Biosynthesis and processing of pro-C3, a precursor of the third component of complement in rat hepatocytes: effect of secretion-blocking agents

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Biosynthesis and intracellular processing of the third component (C3) of complement were studied in cultured rat hepatocytes. In the control cells, the complement C3 was synthesized as a pro-form, a single polypeptide chain comprising both the α- and β-subunits. Although the cleavage of the pro-form into the subunits was not clearly demonstrable within the cells during pulse-chase periods, all the secreted C3 was the mature processed form. The cells were treated with secretion-blocking agents with different modes of action, colchicine and monensin. Colchicine caused an accumulation of the processed C3 within the cells, whereas monensin blocked the secretion without a significant accumulation of the processed form. The results indicate that the conversion of the C3 pro-form into the subunits takes place in the secretory vesicles just before the secretion.

Complement C3 *Pro-form biosynthesis* *Proteolytic processing* *Cultured rat hepatocyte* *Colchicine* *Monensin*

1. INTRODUCTION

The third component of complement, C3, plays a central role in the complement system by participating in both classical and alternative pathways of complement activation [1,2]. The protein is the most abundant of all the complement proteins, and synthesized mainly in the liver by parenchymal cells [3,4], although it is also produced by cells of the macrophage/monocyte series [5,6]. C3 isolated from plasma is a 187 000-dalton glycoprotein composed of disulfide-linked α- and β-chains with M_r = 115 000 and 75 000, respectively [7]. Recently it has been established that C3 is initially synthesized as single precursor, pro-C3 [4,8], and that β-chain is the HN_2-terminal segment of the pro-C3 molecule [9,10]. However, it remains to be determined where the conversion of pro-C3 to the mature form with the subunits takes place during the intracellular transport.

The use of drugs affecting the secretory process at distinct sites in the cell proves valuable for more detailed studies of specific steps in secretion [11,12]. Both the carboxylic ionophore monensin [11,12] and the anti-microtubular alkaloid colchicine [13,14] are known to markedly inhibit the protein secretion in a variety of cells. Recent observations have suggested that monensin blocks the transport step from the *cis* elements to the *trans* elements of the Golgi complex [11,12,15], while colchicine and other microtubule-affecting agents primarily block the secretory process beyond the *trans* Golgi [12,16].

Here we report the effects of secretion-blocking agents on the intracellular processing of pro-C3 in cultured rat hepatocytes, demonstrating that its

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Abbreviations: C3, the third component of complement; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis

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conversion to the mature form occurs in the secretory vesicles just before secretion.

2. MATERIALS AND METHODS

2.1. *Materials*

L-[^{35}S]Methionine (1150 Ci/mmol) and EN^{3}HANCE were purchased from New England Nuclear (Boston, MA); colchicine from Merck (Darmstadt, FRG); monensin from Calbiochem-Behring Corp. (La Jolla, CA); collagenase from Wako Junyaku (Osaka, Japan); Eagle's minimum essential medium from Nissui Seiyaku (Tokyo, Japan). Goat anti-rat complement C3 antiserum was obtained from Cappel Laboratories (West Chester, PA).

2.2. *Hepatocyte culture*

Hepatocytes were isolated from adult male Wistar rats, weighing 200–250 g, by the collagenase perfusing method of Seglen [17]. Isolated hepatocytes were cultured as described previously [12,18].

2.3. *Pulse-chase experiments and immunoprecipitation*

Cells after being cultured 24 h were preincubated at 37°C in the absence or presence of colchicine (50 μM) or monensin (1 μM), pulse-labeled for 10 min with [^{35}S]methionine (100 μCi/1.5 ml/dish) and chased as described previously [12,15]. Each drug, when indicated, was present in the medium throughout the pulse-chase periods. At the indicated times, cell lysate and medium were prepared and used for immunoprecipitation as described previously [12,15].

2.4. *Polyacrylamide gel electrophoresis*

Sodium dodecyl sulfate (SDS–polyacrylamide gel–PAGE) electrophoresis was performed on 7.5% polyacrylamide slab gels [19]. After electrophoresis, gels were fixed with 30% methanol–10% trichloroacetic acid–7% acetic acid for 1 h and treated with EN^{3}HANCE, followed by drying and fluorography [20].

