Cholesteryl esters are bound by a peptide-initiation and a peptide-elongation factor

Zdena TUHÁČKOVÁ and Jan HRADEC

Department of Biochemistry, Oncological Institute, 180 00 Prague 8, Czechoslovakia

(Received 30 June 1980/Accepted 10 July 1980)

Significantly higher quantities of cholesteryl 14-methylhexadecanoate than of cholesteryl laurate and cholesteryl palmitate are bound by a homogeneous peptide-initiation factor and purified peptide-elongation factor 1. Cholesteryl 14-methylhexadecanoate may function as a specific allosteric modifier changing the conformation of protein-synthesis factors and thus modulating the activity of their binding sites.

Evidence was provided by several papers from this laboratory that cholesteryl 14-methylhexadecanoate plays a role in protein synthesis [see Hradec (1975) for a review]. This ester is a constituent of amino acid-tRNA ligases (EC 6.1.1.–) (Hradec & Dušek, 1969), both peptide-elongation factors (Hradec *et al.*, 1971) and ribosomes (Hradec *et al.*, 1974), and its presence is apparently essential for the normal function of these preparations.

Experiments reported in the present paper were performed to elucidate the molecular interaction of labelled cholesteryl 14-methylhexadecanoate and of some other cholesteryl esters with a homogeneous peptide-initiation factor and purified peptide-elongation factor 1.

Materials and methods

Sources of animals, chemicals and radiochemicals have been described by Hradec *et al.* (1978) and Pohlreich *et al.* (1979). Radioactive cholesteryl esters were synthesized from 14-methylhexadecanoic acid (Helmich & Hradec, 1980a) or lauric acid (dodecanoic acid) or palmitic acid (hexadecanoic acid) (Serva Feinbiochemica, Heidelberg, West Germany) and [$G-^3H$]cholesterol or [$4-^{14}C$]cholesterol as described by Helmich & Hradec (1980b). The final preparations of [^3H]cholesteryl esters had a specific radioactivity of 8.8 Ci/mmol, and those of [^{14}C]cholesteryl esters 59 mCi/mmol, and were diluted with corresponding non-labelled cholesteryl esters as indicated for individual experiments.

The protein factor binding initiator tRNA was purified to electrophoretic homogeneity from rat liver as described by Pohlreich *et al.* (1979), and the peptide-elongation factor 1 was purified 93-fold from

rabbit reticulocytes as described by Kemper *et al.* (1976).

Incubation mixtures for the assay of binding of labelled cholesteryl esters contained, in a final volume of 0.1 ml: 100 mM-Tris/HCl buffer, pH 6.0 (unless otherwise stated), 5 mM-KCl and amounts of protein-synthesis factors and cholesteryl esters (in 3–5 μ l of acetone) as indicated for individual experiments, and were incubated at 37°C for 20 min unless otherwise stated.

After the incubation, 75 μ l portions of incubation mixtures were plated on discs of Whatman GF 81 glass-fibre paper and filters were immediately put into slots of a Teflon container (Hradec *et al.*, 1971) immersed in ice-cold acetone and left in acetone at 0°C for 5 min. This was followed by an additional washing with acetone at 20°C for 1 min. Filters were dried under an i.r. lamp and placed into scintillation vials with a toluene-based scintillation mixture for the assay of radioactivity.

Results and discussion

Gel filtration on Sephacryl S-200 (superfine grade) of incubation mixtures containing the initiation factor and [^{14}C]cholesteryl 14-methylhexadecanoate revealed that a portion of the input radioactivity becomes bound to the factor and is eluted in fractions corresponding to the elution volume of subunits of the factor (Pohlreich *et al.*, 1979). The quantity of this factor-bound radioactivity was significantly increased if incubation mixtures were supplemented with CTP (Fig. 1). Free unbound cholesteryl 14-methylhexadecanoate, being insoluble in the eluting buffer, did not soak into the column and could be eluted from the top of Sephacryl by organic solvents only.

Fig. 1. Evidence for the binding of cholesteryl 14-methylhexadecanoate by the peptide-initiation factor. Incubation mixtures containing, in a final volume of 1.0 ml, 100 mM-Tris/HCl buffer, pH 6.0, 5 mM-KCl, 1.72 mg of factor protein, approx. 10^8 c.p.m. of [^{14}C]cholesteryl 14-methylhexadecanoate (—○—), or the same plus 2 mM-CTP (·····○·····), were incubated at 37°C for 20 min. Mixtures were chilled in ice and applied on to a column (diameter 8 mm, length 500 mm) of Sephacryl S-200 (superfine grade) equilibrated against the same buffer, and the column was eluted at 4°C at a constant flow rate of 4.5 ml/h maintained by a peristaltic pump. Fractions (0.75 ml) were collected, mixed with 15 ml of a dioxan-based scintillation mixture and assayed for radioactivity. Elution volumes of standard proteins (bovine serum albumin, egg albumin, chymotrypsinogen A and pancreatic ribonuclease) are indicated on the upper scale. V_0 , void volume.

