Biochem. J. (1974) **138**, 147–154
Printed in Great Britain

Influence of Cholesteryl 14-Methylhexadecanoate on some Ribosomal Functions Required for Peptide Elongation

By J. HRADEC and Z. DUŠEK
Department of Biochemistry, Oncological Institute, Prague 8, Czechoslovakia
and O. MACH
Institute of Haematology and Blood Transfusion, Prague 2, Czechoslovakia

(*Received* 10 *July* 1973)

1. Polyribosomes and ribosomal subunits from rat liver were adsorbed on a cellulosic ion-exchange adsorbent, freeze-dried and extracted with organic solvents. The activity of extracted particles in peptide elongation was tested in the presence of purified peptide-elongation factors. 2. Chloroform–methanol mixture (2:1, v/v) extracted 1.87±0.15 pmol of cholesteryl 14-methylhexadecanoate/pmol of the smaller ribosomal subunit and 0.92±0.11 pmol/pmol of the larger subunit. 3. In the presence of transferase I, extracted polyribosomes and 40S subunits bound more phenylalanyl-tRNA than did control non-extracted particles. The same binding as in control mixtures was obtained with extracted particles supplemented with cholesteryl 14-methylhexadecanoate in quantities corresponding to those extracted. 4. The polymerization of phenylalanine was greatly decreased with extracted polyribosomes and subunits and addition of the cholesteryl ester could not fully restore the original activity. 5. Extraction significantly decreased the activity of the P site of peptidyl transferase and normal activity was recovered after the addition of the ester. The A site of peptidyl transferase in extracted polyribosomes showed an increased activity when compared with non-extracted polyribosomes. 6. Cholesteryl 14-methylhexadecanoate apparently affects the function of the ribosomal A site and peptidyl transferase site and probably also that of the guanosine triphosphatase site and P site. The presence of different amounts of the ester in polyribosomes may be one of the mechanisms modulating peptide elongation at the ribosomal level.

Polypeptide-chain elongation on ribosomes involves three principal steps: binding of aminoacyl-tRNA, peptidyl transfer and translocation of peptidyl-tRNA and mRNA (Siler & Moldave, 1971).

The binding of aminoacyl-tRNA to ribosomes requires a supernatant factor, transferase I, and GTP (McKeehan & Hardesty, 1969). Results of Siler & Moldave (1969) indicate that the binding of aminoacyl-tRNA occurs at the A site according to Watson's (1964) model of ribosome function. During the peptidyl-transfer step the peptidyl moiety of peptidyl-tRNA is transferred to aminoacyl-tRNA at the A site. This reaction is catalysed by peptidyl transferase, which is an integral component of the larger ribosomal subunit (Vazquez *et al.*, 1969). Peptide elongation is completed by the translocation of peptidyl-tRNA from the A to the P site of ribosomes. This step requires the presence of an additional supernatant factor, transferase II, and GTP (Skogerson & Moldave, 1968).

Evidence has been presented by Hradec *et al.* (1971) that cholesteryl 14-methylhexadecanoate is present in the molecules of transferases I and II and is apparently essential for the normal function of these peptide-elongation factors. Further experiments indicated that the ribosome itself may be involved in the effect of this cholesteryl ester on peptide elongation (Hradec, 1972).

In experiments described in the present paper ribosomes were extracted with organic solvents and the activity of such extracted particles was tested in reactions involved in peptide elongation. Evidence is presented suggesting that cholesteryl 14-methylhexadecanoate interferes with peptide elongation and that this ester may have a regulatory function in some of the reactions involved.

Materials and Methods

Biological materials

Wistar rats of both sexes, weighing 150–200g, bred in this laboratory and kept on a standard diet, were used as the source of rat liver tissue.

Chemicals

GTP (sodium salt) was purchased from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. Poly(U) and tRNA from *Escherichia coli* B were products of Calbiochem, Los Angeles, Calif., U.S.A.

Puromycin hydrochloride was purchased from Serva, Heidelberg, Germany. All solvents were redistilled before use. Cholesteryl 14-methylhexadecanoate was synthesized as described by Hradec & Dolejš (1968) and Hradec & Dušek (1969). ECTHAM-cellulose [a cellulosic ion-exchange adsorbent, named by combining letters selected from the names of the reactants, epichlorohydrin and Tris (Peterson & Kuff, 1969)] was prepared from Whatman CC 31 cellulose by the method of Peterson & Kuff (1969).

