**Communication**

**In Vitro Conversion of Proapo protein A-I to Apoprotein A-I**

PARTIAL CHARACTERIZATION OF AN EXTRACELLULAR ENZYME ACTIVITY

(Received for publication, June 30, 1983)

Celina Edelstein‡, Jeffrey I. Gordon‡, Kathy Toscas‡, Harold F. Sims‡, Arnold W. Strauss‡, and Angelo M. Scam‡

From the Departments of ‡Medicine and §Biochemistry, University of Chicago, Pritzker School of Medicine, Chicago, Illinois 60637 and the Departments of ‡Biological Chemistry, §Medicine, and ‡Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110

Previous studies have established that human hepatocellular carcinoma cells (Hep G2) secrete into serum-free medium the pro form of apolipoprotein A-I (proapo-A-I) suggesting that its conversion to mature apo-A-I occurs after secretion. In order to assess the mode and site of proapo-A-I to apo-A-I conversion, we incubated the medium from [3H]proline-labeled Hep G2 cells with either human plasma, serum, lymph, or fractions thereof obtained by density gradient ultracentrifugation. The conversion was monitored by two-dimensional gel electrophoresis and by Edman degradation. Human plasma, serum, or mesenteric lymph all induced proapo-A-I to apo-A-I conversion; this was time dependent, unaffected by the serine protease inhibitor phenylmethylsulfonyl fluoride and inhibited by EDTA. Purified radiolabeled proapo-A-I bound to lymph chylomicrons and plasma high density lipoproteins. The converting enzyme was associated with both of these particles. Activity was also found in the d > 1.21-g/ml fraction and may have been derived from high density lipoprotein after displacement by high salts and/or ultracentrifugal force.

We conclude that the conversion of proapo-A-I to apo-A-I occurs extracellularly and is probably effected by a metallo-enzyme which may act at the amphiphilic surface of either chylomicrons or high density lipoproteins.

---

Previous studies on the biosynthesis and processing of human apo-A-I have shown that this protein is synthesized as a preproapo protein with a 24-amino acid NH₂-terminal extension (1–3). The presegment, 18 amino acid residues long, is cleaved co-translationally by signal peptidase. The remaining proprotein containing an hexapeptide prosegment covalently linked to the NH₂ terminus of mature apo-A-I is secreted into the medium. Lack of intracellular proapo-A-I cleavage may be due to the presence of a Gln-Gln dipeptide at the COOH-end of this prosegment. This feature distinguishes it from the dibasic residues present at the COOH terminus of most vertebrate prosegments which are cleaved during secretion (4). We previously performed two-dimensional gel electrophoresis on the purified proapo protein and found that it represented the more basic isoform 2. By contrast, mature apo-A-I in circulating plasma (2, 5) consists primarily of the more acidic isoform 4. Based on these observations, we have suggested that a converting enzyme system is located extracellularly and plays an active role in the post-translational proteolytic processing of proapo-A-I to mature apo-A-I. In order to test this hypothesis and better characterize this proteolytic activity, we have embarked on studies in which we have followed the events attending the incubation of radiolabeled proapo-A-I obtained from the culture medium of Hep G2 cells with human plasma, serum, lymph, or fractions thereof under various experimental conditions. The results of these studies are the subject of this report.

**EXPERIMENTAL PROCEDURES**

The techniques of isolation of human proapo-A-I from the medium of cultured Hep G2 cells have been described previously (1). Automated NH₂-terminal Edman degradation analyses were performed using a 0.35 M Quadrol program (6, 7) and a Beckman 890 C Sequencer.

Two-dimensional Polyacrylamide Gel Electrophoresis—Radiolabeled proteins present in the incubation medium were analyzed by two-dimensional polyacrylamide gel electrophoresis using the conditions detailed by O'Farrel (8) and modified later by Lester et al. (9). Stained gels containing 'H-labeled proteins were subjected to fluorography.

