Peptide Elongation Enzymes in Tumor Cells and Mouse Liver*

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ABSTRACT: *In vitro* protein synthesis in mouse Ehrlich and rat Novikoff ascites tumors has been investigated with respect to peptide elongation. The supernatant enzymes involved in peptide elongation in both tumor systems can be resolved into two complementary fractions, T_1 and T_2, by Sephadex G-200 gel filtration. The crude ribosomal preparation of Ehrlich ascites cells contains saturating amounts of T_1 and T_2. T_1 can be removed from the ribosomes by deoxycholate treatment. Removal of T_2 required consecutive washings of the ribosomes with deoxycholate and NH_4Cl. T_1 catalyzes the binding of [^{3}H]Phe-tRNA to the poly(U)–ribosome complex. The binding reaction requires GTP; GDPCP and GDP are not effective. T_2 is required in the extension of the peptide chain. Interchangeability of the tumor enzymes with those obtained from normal mouse liver has been studied. Combinations of T_1 and T_2 from heterologous sources were equally effective as those from homologous sources in catalyzing the polymerization of phenylalanine. Ribosomes prepared from Ehrlich ascites tumor were equally effective as those prepared from mouse liver in supporting peptide elongation in the presence of any combinations of T_1 and T_2 prepared from these three sources.

In normal mammalian tissues and microbial systems, at least two complementary supernatant enzymes are required for peptide elongation in protein synthesis (Lipmann, 1969). The mechanism of protein synthesis in tumor cells, however, has not been studied extensively. Previous attempts to demonstrate the presence of complementary peptide elongation enzymes in Novikoff ascites cells yielded no definitive results (Griffin, 1967). It is not certain, therefore, whether there are substantial differences in protein synthesis between normal mammalian tissues and malignant tumor cells.

The present communication reports the fractionation and characterization of complementary supernatant enzymes for peptide elongation of mouse Ehrlich and rat Novikoff ascites tumors. The interchangeability of the tumor enzymes with those obtained from normal mouse liver has also been studied.

Methods

Materials. [^{3}H]Phenylalanine was obtained from Schwarz Co. Poly(U), ATP, and GTP were purchased from Miles Co. [r-^{32}P]GTP was prepared according to Conway and Lipmann (1964).

Tumor Strains. Mouse Ehrlich ascites tumor was obtained from Dr. P. Zamecnik, Harvard Medical School, and rat Novikoff ascites tumor from Arthur D. Little Co. The cell lines were maintained in this laboratory by a 7-day serial transplantation in 10-week-old Charles River white mice and 120- to 180-g albino rats, for Ehrlich and Novikoff ascites, respectively.

Preparation and Fractionation of Cell Homogenate. Tumor cells collected from the peritoneal cavities of mice or rats were washed twice in five volumes of distilled water and twice in a medium consisting of 0.02 M potassium phosphate (pH 7.4), 0.025 M KCl, 0.035 M $KHCO_3$, 4 mM $MgCl_2$, and 0.28 M sucrose (medium A). Each washing was followed by centrifugation at 5000*g* for 5 min. The final packed cells were suspended in three volumes (w/v) of medium A and disrupted by sonication (Biosonik II, Bronwill Scientific) at maximum intensity for 20 sec. Cell homogenates were centrifuged at 27,000*g* for 30 min. The supernatant liquid was collected by decantation and subjected to high-speed centrifugation (78,000*g*) for 3 hr. The pellet and the supernatant were used for the preparation of ribosomes and enzymes, respectively.