Radiochemicals

L-[U-^{3}H]Phenylalanine (12.5Ci/mmol) was a product of The Radiochemical Centre, Amersham, Bucks., U.K. [8-^{3}H]GTP (tetrasodium salt) (10Ci/mmol) was purchased from NEN Chemicals, Dreieichenhain, W. Germany. [^{3}H]Phenylalanyl-tRNA was prepared by charging the tRNA of *E. coli* with labelled phenylalanine in the presence of aminoacyl-tRNA synthetases from *E. coli* and necessary cofactors as described by Matthaei *et al.* (1966). The final preparations contained 2.1–2.6μmol of phenylalanine/mmol of tRNA. Acetylphenylalanyl-tRNA was prepared by acetylation of these preparations (Komárková & Hradec, 1972).

Preparation of subcellular fractions

Postmitochondrial supernatants from rat liver were prepared as described by Hradec *et al.* (1971). Supernatants were adjusted to a final concentration of 1.2% with respect to sodium deoxycholate. Mixtures were stirred for an additional 10min and centrifuged at 3°C and 110000*g* for 90min in an 8×35ml rotor of a VAC 601 ultracentrifuge (H. Janetzki, Leipzig, German Democratic Republic). Supernatants were decanted and polyribosomal pellets were carefully rinsed with the homogenization medium. Pellets obtained in this way are further designated as polyribosomes. For the preparation of ribosomal subunits, polyribosomal pellets were suspended by gentle homogenization in a medium containing 50mM-Tris–HCl buffer, pH7.5, 2.5mM-$MgCl_2$, 250mM-KCl and 7mM-2-mercaptoethanol and the suspension was diluted with the same buffer to the final concentration of 30–50mg of ribosomes/ml. Then 10mM-puromycin was added to give a final concentration of 0.05mM and mixtures were incubated for 45min at 37°C (Borghetti *et al.*, 1970). After the incubation, mixtures were chilled in ice and diluted with the same buffer to a final concentration of 23–29mg of ribosomes/ml. Ribosomal subunits from 20ml of these suspensions were then separated by zonal centrifugation through 10–30% (w/v) linear gradients of sucrose, buffered as described by Falvey & Staehelin (1970). The centrifugation was carried out in a B XIV Ti rotor of a M.S.E. Superspeed 65 ultracentrifuge at 5°C and 36000rev./min for 4h. After centrifugation, the rotor contents were passed through a Unicam SP.800 recording spectrophotometer and fractions (15ml) were collected. Fractions containing 40S and 60S subunits were pooled and precipitated with ethanol as described by Falvey & Staehelin (1970). Both peptide-elongation factors were purified from rat liver cytosol as described by Hradec *et al.* (1971). Sucrose-gradient analysis of polyribosomes and ribosomal subunits was performed as described by Komárková & Hradec (1972).

Extraction of polyribosomes and ribosomal subunits with organic solvents

Pellets of polyribosomes and ribosomal subunits were suspended by gentle homogenization in 5mM-Tris–HCl buffer, pH7.5, containing 0.5mM-$MgCl_2$ and added to a suspension of ECTHAM-cellulose equilibrated against the same buffer. Mixtures were stirred for about 2h and then centrifuged at low speed. Supernatants were rejected, the ECTHAM-cellulose was washed with the same buffer and the washings were rejected. All these operations were performed at 0–5°C. Ribosomes adsorbed on the ion-exchanger were then freeze-dried. Extraction of ribosomes was performed as described by Hradec & Dušek (1969). For the elution of particles from the adsorbent, the material was suspended in 50mM-Tris–HCl buffer, pH7.5, containing 10mM-$MgCl_2$, 300mM-KCl and 7mM-2-mercaptoethanol and the suspension was stirred for 2h. The suspension was then centrifuged at low speed and the ion-exchanger was washed twice with the elution buffer. The eluate and washings were pooled and polyribosomes or ribosomal subunits were precipitated with precooled ethanol as described by Falvey & Staehelin (1970). All these operations were performed at 0–5°C. Pellets of polyribosomes or subunits were suspended in the homogenization buffer and stored at −80°C.

Incubation procedures

Mixtures for the assay of phenylalanyl-tRNA binding to ribosomes and poly(U)-directed phenylalanine polymerization were prepared and incubated as described by Hradec *et al.* (1971). The active site of peptidyl transferase is composed of two binding sites. The donor or peptidyl site attaches tRNA from which the peptide is transferred during the reaction. The acceptor site binds tRNA carrying the amino acid residue which will accept the peptide (Rychlík *et al.*, 1969). Incubation mixtures for the assay of the activity of both these binding sites were composed essentially as described by I. Rychlík (personal communication). A detailed description of the procedures used was given by Komárková & Hradec (1972). The

activity of the aminoacyl-tRNA binding site was assayed in mixtures containing, in a final volume of 0.1ml: 50mM-Tris–HCl, pH7.5, 10mM-$MgCl_2$, 100mM-NH_4Cl, 10μg of poly(U) and amounts of *N*-acetylphenylalanyl-tRNA and polyribosomes as indicated. They were preincubated at 37°C for 30min and then puromycin was added to a final concentration of 0.1mM and the incubation was continued for another 60min. The activity of the binding site of peptidyl transferase for peptidyl-tRNA was tested in reaction mixtures containing, in a final volume of 0.1ml: 50mM-Tris–HCl, pH7.5, 40mM-$MgCl_2$, 400mM-KCl, 10mM-puromycin, 30μl of acetone and quantities of acetyl[^{3}H]phenylalanyl-tRNA and ribosomes as indicated; incubation was at 37°C for 70min. All incubation mixtures contained the same quantities of extracted as of non-extracted particles. The transferase II-dependent binding of GTP to ribosomes was assayed by the method of Bermek & Matthaei (1971).

