Biochem. J. (1971) **123**, 959–966
Printed in Great Britain

The Role of Cholesteryl 14-Methylhexadecanoate in Peptide Elongation Reactions

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(*Received 9 March* 1971)

1. Peptide-elongation factors were purified from rat liver and human tonsils and the contents of cholesteryl 14-methylhexadecanoate were determined in fractions obtained during enzyme purification. The relative contents of this compound in purified enzyme preparations was several times higher than that in the crude starting material. Elongation factors from human tonsils contained a significantly larger quantity of the cholesteryl ester than enzyme from rat liver. 2. Transfer enzymes extracted with various organic solvents showed variable decreased activities in both binding and peptidization assay. The decrease of enzymic activity was proportional to the amount of cholesteryl 14-methylhexadecanoate extracted from a given enzymic preparation. In systems containing both extracted elongation factors the polyphenylalanine synthesis was limited by the residual activity of the less active transfer factor. 3. The original enzymic activity of extracted transferases was fully recovered by the addition of pure cholesteryl 14-methylhexadecanoate in quantities corresponding to those extracted. 4. Increase of the relative contents of this cholesteryl ester during enzyme purification, decrease of the enzymic activity after the extraction and its recovery by the addition of this compound indicates that the presence of this ester in elongation factors is essential for the normal function of these enzymes.

Cholesteryl 14-methylhexadecanoate seems to play an important role in protein synthesis. This compound stimulates the incorporation of labelled amino acids into tRNA of rat liver *in vitro* (Hradec & Dolejš, 1968). Purified aminoacyl-tRNA synthetases from mammalian tissues lost all or most of their activity after extraction with organic solvents (Hradec & Dušek, 1969). Only about 50% of the normal charging of tRNA was obtained with extracted pH 5 enzymes from rat liver (Hradec & Dušek, 1968*b*). In both these instances, a complete reactivation of extracted enzymes could be induced by the addition of cholesteryl 14-methylhexadecanoate into incubation mixtures. These results indicated that the presence of this ester in the molecule of aminoacyl-tRNA synthetases is apparently essential for their normal function. However, in contrast with only partially deactivated pH 5 enzymes no incorporation of labelled amino acids into ribosomal proteins was found in reaction mixtures containing extracted cell sap (Hradec & Dušek, 1968*b*) although normal incorporation was again obtained after the addition of cholesteryl 14-methylhexadecanoate. This apparent discrepancy indicated that the ester affects not only the function of aminoacyl-tRNA ligases but also that of some other soluble enzymes involved in protein synthesis.

Enzymes participating in protein synthesis that are present in the soluble fraction of the cell may be divided into three main groups: those required for peptide-chain initiation, elongation and termination (Matthaei *et al.* 1968). Relatively little is known about the nature and formation of the initiation complex in mammalian cells (Rahaminoff & Arnstein, 1969) or, in particular, about the termination processes. However, increasing knowledge has been gained during the past few years about factors required for peptide-chain elongation (Hardesty, Culp & McKeenan, 1969). The formation of aminoacyl-tRNA is followed by binding of aminoacyl-tRNA to the acceptor and apparently two additional decoding sites on the ribosome (Swan *et al.* 1969). Transferase I is engaged in this process

(Ibuki & Moldave, 1968). Suitable methods were described for the purification of this binding enzyme from rabbit reticulocytes (McKeenan & Hardesty, 1969), rat liver (Schneir & Moldave, 1968) and calf liver (Klink, Kramer, Nour & Peterson, 1967). The function of this enzyme resembles that of T_u and T_s factors from *Escherichia coli* (Lucas-Lenard & Lipmann, 1966).

The next steps in peptide-chain elongation are presented by peptidyl transfer and by translocation of peptidyl-tRNA from the acceptor to the donor site of the ribosome. For these steps the peptidyl transferase of the larger ribosomal subunit (Vasquez, Battaner, Neth, Heller & Monro, 1969) and transferase II (Skogerson & Moldave, 1968) or translocase (Siler & Moldave, 1969) are required. The latter enzyme is apparently identical with the G factor described in *E. coli* (Nishizuka & Lipmann, 1966).

In the present paper results of experiments are described in which both transfer factors were isolated from rat liver and human tonsils. The activity of purified transferases extracted with various organic solvents was tested in the binding and peptidization assay, and results on the reactivation of these extracted enzymes with cholesteryl 14-methylhexadecanoate are also given. Preliminary results of this work have been presented (Hradec & Dušek, 1968*a*).