Preparation of Ribosomes. The microsomal pellets derived from 100 g of Ehrlich ascites cells were suspended in 100 ml of medium A by the use of a hand homogenizer. Sodium deoxycholate solution (5%) was added to a final concentration of 0.45% and the suspension was stirred in ice for 20 hr. After centrifugation at 12,000*g* for 10 min to remove the aggregates, 5 ml of the supernatant was layered on a discontinuous sucrose gradient consisting of 8 ml of 0.5 M sucrose over 8 ml of 1.5 M sucrose both in 0.05 M Tris-HCl (pH 7.4) and 0.004 M $MgCl_2$. The tubes were centrifuged in a Beckman 30 rotor at 72,000*g* for 20 hr and the pellets were resuspended in 40 ml of a buffer mixture containing 0.05 M Tris-HCl (pH 7.4), 0.01 M $MgCl_2$, 0.5 M NH_4Cl, and 0.28 M sucrose. The suspension was stirred in ice for another 20 hr. After a preliminary centrifugation the ribosomes were sedimented for 20 hr through another discontinuous sucrose gradient consisting of 8 ml of 0.5 M sucrose over 8 ml of 1.0 M sucrose both in 0.05 M Tris-HCl (pH 7.4), 0.01 M $MgCl_2$, and 0.5 M NH_4Cl. The transparent pellets were resuspended in 0.05 M Tris-HCl (pH 7.4), containing 0.004 M $MgCl_2$, and 0.28 M sucrose and stored in Dry Ice.

Preparation of Supernatant Peptide Elongation Enzymes. PRECIPITATION AT pH 5. The supernatant fraction obtained from high-speed centrifugation was brought to pH 5.2 by dropwise addition of 1 N acetic acid. The residue collected was dissolved in 20 ml of 0.05 M Tris-HCl (pH 7.4) containing 0.15 M KCl, 2 mM β-mercaptoethanol, and 0.1 mM EDTA (medium B) and dialyzed against the same buffer overnight. This pH 5 enzyme fraction was used for the preparation of aa-tRNA.

AMMONIUM SULFATE FRACTIONATION. The pH 5 supernatant was neutralized with 1 N KOH to pH 7.2 and fractionated

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with ammonium sulfate. The precipitate, formed between 33 and 68% saturation for Ehrlich ascites and between 40 and 70% saturation for Novikoff hepatoma and mouse liver, was collected and dissolved in 0.25 M Tris-HCl (pH 7.4), containing 2 mM β-mercaptoethanol. The enzyme preparations were then dialyzed for 4 hr against 200 volumes of the same buffer.

HYDROXYLAPATITE ADSORPTION CHROMATOGRAPHY. The protein concentration of the enzyme solution from ammonium sulfate fractionation was adjusted to 20 mg/ml. Hydroxylapatite suspension in 0.001 M NaCl (Bio-Gel HT, 0.28 g/ml of settled bed, Bio-Rad Laboratories, Richmond, Calif.) was added with stirring. The final ratio was 3.5 mg of gel/mg of protein. The stirring was continued for 1 hr, and the paste was collected by centrifugation. The paste was washed once with 0.05 M Tris-HCl (pH 7.4) and eluted three times with 0.25 M potassium phosphate (pH 6.8). β-Mercaptoethanol at a concentration of 2 mM was included in the washing and eluting media. The eluates were combined and concentrated to less than 10 ml by dialysis in 20% polyethylene glycol 6000 in medium B.

GEL FILTRATION. Sephadex G-200 (40–120 μ, 30–40-ml bed volume/g) was boiled for 5 hr and the fine particles were removed by repeated decantation. The remainder was packed at 4° to form a 2.5 × 80 cm column. The column was equilibrated with medum B and an upward flow at a constant flow rate of 18 ml/hr was maintained. The uniformity of packing and the void volume of the column were determined by Blue Dextran 2000. The concentrated protein solution from hydroxylapatite adsorption was passed through the column and eluted with medium B. The active fractions were pooled, concentrated by dialysis as described before, and stored in Dry Ice.

ION-EXCHANGE CHROMATOGRAPHY. Cellulose phosphate (0.86 mequiv/g) was washed successively in 1 N NaOH, H_2O, 0.1 M potassium phosphate (pH 7.0), and 0.01 M potassium phosphate (pH 7.0). The packed column (1.5 × 20 cm) was further washed with about 3 bed volumes of 0.01 M potassium phosphate (pH 7.0) containing 2 mM β-mercaptoethanol. The protein solution obtained from gel filtration or from hydroxylapatite adsorption was equilibrated with the same buffer by dialysis and then applied to the column. The column was washed with the same buffer until no more ultraviolet-absorbing material could be detected in the effluent. The enzyme protein was eluted by including 0.14 M KCl in the eluent. The active fractions were pooled, concentrated, and stored in Dry Ice.

Preparation of [³H]Phenylalanyl-tRNA. tRNA used in all experiments was isolated from Ehrlich ascites tumor cells. The isolation of tRNA and the preparation of [³H]Phe-tRNA were carried out as described previously (Li and Yu, 1969).