Assay of radioactivity

Samples for the assay of radioactivity were prepared as described by Hradec *et al.* (1971) and Komárková & Hradec (1972). Radioactivity was counted in a Nuclear–Chicago Mark II liquid-scintillation spectrometer. The efficiency was 29% for the samples on nitrocellulose or glass-fibre filters and 48% for samples from the peptidyl-transfer reactions.

Chemical determinations

Protein and RNA were determined as described by Hradec *et al.* (1971). For the quantitative determination of ribosomes, the relationship 1nmol of mammalian ribosomes = 52.5E_{260} units was used (Gros & Matthaei, 1973). For the quantitative determination of cholesteryl 14-methylhexadecanoate the method of Hradec (1968) was used.

Results

Cholesteryl 14-methylhexadecanoate content in ribosomes

When polyribosomes and ribosomal subunits were adsorbed on ECTHAM-cellulose and eluted without extraction with organic solvents nearly 100% recoveries were obtained. Very similar recoveries were obtained with preparations extracted with ether. Polyribosomes and 40S subunits extracted with iso-octane were recovered in about 80% yields, whereas after the extraction with chloroform–methanol (2:1, v/v) only about 60% of these particles could be recovered. The recoveries of extracted 60S subunits were always 10–15% lower than those of similar 40S preparations. The sedimentation pattern of extracted polyribosomes in sucrose gradients was not different from that found with the same particles before adsorption on ECTHAM-cellulose. The same was true for both extracted ribosomal subunits.

Different solvents or solvent mixtures extracted varied quantities of cholesteryl 14-methylhexadecanoate from a given polyribosomal preparation. Rather dissimilar results in this respect were obtained with different batches of polyribosomes extracted with the same solvent. On the other hand, the amount of this ester extracted by a given solvent from different batches of ribosomal subunits was very uniform. A summary of these results is given in Table 1.

Binding of [^{3}H]phenylalanyl-tRNA to extracted polyribosomes and ribosomal subunits

Values obtained for non-enzymic as well as for transferase I-dependent binding of [^{3}H]phenylalanyl-tRNA to polyribosomes or 40S subunits previously adsorbed on ECTHAM-cellulose and freeze-dried were not different from those with control preparations. Non-enzymic binding of phenylalanyl-tRNA in the presence of polyribosomes and 40S subunits extracted with iso-octane or chloroform–methanol was the same as with control non-extracted particles.

In the presence of transferase I, the poly(U)-dependent binding of phenylalanyl-tRNA to polyribosomes extracted with iso-octane was increased to 125–130% of the control value. However, polyribosomes extracted with chloroform–methanol did not show such an increased activity. The extraction of 40S subunits with organic solvents significantly increased their ability to bind phenylalanyl-tRNA. This effect was even more pronounced if chloroform–methanol was used for the extraction instead of iso-octane (Table 2).

Addition of non-extracted 60S subunits to mixtures containing non-extracted 40S subunits enhanced the phenylalanyl-tRNA binding by about 2-fold. This stimulating effect of the larger subunit on the binding reaction was even more significant if extracted 60S subunits were added to mixtures containing non-extracted 40S particles (Fig. 1). On the other hand, the addition of extracted 60S particles showed an inhibitory effect on the binding reaction compared with the binding of phenylalanyl-tRNA to the smaller subunit alone (Fig. 2). Similarly, an inhibition of the binding reaction was induced by non-extracted 60S subunits in mixtures containing extracted smaller subunits (Table 2).

Addition of cholesteryl 14-methylhexadecanoate to incubation mixtures containing extracted 40S subunits decreased the enzymic binding of phenylalanyl-tRNA to the particle (Table 2). For a complete recovery of the normal activity, the ester had to be added in quantities replacing exactly those extracted.

Table 1. *Extraction of cholesteryl 14-methylhexadecanoate from polyribosomes and ribosomal subunits with different solvents*

Polyribosomes and ribosomal subunits were isolated and extracted as described in the Materials and Methods section. The ester contents in extracts were determined by the method of Hradec (1968). All values are pmol of cholesteryl 14-methylhexadecanoate±S.D. (with the number of observations in parentheses) extracted from 1 pmol of ribosomes (contained in polyribosomes) or subunits.