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. Animals were starved for 24h before death. Human tonsils were obtained from cases of tonsillectomy performed for prophylactic reasons. Only tonsils without signs of acute or chronic inflammation were selected.

Chemicals. GTP (sodium salt) was purchased from Koch–Light Laboratories, Colnbrook, Bucks., U.K. Poly(U) was a product of Calbiochem, Los Angeles, Calif., U.S.A. All solvents were redistilled before use. Cholesteryl (+)-14-methylhexadecanoate was synthesized as described earlier (Hradec & Dolejš, 1968; Hradec & Dušek, 1969).

Radiochemicals. L-[U-^{3}H]Phenylalanine (1000mCi/mmol) was a product of The Radiochemical Centre, Amersham, Bucks., U.K.; L-[U-^{14}C]phenylalanine (118 mCi/mmol) was purchased from the Institute for Research, Production and Utilization of Radioisotopes, Prague. The procedure of Moldave (1963) was used for the preparation of labelled rat liver tRNA and human tonsil tRNA as well. The final preparation of [^{3}H]phenylalanyl-tRNA from rat liver contained 5.95μmol of phenylalanine/mmol of tRNA, that of [^{14}C]phenylalanyl-tRNA from human tonsils contained 8.5μmol of phenylalanine/mmol of tRNA.

Isolation and purification of elongation factors. Elongation factors from both rat liver and human tonsils were prepared essentially by the methods of Bermek, Krämer, Mönkemeyer & Matthaei (1970) and Bermek & Matthaei (1970*a*). In this procedure both factors are precipitated from the postmicrosomal supernatant (fraction II) by $(NH_4)_2SO_4$ (35–70% of saturation) (fraction III). From this enriched fraction individual elongation factors are separated on a column of Sephadex G-200 (fraction IV). The binding enzyme is further purified by a passage through a Sepharose 4B column (fraction V), the translocase is adsorbed on DEAE-cellulose (Whatman DE-50) and eluted with 200mM-KCl in standard buffer (250mM-sucrose, 50mM-tris–HCl buffer, pH7.4, 0.1mM-EDTA and 7mM-2-mercaptoethanol (fraction V). Both purified enzymes were dialysed against standard buffer, mixed with DEAE-cellulose (Whatman DE-50, equilibrated with standard buffer) and the suspensions were stirred for about 30min. Then the DEAE-cellulose was allowed to settle and the supernatant was decanted. The DEAE-cellulose paste was carefully washed with the standard buffer and finally freeze-dried. The freeze-dried preparations of DEAE-cellulose with adsorbed enzymes could be stored at 0–5°C for several days without appreciable losses of activity. Before use, both enzymes were eluted from DEAE-cellulose with 200mM-KCl in standard buffer. For the extraction with organic solvents, freeze-dried DEAE-cellulose preparations with adsorbed enzymes were placed in extraction cartridges and extracted in a Soxhlet apparatus for the time indicated. Immediately after the extraction, extracted enzymes were dried *in vacuo* (0.05mmHg). These dried preparations were then stored at 0–5°C until used. Enzymes were extracted from DEAE-cellulose as described above. Ribosomes from both rat liver and human tonsils were prepared by the method of Bermek & Matthaei (1970*b*) and Bermek *et al.* (1970). The blank value of these purified ribosomes was very near to the background value.

Incubation procedures. The incubation mixtures for binding assay contained in a final volume of 0.1ml: 50mM-tris–HCl buffer, pH7.4, 55mM-KCl, 6mM-$MgCl_2$, 0.1mM-GTP, 10μg of poly(U), 70–90μg of ribosomes, 32.0μg of [^{3}H]phenylalanyl-tRNA from rat liver or 15.8μg of [^{14}C]phenylalanyl-tRNA from human tonsils and transferase I as indicated. The reaction mixtures for the peptidization consisted of 50mM-tris–HCl buffer, pH7.4, 11mM-$MgCl_2$, 55mM-KCl, 2mM-GTP, 10mM-2-mercaptoethanol, 20μg of poly(U), 75–85μg of ribosomes, 32.0μg of [^{3}H]phenylalanyl-tRNA from rat liver or 15.8μg of [^{14}C]phenylalanyl-tRNA from human tonsils and both transferases as indicated in a total volume of 0.1ml. Both reaction mixtures were incubated for 30min at 37°C. For the binding assay, reaction mixtures after the incubation were diluted with ice-cold buffer (20mM-tris–HCl buffer, pH7.5, 10mM-$MgCl_2$, 20mM-KCl), and filtered through nitrocellulose membranes. From reaction mixtures for polymerization assay, 75μl samples were plated on Whatman GF/A or GF-83 filters and immediately put into one of the 25 slots of a Teflon container (Matthaei, 1971) standing in ice-cold 10% (w/v) trichloroacetic acid. Teflon containers with the samples were then transferred for washing in this order: 10min, 90°C, 5% (w/v) trichloroacetic acid; 2×1min, room temperature, 5% trichloroacetic acid; 2×1min, ethanol–ether (1:1, v/v); 2×1min ether. Filters were dried under an i.r. lamp.