Assay Procedures. POLYPHENYLALANINE FORMATION. Phenylalanine polymerization was assayed by measuring the incorporation of radioactivity into hot trichloroacetic acid insoluble material. The reaction mixture consisted of the following: 0.06 M Tris-HCl (pH 7.4), 8 mM $MgCl_2$, 0.08 M NH_4Cl, 4 mM dithiothreitol, 0.8 mM GTP,[1] 25 μg of poly(U), 15 μg of [³H]Phe-tRNA (2100 cpm, 6.7 pmoles), 0.7 A_{260} unit of ribosomes, and supernatant elongation enzymes. The incubation was carried out in a final volume of 0.1 ml at 37° for 20 min. The reaction was stopped by the addition of 20 volumes of 5% trichloroacetic acid and heated for 15 min at 85°. The mixture was then transferred onto a Millipore filter disk and washed three times with 5% tricloroacetic acid and once with 95% ethanol. The disk was dried and the radioactivity was determined in Bray's solution (Bray, 1960) with a Packard liquid scintillation spectrometer, Model 3320.

BINDING OF aa-tRNA TO THE RIBOSOMES. Enzymatic binding activity was determined according to Nirenberg and Leder (1964). The reaction mixture was the same as that for polyphenylalanine formation, except dithiothreitol and T_2 were omitted and the amount of ribosomes used was doubled. Incubation was carried out at 37° for 20 min, after which 3 ml of a binding wash consisting of 0.05 M Tris-HCl (pH 7.4), 8 mM $MgCl_2$, and 0.08 M NH_4Cl was added to stop the reaction. The reaction mixture was immediately poured over a Millipore filter under gentle suction, followed by washing three times with a binding wash. The filter was dried and counted as described.

GTPase ACTIVITY. Hydrolysis of GTP was determined by the procedure of Conway and Lipmann (1964). The composition of the reaction mixture was similar to that for phenylalanine polymerization except for the omission of poly(U) and [³H]Phe-tRNA and substitution of 0.0586 mM [³²P]GTP for 0.8 mM GTP. The final volume of the incubation mixture was 0.5 ml and the reaction was conducted at 37° for 10 min.

Molecular Weight Determination by Sucrose Gradient Centrifugation. About 1.0 mg of each of the T_1 and T_2 enzymes in medium B was mixed, made up to 0.174 ml, and layered on a 4.2-ml preformed gradient consisting of 5–20% sucrose in medium B. The tubes were centrifuged in an SW-39 rotor for 12 hr. Fractions of approximately 0.14 ml were collected and assayed for peptide elongation in the presence of excess amount of the complementary enzyme. Beef catalase (mol wt 250,000) and yeast alcohol dehydrogenase (mol wt 150,000) were used as references. Estimation of molecular weights of the T_1 and T_2 enzymes were made according to Martin and Ames (1961).

Results

Purification of Ribosomes. As shown in Table I, prolonged stirring of crude ribosomes in 0.45% sodium deoxycholate was very effective in removing endogenous mRNA and contaminating supernatant enzymes from the ribosomes, although T_2 activity was still appreciable. If is of interest that if the GTPase assay was used, this residual T_2 activity was almost beyond detection. Additional high salt treatment further reduced T_2 activity of the ribosomes to a low level. A summary of ribosome purification in terms of spectral ratio and yield is also given in Table I.

Resolution and Purification of the Supernatant Complementary Enzymes of Ehrlich Ascites Cells. The resolution of the supernatant enzymes is shown in Figure 1. The polyphenylalanine-synthesizing activity of the individual fractions was low; however, when the assays were carried out in the presence of a fraction near elution volume 250 ml, an activity peak was observed between elution volume 140 and 170 ml. A second peak was located between elution volume 210 and 280 ml when aliquots near elution volume 150 ml were included in the incubation mixture. The resolution pattern is quite similar to those obtained from normal mammalian tissues (Gasior and Moldave, 1965). As will be shown later, the first enzyme fraction is capable of binding [³H]Phe-tRNA to the ribosomes while the second is not. It appears justified to designate these fractions as T_1 and T_2, respectively,

[1] Abbreviation used that is not listed in *Biochemistry* 5, 1445 (1966), is: GDPCP, the analog of GTP, 5′-β-γ-methylene-GTP.