3. RESULTS AND DISCUSSION

3.1. *Time course of secretion of complement C3*

When hepatocytes in control culture were pulse-labeled with [^{35}S]methionine and chased, secretion of the labeled C3 started at 30 min and reached more than 80% of the total labeled C3 by 3 h of chase, as shown in fig.1. The lag time of 30 min for C3 secretion is significantly longer than that for albumin [12,18], α_1-protease inhibitor [12,15] and haptoglobin [21]; the secretion of the latter three proteins started at 10 min under the same conditions of experiments. When the cells were treated with monensin at 1 μM, the secretion was completely blocked up to 1 h, after that the protein was secreted at a similar rate with that in the control cells, reaching about 76% of the normally secreted level compared at 3 h of chase. The inhibitory effect by colchicine at 50 μM was a little weak as compared with that by monensin at 1 μM (fig.1).

3.2. *Analysis of newly synthesized C3 by SDS–PAGE electrophoresis*

Immunoprecipitates of intracellular and medium C3 prepared from the control cells were analyzed by SDS–PAGE followed by fluorography. As shown in fig.2, only a single band was observed in the cells throughout pulse-chase periods (lanes 1–5). Since its apparent molecular weight was estimated to be about 190000, this

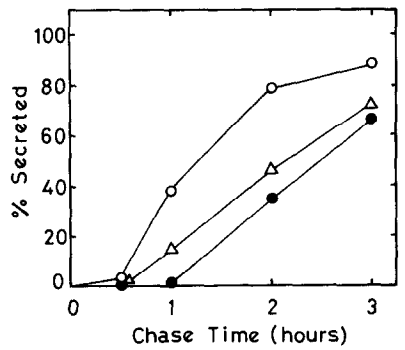

Fig. 1. Time course of secretion of complement C3 in primary culture of rat hepatocytes. Hepatocytes were pulse-labeled for 10 min with [^{35}S]methionine and chased in the absence (○) or presence of colchicine at 50 μM (△) or monensin at 1 μM (●) as described in section 2. At the indicated times, immunoprecipitates of the C3 were prepared from cell lysates and medium, and determined for radioactivity. Values are expressed as percentages of the radioactivity secreted of the total radioactivity incorporated into the complement C3 during 10 min pulse.

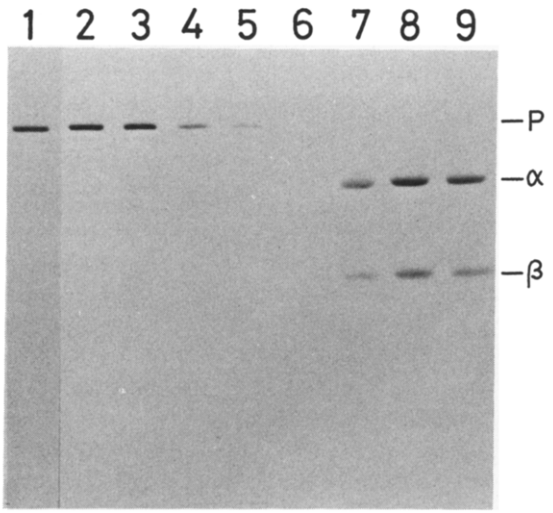

Fig. 2. SDS-page electrophoresis of ^{35}S-labeled complement C3 synthesized by the control cells. Cells were pulse-labeled with [^{35}S]methionine and chased as shown in fig. 1. Immunoprecipitates of C3 prepared from cell lysates (lanes 1–5) and medium (lanes 6–9) were subjected to SDS-PAGE (7.5%), followed by fluorography. Lane 1, no chase; lanes 2 and 6, chase 30 min; lanes 3 and 7, 1 h; lanes 4 and 8, 2 h; lanes 5 and 9, 3 h. P, α and β denote pro-form, α and β-subunits, respectively, of complement C3.

band corresponds to the pro-form of C3 as detected in guinea pig liver [4] and rabbit liver [8]. In the medium, by contrast, two major bands corresponding to α-chain (M_r = 115 000) and β-chain (75 000) were detected at 1 h of chase and accumulated thereafter (lanes 6–9). No pro-form was detectable in the medium. The results demonstrate that the newly synthesized complement C3 was secreted into the medium after all the pro-form was converted to the serum-form with the α- and β-chains within the cells. It is of interest to note that while the conversion of pro-C3 to the serum-form had to be completed before secretion, no significant amount of the processed form was detectable in the cells during all the periods of chase (fig.2, lanes 1–5). This is a contrasting finding as compared with the previous results obtained for the proalbumin processing, in which the processed serum-form was clearly detected within the cells as early as 20 min of chase under the same conditions employed here [16,18].