Maximum binding of all labelled cholesteryl esters tested was obtained after 20 min at 37°C with the initiation factor, and after more than 30 min with the elongation factor. No binding occurred at 0°C , and only approx. 20% of cholesteryl 14-methylhexadecanoate became bound during the same period at 50°C . Labelled free cholesterol was not bound by either protein-synthesis factor at any temperature.

With the same quantity of the initiation or elongation factor, the extent of binding was proportional to the amount of cholesteryl 14-methyl-

hexadecanoate added, and a characteristic sigmoid saturation curve was obtained for this dependence. However, a hyperbolic saturation curve resulted from the addition of increasing amounts of cholesteryl laurate or cholesteryl palmitate to both protein-synthesis factors (Fig. 2). A similar dependence was obtained with incubation mixtures containing constant amounts of labelled cholesteryl esters (30 pmol of ^3H -labelled esters) into which increasing amounts of protein-synthesis factors were added. In the presence of saturating amounts of peptide-initiation (approx. $180\mu\text{g}$) or -elongation factors (approx. $50\mu\text{g}$), 4 times more cholesteryl 14-methylhexadecanoate than cholesteryl laurate or palmitate became bound to both protein-synthesis factors.

The binding of cholesteryl esters to both protein-synthesis factors showed a rather narrow pH optimum at 6.0. The binding of cholesteryl 14-methylhexadecanoate by the initiation factor was significantly enhanced if incubation mixtures were supplemented with [^{35}S]methionyl-tRNA in amounts used for the testing of the formation of the binary complex of the factor (Pohlreich *et al.*, 1979). In the presence of initiator tRNA, binding of cholesteryl 14-methylhexadecanoate was demonstrated also at pH 4.0, although no binding occurred at this pH in the absence of initiator tRNA. A similar stimulation of the binding of this ester was found if incubation mixtures with the elongation factors were supplemented with substrates required for the formation of the ternary complex of this factor, i.e. [^3H]phenylalanyl-tRNA and GTP (Hradec, 1972). The presence of aminoacyl-tRNA stimulated the binding of cholesteryl 14-methylhexadecanoate also at pH 7.5 (Fig. 3). The binding of cholesteryl laurate and cholesteryl palmitate by both protein-synthesis factors was not affected by aminoacyl-tRNA.

The binding of cholesteryl 14-methylhexadecanoate by both protein-synthesis factors was inhibited by approx. 70% by the addition of saturating amounts of cholesteryl laurate or cholesteryl palmitate. This partial inhibition could be fully abolished by the addition of further quantities of cholesteryl 14-methylhexadecanoate. This seems to indicate a partial competitive nature of the inhibition and suggests that cholesteryl 14-methylhexadecanoate becomes bound to binding sites different from those for the other cholesteryl esters. Such a partial inhibition has been described for other enzymic systems by Fromm (1975) and Segel (1975).

Although the peptide-initiation factor used in this study is obviously different from the initiation factor eIF-2 (Anderson *et al.*, 1977), its apparent role in protein synthesis has been demonstrated (Pohlreich *et al.*, 1979), and since it naturally contains cholesteryl 14-methylhexadecanoate (Z.

Fig. 2. Dependence of the binding of cholesteryl esters by the peptide-initiation factor (a) and -elongation factors (b) on the amount of the ester added

Incubation mixture contained 22 μg of the peptide-initiation factor or 16 μg of the peptide-elongation factor and quantities of [^{14}C]cholesteryl 14-methylhexadecanoate (O), cholesteryl laurate (●) or cholesteryl palmitate (▲) as indicated.

Fig. 3. Effect of aminoacyl-tRNA on the binding of cholesteryl 14-methylhexadecanoate by the initiation (a) and elongation factors (b)

Incubation mixtures contained (a) 100 mM-Tris/HCl buffer, pH 6.0 (O, Δ), or 20 mM-potassium acetate buffer, pH 4.0 (●), 5 mM-KCl, 13 μg of the factor, and quantities of [^3H]cholesteryl 14-methylhexadecanoate as indicated, and were incubated without (Δ) or with 10 pmol of [^{35}S]methionyl-tRNA (O, ●), or (b) 100 mM-Tris/HCl buffer, pH 6.0 (O, Δ), or 10 mM-Tris/HCl buffer, pH 7.5 (●), 5 mM-KCl, 0.2 mM-GTP, 10.8 μg of the factor, and quantities of [^{14}C]cholesteryl 14-methylhexadecanoate as indicated, and were incubated without (Δ) or with 30 pmol of [^3H]phenylalanyl-tRNA (O, ●).

