation which corresponded to a molecular weight of 50,000, but in addition, we have observed a mixed trimer of molecular weight 73,500. We conclude that these apolipoproteins possess an affinity for each other which may be a significant factor in lipoprotein structure, but the specificity of this interaction remains to be investigated.

ACKNOWLEDGEMENTS: This work was supported by Research Grant HL 14236 (SCOR) from the National Institutes of Health and by Training Grant HL 5273 from the U.S. Public Health Service.

REFERENCES

1. Gwynne, J., Brewer, B., Jr., and Edelhoch, H. (1974) J. Biol. Chem. 249, 2411-2416.
2. Stone, W.L, and Reynolds, J.A. (1975) J. Biol. Chem. 250,8045-8048.
3. Vitello, L.B., and Scanu, A.M. (1976) J. Biol. Chem. 251,1131-1136.
4. Vitello, L.B., and Scanu, A.M. (1976) Biochemistry 15,1161-1165.
5. Davies, G.E., and Stark, G.R. (1970) Proc. Nat. Acad. Sci. (U.S.) 66, 651-656.
6. Edelstein,C., Lim, C.T., and Scanu, A.M. (1972) J. Biol. Chem. 247, 5842-5849.

7. Scanu, A.M., Lim, C.T., and Edelstein, C. (1972) J. Biol. Chem. 247, 5850-5855.
8. Grow, T.E., and Fried, M. (1975) Biochem. Biophys. Res. Comm. 66,1447-1455.
9. Havel, R., Eder, H., and Bragdon, J. (1955) J. Clin. Invest. 34,1345-1353.
10. Brown, W., Levy, R., and Fredrickson, D. (1969) J. Biol. Chem. 244, 5687-5694.
11. Hitchock, S.E. (1975) Biochemistry 14,5162-5167.
12. Shapiro, A., Vinuela, E., and Maizel, J., Jr. (1967) Biochem. Biophys. Res. Comm. 28,815-820.
13. Maizel, J.V., Jr. (1971) Methods in Virology, V.K. Maramorosch and H. Koprowski, eds., Academic Press, N.Y., pp. 179-245.
14. Swaney, J.B., and Kuehl, K. (unpublished).
15. Reynolds, J.A., and Simon, R.H. (1974) J. Biol. Chem. 249,3937-3940.