3.3. *Effects of secretion-blocking agents on the processing of pro-C3*

The carboxylic ionophore monensin has been demonstrated to block the secretory process at the Golgi complex [11]. We previously reported that the drug inhibits not only secretion but also proteolytic processing of proalbumin [12,15,16] as well as terminal glycosylation of the oligosaccharide chains of α_1-protease inhibitor [12] and haptoglobin [21]. On the other hand, the antimicrotubular agent colchicine did not significantly block these processings but caused an accumulation of the processed proteins [12,18]. Taking together other available evidence, we have concluded that monensin exerts its effect by blocking the intracellular transport of secretory proteins from the *cis* elements to the *trans* Golgi, while colchicine primarily blocks the transport from the *trans* Golgi to the cell surface.

To precisely determine the intracellular conversion site of pro-C3, we examined the effects of monensin and colchicine on the C3 processing. As shown in fig. 3A, monensin caused an accumulation of pro-C3, not the processed form, within the cells for a prolonged period of chase (lanes 1–5), and as a result of delayed secretion there appeared in the medium a significant amount of pro-C3 in addition to the processed form (lanes 8 and 9), as observed in a case of albumin [15]. Treatment of the cells with colchicine, for the first time, brought about a detectable accumulation of the processed form with the α- and β-chains in the cells (Fig. 3B, lanes 3–5). Based on evidence for the blocking sites by the two drugs [11,12], these results strongly support the conclusion that the conversion of pro-C3 to the mature form takes place within the secretory vesicles just before the secretion.

Recently Domdey et al. [10] have reported the amino acid sequence of selected domains of the mouse C3 molecule which was predicted by nucleotide sequence data from cDNA clones. The data established the order of subunits in pro-C3 to be NH_2-β-α-COOH and also indicated the presence of four arginine residues located between the COOH terminus of the β and the NH_2 *terminus of the* α subunits in pro-C3 [10]. Thus, it is likely that the proteolytic conversion of pro-C3 to the serum form would be initiated by trypsin-like protease, followed by removal of arginine residues by carboxypeptidase B-like protease, as commonly