Particles extracted with ...	Cholesteryl 14-methylhexadecanoate		
	Ether	Iso-octane	Chloroform–methanol
Polyribosomes	1.52±0.84 (3)	2.32±1.05 (3)	3.67±1.48 (3)
40S subunits	0.84±0.14 (3)	1.55±0.09 (4)	1.87±0.15 (4)
60S subunits	0.45±0.10 (3)	0.59±0.12 (4)	0.92±0.11 (4)

Table 2. *Binding of [^{3}H]phenylalanyl-tRNA to extracted polyribosomes and ribosomal subunits*

The particles were isolated and extracted with organic solvents as described in the Materials and Methods section. The type of extraction is indicated in parentheses. Incubation mixtures contained 150 μg of polyribosomes or 22 μg of 40S subunits and (where indicated) 45 μg of non-extracted 60S subunits, the quantity of aminoacyl-tRNA containing 1.7–1.9 pmol of [^{3}H]phenylalanine, saturating quantities of transferase I (2–3 μg of protein) and the other components described by Hradec *et al.* (1971). Mixtures were incubated and radioactivity was assayed as given in the same paper. Cholesteryl 14-methylhexadecanoate was added to mixtures containing extracted particles (where indicated) in quantities replacing exactly those extracted.

System	[^{3}H]Phenylalanine bound (pmol/incubation mixture)	
	No ester added	Ester replaced
Polyribosomes		
(non-extracted)	0.41	—
(iso-octane)	0.52	0.40
(chloroform–methanol)	0.40	0.41
40S subunits		
(non-extracted)	0.27	—
(iso-octane)	0.38	0.29
(chloroform–methanol)	0.41	0.25
(non-extracted)+60S subunits	0.53	—
(iso-octane)+60S subunits	0.08	0.54
(chloroform–methanol)+60S subunits	0.11	0.49

Lower or higher amounts of the ester did not affect the enhanced activity of extracted smaller subunits (Fig. 3).

Polymerization of [^{3}H]phenylalanine

Polyphenylalanine synthesis was significantly inhibited in incubation mixtures containing extracted polyribosomes compared with control non-extracted

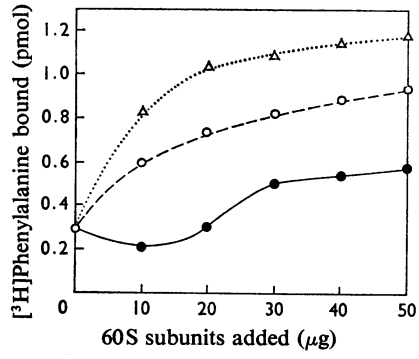

Fig. 1. *Effect of non-extracted* (●) *and extracted* (○, *iso-octane*; △, *chloroform–methanol*, 2:1, *v*/*v*) *60S subunits on the binding of phenylalanyl-tRNA by non-extracted 40S subunits*

Incubation mixtures contained 22 μg of 40S subunits and other components described in the Materials and Methods section.

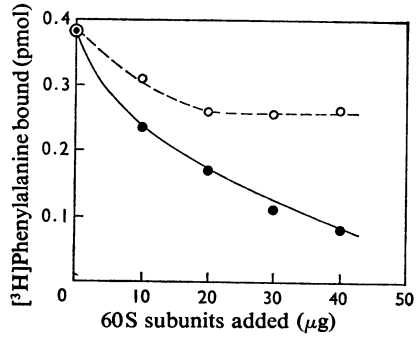

Fig. 2. *Effect of non-extracted 60S subunits* (●) *and the same particles extracted with iso-octane* (○) *on the binding of phenylalanyl-tRNA by 40S subunits extracted with iso-octane*

Composition of incubation mixtures is indicated in the legend for Fig. 1.

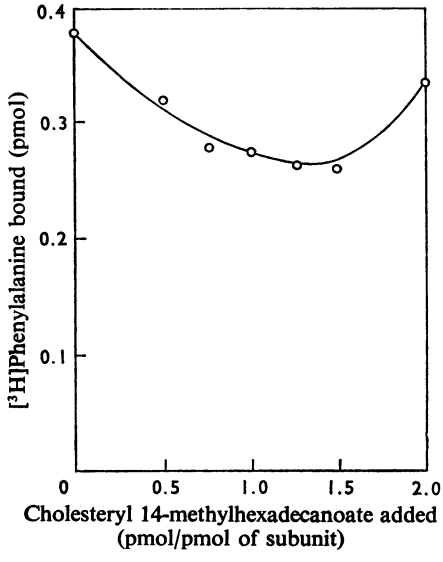

Fig. 3. *Effect of cholesteryl 14-methylhexadecanoate on the binding of phenylalanyl-tRNA by extracted 40S subunits*

Incubation mixtures contained 25 μg of subunits extracted with iso-octane (1.46pmol of the ester/pmol of the 40S subunit was removed). In control incubation mixtures containing non-extracted subunits 0.27pmol of phenylalanine was bound.