Assay of radioactivity. Dried filters were placed into scintillation vials containing 2–5ml of the SLT-31 scintillation mixture (TESLA. Přemyšlení, Czechoslo-

vakia) and the radioactivity was counted in a Packard Tri-Carb scintillation spectrometer (efficiency for ^3H 25.1%) or in the NZB 315 Liquid-Scintillation Counter (TESLA, Přemyšlení, Czechoslovakia) (efficiency for ^{14}C 76%, for ^3H 13.4%).

Chemical determinations. Protein and RNA were determined by the method of Warburg & Christian (1942). For the quantitative determination cholesteryl 14-methylhexadecanoate in enzymes and extracts of enzymes the method of Hradec (1968) was used.

RESULTS

Cholesteryl 14-methylhexadecanoate contents in elongation factors. The relative contents of the ester increased during the purification of both transfer factors from rat liver. However, no exact correlation was found between the specific activity and relative cholesteryl 14-methylhexadecanoate contents during the whole course of purification. For instance, the relative content of cholesteryl 14-methylhexadecanoate in translocase after Sephadex G-200 chromatography was lower than that in the less purified fraction obtained by ammonium sulphate precipitation. However, there was a rough proportionality between increased relative cholesteryl 14-methylhexadecanoate contents and the specific activity of both elongation factors at the ultimate steps of purification. A typical result with rat liver enzymes is given in Table 1.

Very similar results were obtained with both elongation factors from human tonsils. However, some differences were found indicating that these two types of starting material are different as far as the content of cholesteryl 14-methylhexadecanoate is concerned.

All fractions obtained during the purification of elongation factors from human tonsils contained much larger relative quantities of the ester than corresponding preparations from rat liver. On their final purification steps, tonsil enzymes had almost twice as much ester as similar fractions from rat liver. Unlike rat liver transfer factors, no increase of relative ester contents was found in the ammonium sulphate precipitate of both enzymes when compared with the postmicrosomal supernatant although the specific activity increased about fivefold after this step. Results with human elongation factors are demonstrated in Table 2.

Activity of extracted binding enzyme. Adsorption of the purified binding enzymes with subsequent freeze-drying had apparently no effect on the activity of such preparations. After elution from DEAE-cellulose such enzymes had the same activity as preparations not treated in this way.

As revealed by the binding assay, extracted preparations of transferase I from rat liver showed a decreased activity when compared with enzymes freeze-dried only. The different organic solvents extracted different quantities of cholesteryl 14-methylhexadecanoate from a given enzyme preparation. Enzymes extracted with different organic solvents had lower activities than the original enzyme. Iso-octane extracted the highest quantity of cholesteryl 14-methylhexadecanoate from freeze-dried preparations of the binding enzyme (85–95% of the total amount present, five different batches). These enzymes retained very low residual activities. On the other hand, all other organic solvents used for the extraction were less active. Ethyl ether extracted only about 40–60% of the total ester present in enzyme preparations and the enzyme activity of these preparations (four batches) was decreased to about 50% of the original value. Results obtained with one particular batch of the binding enzyme extracted with various solvents are given in Table 3.

Almost all extracted enzyme preparations could be fully reactivated by the addition of pure cholesteryl 14-methylhexadecanoate in quantities corresponding to those that had been extracted from a given enzyme preparation (Table 3).

Table 1. *Relative purification and cholesteryl methylhexadecanoate contents of fractions obtained during the purification of peptide elongation factors from rat liver*

Both enzymes were purified by the method of Bermek *et al.* (1970) and Bermek & Matthaei (1970*a*). Values for relative purification are based on results in polymerization assays. Cholesteryl methylhexadecanoate contents were determined by the method of Hradec (1968) and are given in nmol/mg of protein.