Tuháčková, unpublished work) it appeared to be a preparation suitable for our studies. The peptide-elongation factor 1 was only partially purified and is therefore certain to contain many contaminating

proteins. However, this preparation was used by Kemper *et al.* (1976) for their studies on the role of lipids in the function of this factor, and thus this preparation seemed to have certain advantages over

the other even more purified preparations of this factor.

There are apparently fundamental differences between the binding by protein-synthesis factors of cholesteryl 14-methylhexadecanoate and the other esters of cholesterol. Significantly higher quantities of the former ester are bound by both protein-synthesis factors than of cholesteryl laurate or cholesteryl palmitate. Quite dissimilar types of saturation curves were obtained for cholesteryl 14-methylhexadecanoate than for the other esters. In particular, only the binding of cholesteryl 14-methylhexadecanoate was significantly enhanced by substrates of both protein-synthesis factors (initiator or aminoacyl-tRNA). In agreement with our earlier results (Hradec & Sommerau, 1970), these findings indicate a specific role of cholesteryl 14-methylhexadecanoate in protein synthesis.

Both protein-synthesis factors used in the present study have high molecular weights and subunit structure, which are properties of most allosteric enzymes (Segel, 1975). The sigmoid saturation curve of cholesteryl 14-methylhexadecanoate may be an expression of a homotropic interaction between this ester originally present in the molecule of the factor (Hradec, 1972) and that newly bound by the factor. A non-hyperbolic sigmoid saturation curve is often dependent on allosteric modifiers (Sanwal, 1970). Cholesteryl 14-methylhexadecanoate as a normal constituent of both protein-synthesis factors significantly influences their function (Hradec, 1972; Z. Tuháčková, unpublished work), but bears no structural resemblance to their natural substrates and can be thus considered to be an allosteric modulator (Monod *et al.*, 1963). It may induce conformational changes of the tertiary or quaternary structure of proteins resulting in a modification of the binding sites of protein-synthesis factors for their

substrates, as with allosteric modifiers in other enzymic systems (Benesch & Benesch, 1969).

The excellent technical assistance of Mrs. V. Haufová is gratefully acknowledged.

References

- Anderson, W. F., Bosch, L., Cohn, W. E., Lodish, H., Merrick, W. C., Weissbach, H., Wittmann, H. C. & Wool, I. G. (1977) *FEBS Lett.* **76**, 1–10
- Benesch, R. & Benesch, R. E. (1969) *Nature (London)* **221**, 618–622
- Fromm, H. J. (1975) *Initial Rate Enzyme Kinetics*, pp. 86–88, Springer, Berlin
- Helmich, O. & Hradec, J. (1980a) *J. Chromatogr.* **193**, 153–156
- Helmich, O. & Hradec, J. (1980b) *J. Labelled Compd. Radiopharm.* in the press
- Hradec, J. (1972) *Biochem. J.* **126**, 1225–1229
- Hradec, J. (1975) in *Lipids and Tumors* (Carroll, K. K., ed.), pp. 197–227, S. Karger, Basel
- Hradec, J. & Dušek, Z. (1969) *Biochem. J.* **115**, 873–880
- Hradec, J. & Sommerau, J. (1970) *FEBS Lett.* **9**, 161–162
- Hradec, J., Dušek, Z., Bermek, E. & Matthaei, H. (1971) *Biochem. J.* **123**, 959–966
- Hradec, J., Dušek, Z. & Mach, O. (1974) *Biochem. J.* **138**, 147–154
- Hradec, J., Tuháčková, Z. & Dušek, Z. (1978) *Biochem. J.* **172**, 9–13
- Kemper, W. M., Merrick, W. C., Redfield, B., Liu, C. K. & Weissbach, H. (1976) *Arch. Biochem. Biophys.* **174**, 603–612
- Monod, J., Changeux, J. P. & Jacob, F. (1963) *J. Mol. Biol.* **6**, 306–320
- Pohlreich, P., Kříž, O., Tuháčková, Z., Dušek, Z. & Hradec, J. (1979) *Biochem. J.* **177**, 707–719
- Sanwal, B. D. (1970) *Bacteriol. Rev.* **34**, 20–36
- Segel, J. H. (1975) *Enzyme Kinetics*, pp. 161–166, 346–464, J. Wiley and Sons, New York