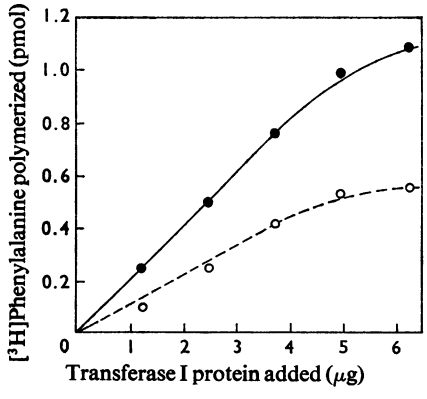

Fig. 4. *Polymerization of [3H]phenylalanine with non-extracted polyribosomes* (●) *and particles extracted with iso-octane* (○)

Incubation mixtures contained transferase I as indicated, saturating quantities of transferase II (12 μg of protein), 120 μg of ribosomes, amounts of aminoacyl-tRNA containing 2.2pmol of [3H]phenylalanine and other components described in the Materials and Methods section.

particles (Fig. 4). A similar decrease in polyphenylalanine synthesis was found in mixtures containing extracted ribosomal subunits. In systems containing combinations of non-extracted and extracted subunits, extracted 60S subunits seemed to be more inhibitory than extracted smaller subunits (Table 3).

Addition of cholesteryl 14-methylhexadecanoate to incubation mixtures containing extracted polyribosomes stimulated the polyphenylalanine synthesis, and values corresponding to 60–80% of the control value were obtained if the ester was added in quantities corresponding to those that had been extracted from a given polyribosome preparation. Similar stimulations were also found after the addition of the ester to incubation mixtures with extracted ribosomal subunits (Table 3).

Transferase II-catalysed binding of GTP to polyribosomes

In the presence of translocase the binding of GTP to extracted polyribosomes was increased compared with the same non-extracted preparations (Fig. 5). If cholesteryl 14-methylhexadecanoate was added to mixtures containing polyribosomes, similar values were obtained for the nucleotide binding to extracted and to non-extracted polyribosomes.

Peptidyl transfer with extracted polyribosomes and 60S subunits

Extraction of polyribosomes with organic solvents significantly decreased the activity of the P site of peptidyl transferase as shown in Fig. 6. Closely similar results were also obtained with extracted 60S subunits. Addition of cholesteryl 14-methylhexadecanoate to mixtures containing extracted particles significantly enhanced the acetylphenylalanyl-puromycin formation and values corresponding to 80–100% of the control value (with the same non-extracted particles) were obtained if the ester was added in quantities corresponding to those that had been removed by the extraction (Table 4).

On the other hand, polyribosomes extracted with organic solvents showed an increased activity of the A site of peptidyl transferase when compared with control non-extracted particles. This is apparent from saturation curves for non-extracted and extracted polyribosomes (Fig. 7). In the presence of cholesteryl 14-methylhexadecanoate, however, extracted polyribosomes showed an activity comparable with that of control non-extracted particles.

Discussion

In our experiments we were able to confirm the results of Kedes *et al.* (1969) that treatment of ribosomes with ECTHAM-cellulose does not alter the activity of these particles in protein synthesis.

Table 3. *Polymerization of [^{3}H]phenylalanine with extracted ribosomal subunits*

The particles were isolated and extracted with iso-octane as described in the Materials and Methods section. Incubation mixtures contained quantities of subunits and aminoacyl-tRNA indicated in legends for Table 2 and all other components described by Hradec *et al.* (1971). They were incubated and the radioactivity was assayed as described in the same paper. Cholesteryl 14-methylhexadecanoate was added to mixtures containing extracted particles in quantities exactly replacing those extracted.

System	[^{3}H]Phenylalanine polymerized (pmol/reaction mixture)	
	Ester extracted	Ester replaced
40S subunits (non-extracted) +60S subunits (non-extracted)	0.54	—
40S subunits (non-extracted) +60S subunits (iso-octane)	0.25	0.41
40S subunits (iso-octane) +60S subunits (non-extracted)	0.34	0.47
40S subunits (iso-octane) +60S subunits (iso-octane)	0.19	0.33