Preparation of Samples for Incubation with Hep G2 Medium—Blood was collected from fasting normal subjects in lithium heparin coated tubes. The plasma was separated by centrifugation (10). Serum was obtained from the same fasted subjects following blood clotting using identical centrifugal conditions. Retropitoneal lymph of mesenteric origin was collected during abdominal surgery on a normalipemic patient with an aortic aneurism. Red cells were sedimented by centrifugation (10) and chylomicrons were separated by ultracentrifugation at 100,000 × g for 30 min at 4°C. The top floating chylomicrons were removed, overlayed with 0.15 M NaCl, pH 7.0, 0.1 M KCl, and refloated under the same conditions. In the case of plasma, top and bottom d 1.21-g/ml fractions were prepared by adjusting the density to 1.21 g/ml with solid NaBr and centrifuging at 220,000 × g for 24 h at 10°C. The floating top 1-ml fraction and the bottom 1-ml fraction were removed and dialyzed at 4°C against 0.1 M NaCl, pH 7.0, before use. Incubation with Hep G2 Medium—Hep G2 medium containing radiolabeled proapo-A-I was incubated with plasma, serum, lymph, or their ultracentrifugal fractions in a shaking water bath at 37°C under the conditions specified under “Results.” For two-dimensional gel electrophoresis, the reaction was stopped by adding one-half volume of 10 mM Tris buffer (9). The incubated products were also subjected to Edman degradation following immunoadsorption with monoclonal antisera (1). Apo-A-I was run as a marker protein with each sample to permit a comparison among gels.

Preparation of HDL and Apo-A-I—HDL was prepared by conventional ultracentrifugal methods (10). Apo-A-I was purified by size...
exclusion high performance liquid chromatography as previously described (11).

Binding Studies of Purified Proapo-A-I with Serum or Isolated Lipoproteins—[3H]Proline-labeled proapo-A-I (10,000 dpm) was incubated with either whole serum or HDLs at room temperature for 30 min with gentle stirring in 0.15 M NaCl, 1 mM EDTA, and 1 mM PMSF. In general, 1 to 3 mg of HDLs proteins or 0.5 to 1 ml of serum were utilized per incubation. Each sample was then adjusted to 1 ml with 0.15 M NaCl, 1 mM EDTA, pH 7.0, and separated by density gradient ultracentrifugation.

Density Gradient Ultracentrifugation—Density gradients were prepared as previously described (12). The gradients were collected in volumes of 0.4 ml/fraction and the absorbance monitored at 280 nm. Aliquots of 100 µl were diluted with 10 ml of scintillation fluid and counted.

RESULTS

Incubation Studies of [3H]Proline-labeled Hep G2 Medium with Plasma, Serum, and Lymph—The incubation products were analyzed by two-dimensional gel electrophoresis. Fig. 1, A and B, show the fluorographs of radiolabeled proapo-A-I present in Hep G2 medium before and after 6-h incubation at 37 °C in the absence of plasma. Compared to the isoform pattern of the mature apo-A-I shown in Fig. 1N, proapo-A-I only exhibits the more basic isoforms 2 and 3. Incubation of medium containing radiolabeled proapo-A-I with plasma for up to 6 h resulted in partial conversion of isoforms 2 and 3 into isoforms 4 and 5 (Fig. 1, C, D and E). The higher molecular weight component above isoform 4 in Fig. 1C was seen only occasionally. Its identity is unknown, but has also been observed previously in Hep G2 medium (13). Based on densitometric tracings of the fluorographs, the extent of conversion of isoforms 2 and 3 to 4 and 5 after a 1- and 6-h incubation with plasma was 8 and 25%, respectively. Automated sequential Edman degradation of [3H]proline-labeled proapo-A-I after its immunoadsorption from the incubation mixture corroborated these results (Fig. 2). The distribution of [3H]proline residues in the immunoadsorbed product obtained from the Hep G2 medium which had not been incubated with plasma, revealed peaks at proline positions 9, 10, and 13. This agreed precisely with the known NH2-terminal proapo-A-I sequence (1, 2). As shown at the bottom of Fig. 2, incubation of [3H]proline-labeled Hep G2 medium with plasma for 6 h generated peaks of radioactivity at cycles 3, 4, and 7, as well as 9 and 10. The [3H]prolines observed in positions 3, 4, and 7 correspond to the sequence of mature apo-A-I described previously (14). The [3H]prolines appearing in positions 9 and 10 (Fig. 2, bottom) are contributed by the proapo-A-I that remained unconverted. From these data, we conclude that conversion of isoforms 2 and 3 to 4 and 5 reflects proteolytic removal of the hexapeptide prosegment. Based on the [3H]proline peaks at cycles 3, 4, and 7, compared to 9 and 10 there was 30% conversion. This is an agreement with data obtained from densitometric tracings of the fluorographs.