		Relative purification		Cholesteryl methylhexadecanoate contents	
No.	Fraction	Transferase I	Transferase II	Transferase I	Transferase II
II	Postmicrosomal supernatant	1		0.135	
III	35–70%-satd.-$(NH_4)_2SO_4$ precipitate	5		0.635	
IV	Sephadex G-200 eluate	38	22	1.300	0.525
V	Sepharose 4B or DEAE-cellulose eluate	108	81	4.470	2.210

Table 2. *Relative purification and cholesteryl methylhexadecanoate contents of fractions obtained during the purification of peptide elongation factors from human tonsils*

Both enzymes were purified by the method of Bermek *et al.* (1970) and Bermek & Matthaei (1970*a*). Values for the relative purification are based on results of polymerization assays. Cholesteryl methylhexadecanoate contents were determined by the method of Hradec (1968) and are given in nmol/mg of protein.

No.	Fraction	Relative purification		Cholesteryl methylhexadecanoate contents	
		Transferase I	Transferase II	Transferase I	Transferase II
II	Postmicrosomal supernatant	1		0.492	
III	35–70%-satd.-$(NH_4)_2SO_4$ precipitate	4.8		0.472	
IV	Sephadex G-200 eluate	40	25	2.98	1.40
V	Sepharose 4B or DEAE-cellulose eluate	115	87	8.00	3.56

Table 3. *Effect of the extraction with various organic solvents on the activity of rat liver transferase I in the binding assay*

Enzymes of the same batch were extracted for 2 h with the solvents indicated. Incubation mixtures contained 1.25 μg of enzyme fraction V proteins and all other components described for the standard binding assay. Cholesteryl methyhexadecanoate contents in extracts are expressed as nmol extracted/mg of protein. Cholesteryl methylhexadecanoate (ester) was added to extracted enzymes in quantities replacing exactly the amounts removed by the extraction.

Solvent	Cholesteryl methylhexadecanoate extracted (nmol/mg of protein)	[^{3}H]Phenylalanine bound (pmol)	
		Ester extracted	Ester replaced
Ethyl ether	2.20	5.7	11.8
Chloroform	3.58	3.9	13.8
Iso-octane	4.26	1.7	11.0
Carbon tetrachloride	3.08	4.6	11.6
Benzene	2.06	5.8	11.3
Control value (unextracted enzyme)	—	11.6	

Essentially the same results were obtained in the binding assay with extracted binding enzymes isolated from human tonsils.

Similarly in peptidization experiments in which extracted binding enzymes were used together with non-extracted translocases, variable decreases of the enzymic activity were found with enzymes extracted with different organic solvents. Enzyme preparations could be again fully reactivated by the addition of cholesteryl 14-methylhexadecanoate in quantities corresponding to those extracted. Higher or lower amounts of this compound were less effective. Moreover, a direct proportionality was found between the quantity of cholesteryl 14-methylhexadecanoate extracted from a given enzyme preparation and the decrease of its activity (Fig. 1). Saturating quantities of extracted binding enzymes in the peptidization assay were considerably higher than those of non-extracted preparations (Fig. 2).

Results obtained with extracted human transferase I preparations in the peptidization assay were very similar to those described with the binding assay. Different organic solvents caused various decreases of the enzymic activity and a complete reactivation could be obtained after replacement of the quantities of cholesteryl 14-methylhexadecanoate extracted (Table 4).

Activity of extracted translocases. Unlike the binding enzyme, translocase was apparently severely damaged by both the adsorption on DEAE-cellulose and the subsequent freeze-drying. Preparations of translocase treated in this way showed on the average only about 40–50% of the activity of the same preparations not subjected to DEAE-cellulose treatment and freeze-drying. Moreover, sucrose had apparently a protective effect on translocase activity. Preparations of translocase treated with DEAE-cellulose in standard buffer without sucrose lost almost all of their original activity.