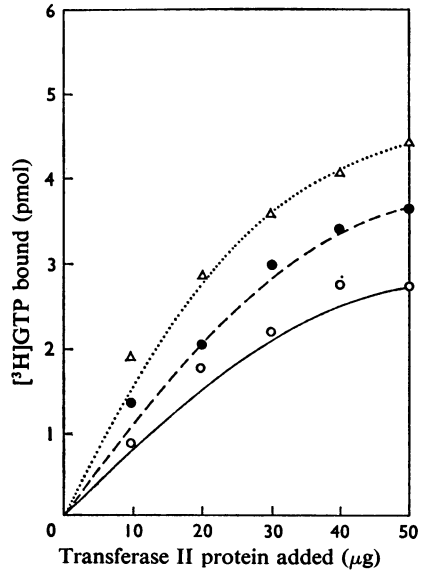

Fig. 5. *Transferase II-dependent binding of GTP to non-extracted polyribosomes* (○) *and particles extracted with ether* (●) *and iso-octane* (△)

Incubation mixtures were prepared and incubated as described by Bermek & Matthaei (1971).

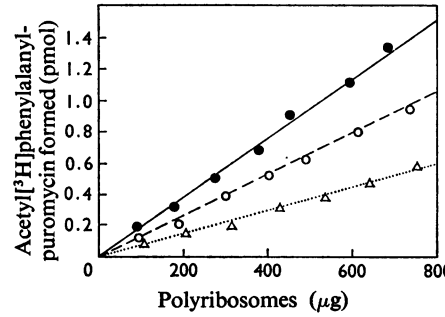

Fig. 6. *Peptidyl transfer with non-extracted polyribosomes* (●) *and polyribosomes extracted with ether* (○) *or iso-octane* (△)

Incubation mixtures for the assay of the activity of the P site of peptidyl transferase contained in the final volume of 0.1ml: 50mM-Tris–HCl, pH7.5, 40mM-$MgCl_2$, 400mM-KCl, 10mM-puromycin, 30μl of acetone, quantities of aminoacyl-tRNA containing 2.3pmol of *N*-acetylphenylalanine and polyribosomes as indicated; incubation was at 37°C for 70min. The quantity of acetylphenylalanyl-puromycin formed was assayed as described by Komárková & Hradec (1972).

Table 4. *Activity of the P site of peptidyl transferase in extracted polyribosomes and 60S subunits*

The particles were isolated and extracted with organic solvents as described in the Materials and Methods section. The solvent used for extraction is indicated in parentheses. Incubation mixtures contained in the final volume of 0.1ml: 50mM-Tris–HCl, pH7.5, 40mM-$MgCl_2$, 400mM-KCl, 10mM-puromycin, 30μl of acetone, 405μg of polyribosomes (or 60μg of 60S subunits) and quantities of aminoacyl-tRNA containing 2.3pmol of *N*-acetyl-[^{3}H]-phenylalanine; incubation was at 37°C for 70min. The quantity of acetylphenylalanyl-puromycin formed was assayed as described by Komárková & Hradec (1972). Cholesteryl 14-methylhexadecanoate was added to mixtures containing extracted particles in quantities exactly replacing those extracted.

System	*N*-Acetyl[^{3}H]phenylalanyl-puromycin formed (pmol/incubation mixture)	
	Ester extracted	Ester replaced
Polyribosomes		
(non-extracted)	0.64	—
(ether)	0.50	0.66
(iso-octane)	0.29	0.61
60S subunits		
(non-extracted)	0.28	—
(ether)	0.21	0.30
(iso-octane)	0.12	0.29

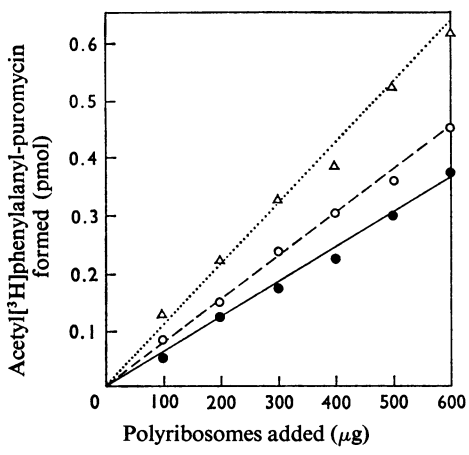

Fig. 7. *Activity of the A site of peptidyl transferase in non-extracted polyribosomes* (●) *and particles extracted with ether* (○) *and iso-octane* (△)

Incubation mixtures contained in the final volume of 0.1ml: 50mM-Tris–HCl, pH7.5, 10mM-$MgCl_2$, 100mM-NH_4Cl, 10μg of poly(U), quantities of aminoacyl-tRNA containing 1.2pmol of *N*-acetylphenylalanine and polyribosomes as indicated. They were preincubated at 37°C for 30min and then puromycin was added to a final concentration of 0.1mM and the incubation was continued for another 60min. The quantity of acetylphenylalanyl-puromycin formed was assayed as described by Komárková & Hradec (1972).