The presence of 1 mM PMSF in the incubation reaction mixture containing Hep G2 medium and plasma had no effect on conversion. However, addition of 1 mM EDTA blocked the conversion completely (Fig. 1F). Like plasma, serum incubated with [3H]proline-labeled Hep G2 medium for 6 h at 37 °C resulted in the appearance of isoform 4 (Fig. 1G). Incubation of [3H]proline-labeled Hep G2 medium with mesenteric lymph for 1 h (Fig. 1H) and 6 h (Fig. 1I) resulted in 11 and 24% conversion, respectively. Thus, plasma, serum, and lymph appear to contain a common proteolytic activity capable of converting proapo-A-I to apo-A-I.

Incubation Studies of [3H]Proline-labeled Hep G2 Medium with d 1.21-g/ml Ultracentrifugal Fractions and Chylomicrons—The incubation of the radiolabeled medium with the d 1.21 g/ml top (Fig. 1J) and bottom (Fig. 1K) fractions from plasma showed that both were effective in producing isoform 4. The top (30 µg of protein) and bottom fractions (300 µg of protein) caused 19 and 10% isoform conversion, respectively. Isoform conversion was consistently found with the bottom fraction. With the top fraction, the conversion was observed in two out of three preparations studied; activity also was found with HDLs (Fig. 1L). Processing by chylomicron (Fig. 1M) was dose-dependent within the protein concentration range studied (77 to 350 µg/incubation; data not shown).

Interaction of [3H]Proline-labeled Proapo-A-I with Serum and HDLs.—All of the following studies were conducted in the presence of 1 mM EDTA to specifically inhibit the conversion of proapo-A-I to mature apo-A-I. Purified [3H]proline-labeled

FIG. 1. Two-dimensional gel fluorographs of incubated mixtures of radiolabeled Hep G2 medium with plasma, serum, lymph, and fractions thereof. Each sample was incubated with 30 µl of medium at 37 °C and the reaction was stopped with 15 µl of lysis buffer and frozen at −70 °C. After incubation, sample M was delipidated with methanol at 4 °C; the precipitate was washed with diethyl ether and centrifuged. The washed precipitate was then dissolved in 30 µl of lysis buffer and frozen at −70 °C until further analysis. A, medium (30 µl); B, medium (30 µl); C, plasma (15 µl); D, plasma (15 µl); E, plasma (15 µl); F, plasma (15 µl) with 1 mM EDTA; G, serum (15 µl); H, lymph (15 µl); I, lymph (15 µl); J, top d 1.21-g/ml plasma fraction (30 µg of protein); K, bottom d 1.21-g/ml plasma fraction (700 µg of protein); L, HDLs (40 µg of protein); M, lymph chylomicrons (350 µg of protein); N, purified apo-A-I run as a standard. This gel was stained with Coomasie blue. Isoforms 2 to 6 migrate from the more basic (left) to the more acidic (right) portion of the gel. In all panels, only the area of the gel in the vicinity of the proteins of interest are shown.
labeled proapo-A-I without lipids sedimented at density 1.28 g/ml. Incubation of proapo-A-I with lymph chylomicrons resulted in 15% of the counts being recovered in the top floating (d 1.006 g/ml) fraction (data not shown). This finding suggested that proapo-A-I also binds to chylomicrons.

**DISCUSSION**

The results of our studies show that when medium containing proapo-A-I secreted from an Hep G2 cell line is incubated with either plasma, serum, or lymph, further proteolytic processing occurs resulting in the conversion of proapo-A-I (isoforms 2 and 3) into mature apo-A-I (isoforms 4 and 5). This observation provides support for our earlier suggestion (1) that the proteolytic cleavage of proapo-A-I is an extracellular event. Under our experimental conditions, the kinetics of formation of apo-A-I, which was on the order of hours, is in agreement with the slow conversion process reported for other proproteins such as proinsulin to insulin and G34 gastrin to gastrin (3). However, we may not have chosen the optimal conditions for the conversion; a complete proapo-A-I to apo-A-I conversion was not achieved even after a 24-h incubation.

Although we have not thoroughly defined the nature of the converting enzyme activity, it is apparent that it does not possess the general characteristics of a serine protease because of the lack of inhibition by PMSF. The fact that this activity was inhibited by EDTA suggests that we may be dealing with a metallo-enzyme. We observed no effect of exogenous Ca²⁺ (up to 10 mM) on the converting activity. However, the fluids that we examined, plasma, serum, or lymph, contain calcium. The effects of other metal ions and chelators have to be investigated.