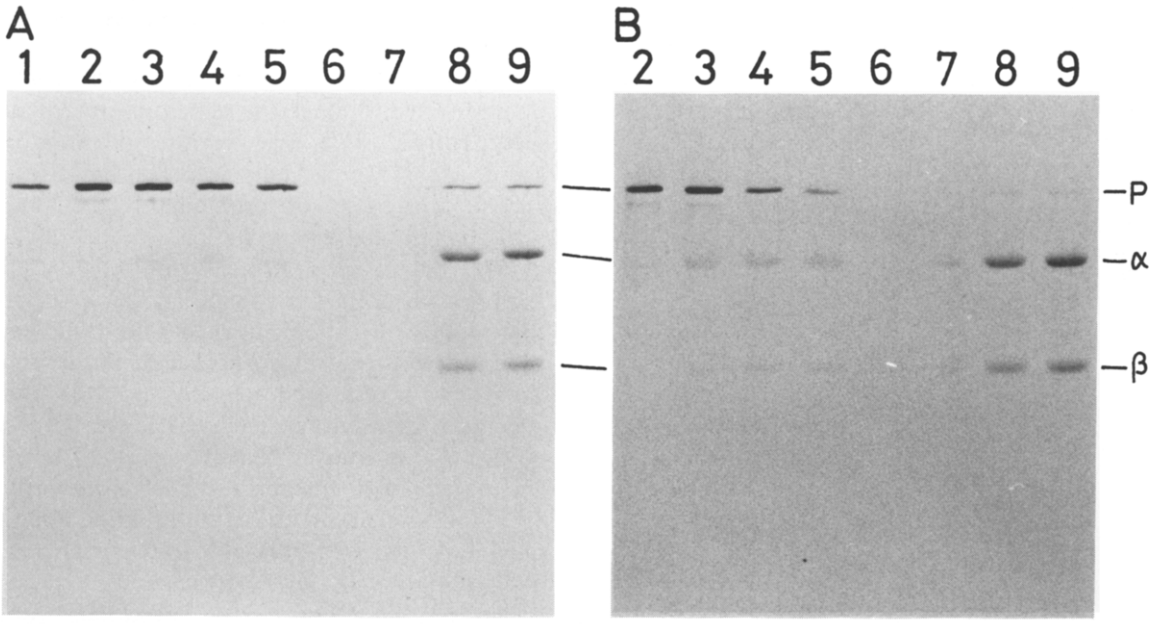

Fig. 3. Effects of secretion-blocking agents on intracellular processing of complement C3. Cells were pulse-labeled and chased in the presence of monensin (A) or colchicine (B) as shown in fig. 1. Immunoprecipitates of C3 prepared from cell lysates (lanes 1–5) and medium (lanes 6–9) were analyzed by SDS-PAGE. Lane 1, no chase; lanes 2 and 6, chase 30 min; lanes 3 and 7, 1 h; lanes 4 and 8, 2 h; lanes 5 and 9, 3 h. Symbols are the same as in fig. 2.

observed for proteolytic processing of other proform proteins [22]. In the hepatocytes, the conversion of proalbumin to the serum albumin has been suggested to be such a case and to occur mainly in the secretory vesicles [12,18,23]. It is therefore expected that the same proteolytic system in the secretory vesicles may function in the conversion of both proalbumin and pro-C3, although some differences found in kinetics of secretion and conversion between the two proteins, as pointed out above, remain to be clarified.

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REFERENCES

[1] Müller-Eberhard, H.J. (1975) Annu. Rev. Biochem. 44, 697–724.
[2] Reid, K.B.M. and Porter, R.R. (1981) Annu. Rev. Biochem. 50, 433–464.
[3] Alper, C.A., Johnson, A.M., Birtch, A.G. and Moore, F.D. (1969) Science 163, 286–288.
[4] Brade, V., Hall, R.E. and Colten, H.R. (1977) J. Exp. Med. 146, 759–765.
[5] Stecher, V.J. and Thorbecke, G.J. (1967) J. Immunol. 99, 643–652.
[6] Bentley, C., Bitter-Suermann, D., Hadding, U. and Brade, V. (1976) Eur. J. Immunol. 6, 393–398.
[7] Bokisch, V.A., Dierich, M.P. and Müller-Eberhard, H.J. (1975) Proc. Natl. Acad. Sci. USA 72, 1989–1993.
[8] Patel, F. and Minta, J.O. (1979) J. Immunol. 122, 1582–1586.
[9] Goldberger, G., Thomas, M.L., Tack, B.F., William, J., Colten, H.R. and Abraham, G.N. (1981) J. Biol. Chem. 256, 12617–12619.
[10] Domdey, H., Wiebauer, K., Kazmaier, M., Müller, V., Odink, K. and Fey, G. (1982) Proc. Natl. Acad. Sci. USA 79, 7619–7623.
[11] Tartakoff, A.M. and Vassalli, P. (1978) J. Cell Biol. 79, 694–707.
[12] Oda, K., Misumi, Y. and Ikehara, Y. (1983) Eur. J. Biochem. 136, 209–216.
[13] LeMarchand, Y., Singh, A., Assimacopoulos-Leannet, F., Orci, L., Rouiller, C. and Jeanrenaud, B. (1973) J. Biol. Chem. 248, 6862–6870.

[14] Redman, C.M., Banerjee, D., Howell, K. and Palade, G.E. (1975) J. Biol. Chem. 66, 42-59.
[15] Misumi, Y., Oda, K., Takami, N. and Ikehara, Y. (1984) in: Protein Transport and Secretion (Oxender, D.L. ed.) Alan R. Liss, Inc., New York (in press).
[16] Oda, K. and Ikehara, Y. (1982) Biochem. Biophys. Res. Commun. 107, 561-567.
[17] Seglen, P.O. (1976) Methods Cell Biol. 13, 29-83.
[18] Oda, K. and Ikehara, Y. (1982) Biochem. Biophys. Res. Commun. 105, 766-772.
[19] Laemmli, U.K. (1970) Nature (London) 227, 680-685.
[20] Bonner, W.M. and Laskey, R.A. (1974) Eur. J. Biochem. 46, 83-88.
[21] Misumi, Y., Tanaka, Y. and Ikehara, Y. (1983) Biochem. Biophys. Res. Commun. 114, 729-736.
[22] Steiner, D.F., Docherty, K. and Carroll, R. (1984) J. Biol. Chem. 24, 121-130.
[23] Ikehara, Y., Oda, K. and Kato, K. (1976) Biochem. Biophys. Res. Commun. 72, 319-326.