Dissimilar values for the content of cholesteryl 14-methylhexadecanoate in polyribosomes are probably due to the fact that these particles contained adsorbed supernatant proteins, since an unwashed polyribosomal fraction was used. It has been demonstrated that washing in high salt concentration may alter the ribosomal structure and split off some ribosomal proteins (Schreier & Staehelin, 1973). Ribosomal subunits that were washed free of protein admixture, by centrifugation through gradients, showed closely similar quantities of extracted ester for different preparations. A relation could be demonstrated between the quantity of the ester extracted and the alteration of activity of such preparations, as in our previous experiments with peptide-elongation factors (Hradec *et al.*, 1971).

There is good evidence that the chloroform–methanol mixture used in our experiments extracts up to 100% of lipids from lipoproteins (Nelson, 1972). Amounts of the ester extracted by this solvent mixture from particles should thus represent the total quantity of this compound present in the particle or be at least near to this value. Assuming molecular weights of 1.5×10^6 for the smaller and 3.0×10^6 for the larger ribosomal subunit (Hamilton *et al.*, 1971), the 40S subunit of rat liver ribosomes seems to contain two cholesteryl 14-methylhexadecanoate molecules and the 60S subunit one molecule of this ester.

Unlike in aminoacyl-tRNA synthetases (Hradec & Dušek, 1969) or in peptide-elongation factors (Hradec *et al.*, 1971), cholesteryl 14-methylhexadecanoate seems to be rather firmly bound to ribosomal structures. Diethyl ether, which efficiently extracts this ester from pH5 enzymes and cell sap (Hradec & Dušek, 1968), is quite ineffective for the extraction of ribosomes. Iso-octane, the most effective solvent for peptide-elongation factors (Hradec *et al.*, 1971), extracts only moderate quantities of the ester from ribosomes.

Results of our experiments indicate that both ribosomal subunits are involved in the binding of phenylalanyl-tRNA to ribosomes. Kaufmann & Zamir (1972) presented evidence that the rather limited binding of aminoacyl-tRNA to the 30S subunit alone (when compared with the binding to the 70S ribosome) results from some deformation of the aminoacyl-tRNA binding site on the smaller ribosomal subunit. Such a deformation may possibly be inhibited by conformational changes in ribosomal structure induced by extraction with organic solvents, and this may then result in the enhanced binding activity of extracted smaller subunits, as found in our experiments. Suzuka *et al.* (1966) suggested that the increased binding of aminoacyl-tRNA to the 30S subunit in the presence of the 50S subunit may be due to a stabilizing effect of the larger subunit on the complex formed between the smaller subunit and aminoacyl-tRNA. Similar evidence has been reported by Kaufmann & Zamir (1972). The larger ribosomal subunit may thus have a regulatory function in the binding reaction, and it seems possible that the extraction of cholesteryl 14-methylhexadecanoate from the 60S subunit may also affect this regulatory function.

It is probable that the ester present in both ribosomal subunits is bound to some ribosomal proteins, since it can bind to enzymic proteins (Hradec *et al.*, 1971). Evidence has been presented by Kurland (1971) that some ribosomal proteins play a structural role in ribosome assembly whereas others are required for ribosomal functions. Thus specific proteins required for the function of the ribosomal A site have been isolated (Randall-Hazelbauer & Kurland, 1972) and a ribosomal protein essential for the function of peptide-elongation factors has been described (Weissbach *et al.*, 1972). It seems possible that cholesteryl 14-methylhexadecanoate is bound to some of these specific functional proteins and may in this way affect the ribosomal activity. Alternatively, the ester could be bound to some other protein, thus affecting the configuration of the ribosome as a whole and hence its function.

Our results indicate that the ester also affects some functions of the larger ribosomal subunit. According to Richman & Bodley (1972), there are at least four different functional regions on the 50S subunit: a portion of the P site, a portion of the A site, peptidyl transferase site and a guanosine triphosphatase site. Our experiments provided good evidence that cholesteryl 14-methylhexadecanoate affects both the function of the A site to which aminoacyl-tRNA becomes bound (Watson, 1964) and the peptidyl transferase site, as indicated by changes in peptidyl transferase activity of extracted ribosomes and 60S subunits. Changes in the binding of GTP to extracted ribosomes indicate that the ester may also affect the guanosine triphosphatase site. Only indirect evidence, however, can be presented for the effect of cholesteryl 14-methylhexadecanoate on the ribosomal P site. Unlike the peptidyl transferase activity, the decreased polyphenylalanine synthesis with extracted ribosomes could not be fully recovered by the addition of appropriate quantities of the ester. Bearing in mind the significant inhibitory effect of extracted 40S subunits on phenylalanine polymerization, it is possible that partially irreversible uncoupling of the ribosomal A and P sites may be induced by the extraction, which affects the translocation step and results in decreased peptide elongation. It has been demonstrated that a change in ribosome conformation occurs during the translocation (Schreier & Noll, 1971). Possibly extracted ribosomes can no longer change their conformation because of structural changes induced by the extraction.