Lymph chylomicrons and plasma HDL proved to be sources of the converting activity, also bound proapo-A-I. Based on the mass of protein present in the conversion assays, it appears that plasma HDL was more active than chylomicrons. From these findings, we may conclude that the interaction between the converting activity and proapo-A-I occurs at the amphiphilic interface of the lipoprotein particle. This conclusion is further supported by our observation that proapo-A-I combined with synthetic mixed discoidal particles of lecithin and cholesterol. However, we did find activity free from the HDL particles. This may be explained by a dissociation of this activity from the lipoprotein surface by the high salt concentrations and/or the ultracentrifugal gravitational force used. It could also account for our observation that top fraction mediated isoform conversion was occasionally absent. Alternatively, we may not be dealing with an ultracentrifugal artifact but rather with an activity which can also exist in a lipoprotein free state but capable of lipid binding when exposed to the Hep G2 medium. We may further speculate that at the time of secretion proapo-A-I is either already bound to lipids or becomes immediately associated with lipoproteins following its entry into the circulation, a step which may be necessary for efficient conversion. We have no information at this time on the source of the enzyme accounting for the converting activity. Its overall role in the events attending lipoprotein interconversions in plasma remains to be elucidated.

**Acknowledgments** — We wish to thank Dr. Eric P. Lester for lending his expertise and laboratory facilities in doing the two-dimensional gel electrophoresis and Rose E. Scott for her valuable help in preparing the manuscript.

---

Footnotes:
1. C. Edelstein, unpublished observation.
2. C. Edelstein, J. I. Gordon, H. F. Sims, A. W. Strauss, and Scanu, A. M., manuscript in preparation.
REFERENCES

1. Gordon, J. I., Sims, H. F., Lenta, S. R., Edelstein, C., Scanu, A. M., and Strauss, A. W. (1983) J. Biol. Chem. 258, 4037-4044
2. Zannis, V. I., Karathanasis, S. K., Keutmann, H. T., Goldberger, G., and Brewlow, J. L. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2574-2578
3. Stoffel, W., Kruger, E., and Deutzmann, R. (1983) Hoppe-Seyler's Z. Physiol. Chem. 364, 227-237
4. Steiner, D. F., Quinn, P. S., Chan, S. J., Marsh, J., and Tager, H. S. (1980) Ann. N. Y. Acad. Sci. 343, 1-16
5. Ghiselli, G., Schaefer, E. J., Law, S., Light, J. A., and Brewer, H. B., Jr. (1983) Clin. Res. 31, 500A
6. Gordon, J. I., Smith, D. P., Alpers, D. H., and Strauss, A. W. (1982) J. Biol. Chem. 257, 8418-8423
7. Thomas, K. A., Silvermann, R. E., Jeng, L., Baglan, N. C., and Bradshaw, R. A. (1981) J. Biol. Chem. 256, 9147-9155
8. O'Farrel, P. H. (1975) J. Biol. Chem. 250, 4007-4021
9. Lester, E. P., Lemkin, P., Lipkin, L., and Cooper, H. L. (1980) Clin. Chem. 26, 1392-1402
10. Scanu, A. M. (1966) J. Lipid Res. 1, 295-306
11. Polacek, D., Edelstein, C., and Scanu, A. M. (1981) Lipids 16, 927-929
12. Nilsson, J., Mannickarottu, V., Edelstein, C., and Scanu, A. M. (1981) Anal. Biochem. 110, 342-348
13. Zannis, V. I., Breslow, J. L., and Katz, A. J. (1980) J. Biol. Chem. 255, 8612-8617
14. Brewer, H. B., Jr., Fairwell, T., Larue, A., Ronan, R., Houser, A., and Bronzert, T. J. (1978) Biochim. Biophys. Res. Commun. 80, 623-630
In vitro conversion of proapoprotein A-I to apoprotein A-I. Partial characterization of an extracellular enzyme activity.
C Edelstein, J I Gordon, K Toscas, H F Sims, A W Strauss and A M Scanu
J. Biol. Chem. 1983, 258:11430-11433.

Access the most updated version of this article at http://www.jbc.org/content/258/19/11430

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/258/19/11430.full.html#ref-list-1