In peptidization experiments with non-extracted binding enzymes together with extracted translocases, results were very similar to those obtained with extracted transferase I. Different organic

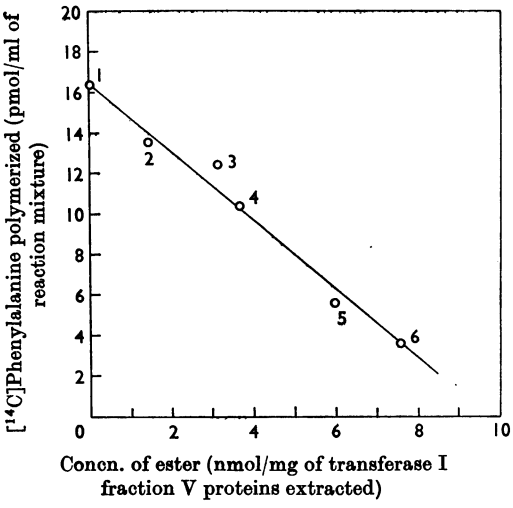

Fig. 1. Relation between the quantity of cholesteryl methylhexadecanoate extracted and the residual activity of extracted transferase I in the polymerization assay. Point (1) represents the activity of control non-extracted binding enzyme of human tonsils, the other enzyme preparations of the same batch were extracted with (2) *n*-pentane, (3) ethyl ether, (4) toluene, (5) benzene and (6) chloroform. The values represent the amount of polymerization in 30 min at 37°C.

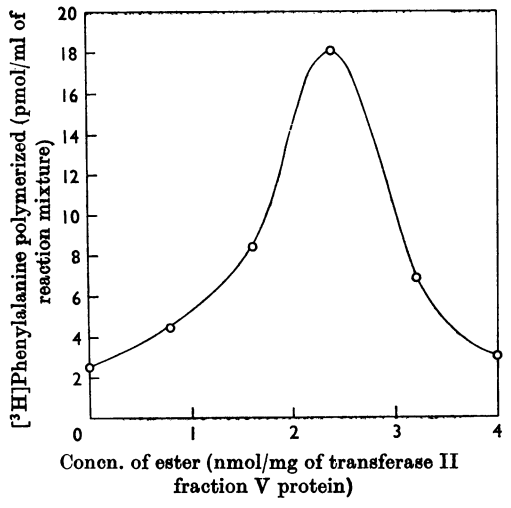

Fig. 2. Effect of different doses of cholesteryl methylhexadecanoate on the activity of extracted rat liver translocase in the polymerization assay. The enzyme was extracted with iso-octane (2.12 nmol of cholesteryl methylhexadecanoate/mg of protein extracted) and tested in the standard polymerization assay together with unextracted rat liver binding enzyme. In control mixtures containing both non-extracted transferases 17.6 pmol of [^{3}H]phenylalanine was polymerized.

solvents again extracted different quantities of cholesteryl 14-methylhexadecanoate from a given preparation of translocase from rat liver. Moreover, such extracted enzymes showed variable decreases of the enzymic activity as shown in Table 5.

It was again possible to restore most of the translocase activity by the addition of ester in quantities corresponding exactly to those extracted. In contrast with the binding enzyme, translocases extracted with chloroform (five different batches) could not be reactivated by cholesteryl 14-methylhexadecanoate probably due to the enzyme denaturation. Optimum amounts of the ester were required for complete reactivation (Fig. 2).

A direct proportionality exists between the quantity of cholesteryl 14-methylhexadecanoate extracted from a given enzyme preparation and the decrease in enzymic activity of such translocases (Fig. 3).

Similarly as with extracted transferase I, higher quantities of extracted translocases were required for maximum incorporation compared with non-extracted enzyme.

Results obtained with extracted translocases isolated from human tonsils were not different from those described with rat liver translocases. Again a decrease of the enzyme activity was found with extracted translocases and their original activity was fully recovered by replacement of pure cholesteryl 14-methylhexadecanoate in the amounts extracted.

Polymerization with both extracted transfer factors. Incubation mixture containing both extracted transfer factors showed decreased polyphenylalanine synthesis when compared with systems composed of non-extracted enzymes. However, the final value was not a summation of the effect of extraction on both individual enzymes. In such systems, polyphenylalanine synthesis was limited rather by the residual activity of the less-active transfer factor.

A complete recovery of the original activity could again be obtained in these systems containing both extracted elongation factors by the addition of appropriate amounts of cholesteryl 14-methylhexadecanoate.

Essentially the same results were obtained with factors isolated from both rat liver and human tonsils. Results of experiments with human elongation factors are given in Table 6.