Enzymes from which cholesteryl 14-methylhexadecanoate has been extracted always show a decreased activity (Hradec & Dušek, 1969; Hradec *et al.*, 1971). On the other hand, extraction of this ester from ribosomes is in some instances followed by an enhanced activity of such particles. This seems to indicate that the ester may have some regulatory role and that some ribosomal functions may be modulated by the quantity of the ester present. Further experiments are required to obtain direct evidence in this respect.

We thank Dr. O. Helmich for the quantitative determination of cholesteryl 14-methylhexadecanoate. The excellent technical assistance of Miss J. Jandová, Mrs. J. Žemlová and Mr. J. Hrubeš is also gratefully acknowledged.

References

Bermek, E. & Matthaei, H. (1971) *Biochemistry* **10**, 4906–4911

Borghetti, A. F., Comi, P., Franchi, R., Gianni, A. M., Giglioni, B., Ottolenghi, S. & Guidotti, G. G. (1970) *Ital. J. Biochem.* **19**, 397–416

Falvey, A. K. & Staehelin, T. (1970) *J. Mol. Biol.* **53**, 1–19

Gros, F. & Matthaei, H. (1973) *Practical Molecular Genetics*, Springer-Verlag, Berlin, Heidelberg and New York, in the press

Hamilton, M. G., Pavlovec, A. & Petermann, M. L. (1971) *Biochemistry* **10**, 3424–3427

Hradec, J. (1968) *J. Chromatogr.* **32**, 511–518

Hradec, J. (1972) *Biochem. J.* **126**, 1225–1229

Hradec, J. & Dolejš, L. (1968) *Biochem. J.* **107**, 129–134

Hradec, J. & Dušek, Z. (1968) *Biochem. J.* **110**, 1–8

Hradec, J. & Dušek, Z. (1969) *Biochem. J.* **115**, 873–880

Hradec, J., Dušek, Z., Bermek, E. & Matthaei, H. (1971) *Biochem. J.* **123**, 959–966

Kaufmann, Y. & Zamir, A. (1972) *J. Mol. Biol.* **69**, 357–372

Kedes, L. H., Kuff, E. L. & Peterson, E. A. (1969) *Biochemistry* **8**, 2923–2929

Komárková, E. & Hradec, J. (1972) *Biochem. J.* **129**, 367–372

Kurland, C. G. (1971) in *Protein Synthesis* (McConkey, E. E., ed.), vol. 1, pp. 179–228, M. Dekker Inc., New York

Matthaei, H., Heller, G., Voigt, H. P., Neth, R., Schöh, G. & Kubler, H. (1966) in *FEBS Symp. Genetic Elements, Warsaw* (Shugar, D., ed.), pp. 233–250, Polish Scientific Publishers, Warsaw; Academic Press, London

McKeehan, W. L. & Hardesty, B. (1969) *J. Biol. Chem.* **244**, 4330–4339

Nelson, G. J. (1972) in *Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism* (Nelson, G. J., ed.), pp. 3–21, Wiley-Interscience Inc., New York

Peterson, E. A. & Kuff, E. L. (1969) *Biochemistry* **8**, 2916–2922

Randall-Hazelbauer, L. L. & Kurland, C. G. (1972) *Mol. Gen. Genet.* **115**, 234–242

Richman, N. & Bodley, J. W. (1972) *Proc. Nat. Acad. Sci. U.S.* **69**, 686–689

Rychlík, I., Černá, J., Chládek, S., Žemlička, J. & Haladová, Z. (1969) *J. Mol. Biol.* **43**, 13–24

Schreier, M. H. & Noll, H. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 805–812

Schreier, M. H. & Staehelin, T. (1973) *J. Mol. Biol.* **73**, 328–349

Siler, J. & Moldave, K. (1969) *Biochim. Biophys. Acta* **195**, 123–129

Siler, J. G. & Moldave, K. (1971) in *Protein Synthesis* (McConkey, E. H., ed.), vol. 1, pp. 121–147, M. Dekker Inc., New York

Skogerson, L. & Moldave, K. (1968) *J. Biol. Chem.* **243**, 5354–5360

Suzuka, I., Kaji, H. & Kaji, A. (1966) *Proc. Nat. Acad. Sci. U.S.* **55**, 1483–1495

Vazquez, D., Battaner, E., Neth, R., Heller, G. & Monro, R. E. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 369–375

Watson, J. D. (1964) *Bull. Soc. Chim. Biol.* **46**, 1399–1425

Weissbach, H., Redfield, B., Yamasaki, B. E., Davis, R. C., Pestka, S. & Brot, N. (1972) *Arch. Biochem. Biophys.* **149**, 110–117