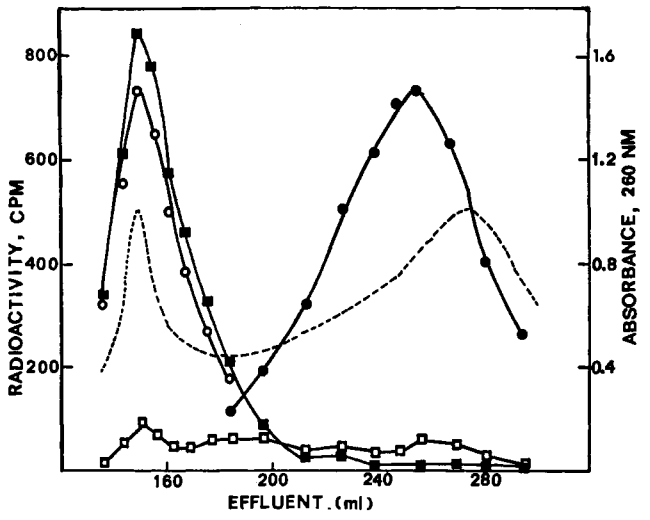

FIGURE 1: Resolution of the peptide elongation enzymes of Ehrlich ascites on Sephadex G-200. The hydroxylapatite eluate (200 mg of protein) was dialyzed, concentrated, and applied to a column of Sephadex G-200 equilibrated with medium B at 4°. No adverse effect was detected when the amount of protein applied was varied from 90 to 240 mg for this column which has a void volume of 151 ml and a total bed volume of 396 ml (2.5 × 80 cm). The column was eluted with medium B and fractions of 5 ml were collected. In each assay, the amount of the eluate was as follows: (□) 10 μl of various fractions alone; (●) 10 μl of the fractions assayed in the presence of 10 μl of the fraction eluted between 155 and 160 ml; (○) 10 μl of the fractions assayed in the presence of 10 μl of the fractions eluted between 255 and 260 ml; and (■) 5 μl of the fractions assayed for binding activity. The protein profile (A_{280}) is indicated by dashed lines. Other details of the assay procedure are as described in Methods.

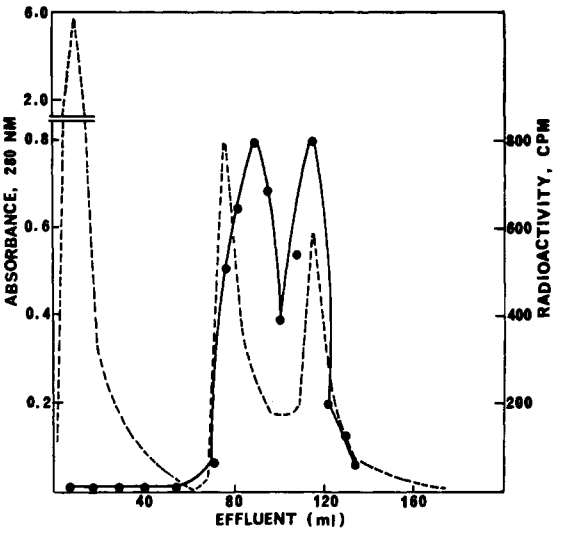

FIGURE 2: Cellulose phosphate chromatography of T_2. A pooled T_2 eluate from gel filtration was dialyzed in 0.01 M potassium phosphate (pH 7.0) containing 2 mM β-mercaptoethanol overnight. The resulting solution which contained 78 mg of protein was applied to a cellulose phosphate column (1.3 × 16 cm) preequilibrated with 0.01 M potassium phosphate (pH 7.0) containing 2 mM β-mercaptoethanol. The column was washed with the same buffer until no A_{280} could be detected. The enzyme was then eluted with 0.15 M KCl in the same buffer. Fractions of 3 ml were collected. Assays for polyphenylalanine formation were conducted according to the procedure stated in Methods with 5 μl of each fraction in the presence of excess T_1 (15 μg). Dashed line, absorbance. Solid line, radioactivity.

following the usage applied to normal mammalian enzyme systems.