DISCUSSION

Unlike most aminoacyl-tRNA synthetases (Hradec & Dušek, 1969), the transfer enzymes appear to be very sensitive to freeze-drying. This

Table 4. *Effect of the extraction with various organic solvents on the activity of human tonsil transferase I in the polymerization assay*

Samples of the same batch were extracted for 2h with the solvents indicated. Incubation mixtures contained 1.95μg of transferase I fraction V proteins, saturating quantities of non-extracted translocase fraction V (12.5μg) and all other components described for the standard polymerization assay. Cholesteryl methylhexadecanoate contents in extracts are given in nmol extracted/mg of enzyme-fraction proteins. In reactivation experiments, cholesteryl methylhexadecanoate (ester) was added to extracted enzymes in quantities replacing exactly the amounts removed by the extraction.

Solvent	Cholesteryl methylhexadecanoate extracted (nmol/mg of protein)	[^{14}C]Phenylalanine polymerized (pmol)	
		Ester extracted	Ester replaced
n-Pentane	0.89	16.50	20.65
Ethyl ether	3.00	8.65	19.25
Toluene	3.42	10.72	19.20
Chloroform	5.00	4.95	20.40
Control value (unextracted enzymes)	—	20.10	

Table 5. *Effect of the extraction with various organic solvents on the activity of rat liver translocases in the polymerization assay*

Samples of the same batch were extracted for 2h with the solvents indicated. Incubation mixtures contained saturating quantities of non-extracted rat liver transferase I fraction V (3.25μg), 9.5μg of transferase II fraction V and the other components for the standard polymerization assay. Cholesteryl methylhexadecanoate contents in extracts are given in nmol extracted/mg of enzyme fractions proteins. Cholesteryl methylhexadecanoate (ester) was added to extracted enzymes in quantities supplementing exactly the amounts extracted.

Solvent	Cholesteryl methylhexadecanoate extracted (nmol/mg of protein)	[^{3}H]Phenylalanine polymerized	
		Ester extracted	Ester replaced
Benzene	1.39	7.50	15.25
Ethyl ether	1.12	8.60	15.05
Isopropyl ether	0.87	11.85	14.70
Iso-octane	2.25	0.15	14.50
Control value (unextracted enzyme)	—	14.80	

fact presented great practical difficulties in our work. In the preliminary experiments (Hradec & Dušek, 1968*a*) in which transfer factors were purified by the method Gasior & Moldave (1965), Klink *et al.* (1967) or Felicetti & Lipmann (1968) even transferase I showed a low stability and most of its activity was lost after adsorption of purified enzymes on DEAE-cellulose with subsequent freeze-drying. No losses of the binding-enzyme activity were found, however, with enzymes prepared by the present procedure (Bermek *et al.* 1970; Bermek & Matthaei, 1970*a*). This apparent difference in the stability of binding enzymes purified by different techniques may be due to the fact that the former procedures use the pH 5 supernatant as the starting material whereas in the method of Bermek & Matthaei (1970*a*) postmicrosomal supernatant is the starting material. It seems possible that the binding enzyme which is stable only in a rather narrow range of pH (McKeenan & Hardesty, 1969) may be damaged by the acidification of the cell sap and thus may become less resistant towards freeze-drying. However, it was not possible to prevent large losses of translocase activity during freeze-drying although these losses were considerably smaller with enzyme prepared by the method of Bermek & Matthaei (1970*a*).

The direct proportionality between the decrease of the cholesteryl 14-methylhexadecanoate content and the loss of enzyme activity in extracted-elongation-factor preparations indicates that the presence of this compound in the molecule of these enzymes is essential for their function. The lack of such a correlation between increased specific activity and ester content of enzyme fraction proteins during the early steps of purification may be due to the presence of less cholesteryl methylhexadecanoate-containing proteins and eventually free cholesteryl 14-methylhexadecanoate. Such results were also obtained in the purification of some aminoacyl-tRNA synthetases (Hradec & Dušek, 1969).

The relative content of cholesteryl 14-methylhexadecanoate in the final preparations of transfer enzymes is significantly lower than that found in most aminoacyl-tRNA ligase preparations (Hradec & Dušek, 1969). The reason for the lower relative content of the ester in elongation enzymes may be that these factors were less purified than ligases used in our previous study. Even larger differences in cholesteryl methylhexadecanoate contents between the preparations of different aminoacyl-tRNA synthetases have been found (Hradec & Dušek, 1969). Further, structural differences between elongation factors and amino acid-activating enzymes may be involved. Such differences may also be the reason why some solvents that very effectively extract cholesteryl methylhexadecanoate from elongation factors (e.g. iso-octane) are not suitable for the extraction of larger quantities of this compound from aminoacyl-tRNA synthetases (Hradec & Dušek, 1969).

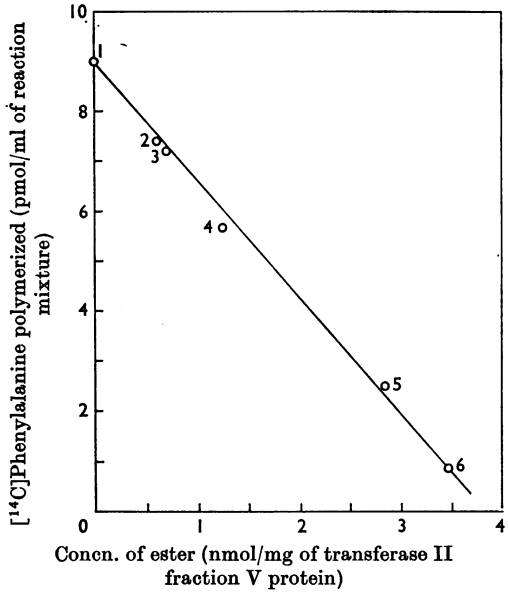

Fig. 3. Relation between the quantity of cholesteryl methylhexadecanoate extracted and the residual activity of extracted transferase II in polymerization assay. Point (1) represents the activity of control unextracted translocase of human tonsils, the other enzyme preparations of the same batch were extracted with (2) *n*-pentane, (3) carbon tetrachloride, (4) toluene, (5) ethyl ether and (6) iso-octane.

Although the present results seem to support the opinion that cholesteryl 14-methylhexadecanoate is essential for processes in normal peptide elongation, the function of this compound is unknown. The relatively low contents of cholesteryl 14-methylhexadecanoate in both aminoacyl-tRNA synthetases and elongation factors may indicate that this compound has a catalytic rather than stoicheiometric function.

Both elongation enzymes probably contain more than one active centre since they apparently catalyse several different reactions. The binding enzyme apparently combines first with GTP (Shorey, Ravel, Garner & Shive, 1969), this complex reacts with aminoacyl-tRNA, and this ternary complex may then ultimately be attached to decoding sites on the ribosome (Swan *et al.* 1969). Further, there seems to be a regulatory site for the binding of nucleotides (Lin, McKeenan, Culp & Hardesty, 1969). The translocase, on the other hand, binds GTP, has guanosine triphosphatase

Table 6. *Polyphenylalanine synthesis with both extracted transfer factors from human tonsils*

Both elongation factors from the same batch were extracted for 2h with the solvents indicated. Incubation mixtures contained 2.10μg of transferase I fraction V, 9.08μg of translocase fraction V and the other components of the standard assay mixture. The residual enzyme activity is expressed as a percentage of the control value, tested in the polymerization assay with the other non-extracted enzyme. Cholesteryl methylhexadecanoate (ester) was replaced in quantities replacing the amounts extracted from both transferases.

Extracting solvent		Residual activity		[14C]Phenylalanine polymerized (pmol)	
Transferase I	Transferase II	Transferase I	Transferase II	Ester extracted	Ester replaced
Ethyl ether	Ethyl ether	73	65	4.34	8.52
n-Pentane	Iso-octane	80	12	1.25	8.35
Chloroform	Ethyl ether	28	60	2.54	8.65
Toluene	Ethyl ether	32	63	2.30	9.30
Isopropyl ether	Iso-octane	85	12	1.78	8.45
Ethyl ether	Isopropyl ether	65	80	4.00	9.05
Benzene	Iso-octane	35	6	0.41	8.24
Control value (unextracted enzymes)		100	100		8.48

activity and is involved in the translocation of peptidyl-tRNA (Moldave, Galasinski, Rao & Siler, 1969). It would be interesting to know whether cholesteryl methylhexadecanoate participates in all these reaction steps or whether its function is limited to one or more individual steps.

Our thanks are due to Mrs Z. Tuháčková, Mrs M. Čechová, Miss J. Maierová and Mrs B. Schmelz for excellent technical assistance. This work was in part supported by the Deutsche Forschungsgemeinschaft.

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