The enzymes used in the present experiments were the pooled active fractions from Sephadex G-200 gel filtration. In some cases, the pooled T_2 fraction was further purified by phosphocellulose chromatography. Approximately 40% of the applied protein was washed out by the loading buffer. Elution of T_2 was achieved by including KCl at 0.15 M in the buffer. The elution pattern is shown in Figure 2. It is interesting to note that two T_2 peaks were eluted by the 0.15 M KCl eluent. The A_{280}/A_{260} values were about 1.8. In terms of specific activity, the ratio of the two peaks was approximately 1:2 with a purification of 1.7- and 3.5-fold, respectively. Total recovery of enzymatic activity was 28%. No difference in molecular weight, when measured by sucrose gradient centrifugation, could be detected between these two peaks. Two forms of T_2 have been reported in yeast (Albrecht *et al.*, 1970).

T_2 can also be purified by DEAE-cellulose column chromatography. The column was in 0.01 M Tris-HCl (pH 7.8) containing 2 mM β-mercaptoethanol. A 0–0.3 M KCl gradient in the same buffer was used as the eluent and T_2 activity emerged as a broad peak centering at 0.15 M KCl.

When the pooled T_1 fraction was applied to ion-exchange chromatography, no T_1 activity was detectable in the eluate. The reason for the instability of T_1 during ion-exchange chromatography remains obscure, although it has been shown that T_1 forms inactive aggregates when ionic strength of buffer falls below 0.1.

Characterization of T_1 Binding of [^{3}H]Phe-tRNA to the Ribosomes. When fractions from Sephadex G-200 gel filtration were analyzed for catalytic activity in the binding of [^{3}H]Phe-tRNA to the ribosomes, the active region coincided with the T_1 activity peak in Figure 1. In addition to T_1, the binding reaction requires GTP, poly(U), and Mg^{2+}, as shown in Table II.

TABLE I: Purification of Ribosomes: Removal of Endogenous mRNA and Supernatant Enzymes from Ribosomes.[a]

Purification Step	A_{260} (%)	A_{260}/A_{280}	Incubn System	[^{3}H]Phe-tRNA Polymerized (cpm)
A. Crude pellet	5300 (100)	1.44	Complete	768
			−Poly(U)	780
			−T_1, T_2	770
B. Deoxycholate treated	1823 (34)	1.78	Complete	760
			−Poly(U)	108
			−T_2	530
			−T_1, T_2	62
C. NH_4Cl treated	1334 (25)	1.58	Complete	725
			−Poly(U)	58
			−T_2	95
			−T_1, T_2	14

[a] Reactions for polymerization were carried out as described in Methods except for the modification indicated. Amount for the supernatant enzymes, whenever used, was: T_1, 10 μg, and T_2, 25 μg. The same amount of ribosomes (0.7 A_{260} unit) was used in all experiments.

TABLE II: Requirements for the Binding of aa-tRNA to the Ribosomes.[a]

Incubation System	[^{3}H]Phe-tRNA Bound (cpm)
Complete	850
$-T_1$	30
−Ribosomes	0
−GTP	35
−GTP + GDPCP	130
−GTP + GDP	133
−Poly(U)	34
$-Mg^{2+}$	0

[a] Assays were done according to the standard procedure specified in Methods unless otherwise indicated. The amount of T_1 used in each assay was 12 μg. The concentration of GDPCP and that of GDP, whenever used, was the same as that of GTP (0.8 mM).

Comparison of analogs of GTP in supporting the binding reaction is also given in Table II. When substituted for GTP, GDPCP, and GDP are only 10% as active in the binding reaction. Similar findings were reported for the reticulocyte system (Lin *et al.*, 1969). Inclusion of a sulfhydryl reagent, dithiothreitol, is without effect in the binding reaction.

Characterization of T_2. Without added T_2, less than 10% of the total bound [^{3}H]Phe-tRNA was hot trichloroacetic acid precipitable. When T_2 was added to the reaction mixture, hot trichloroacetic acid precipitable [^{3}H]Phe-tRNA rose to 57% of the total bound. GTPase activity associated with T_2 was assayed with increasing amounts of T_2. GTPase activity was approximately proportional to the added T_2 up to 10-μg/ml incubation mixture, and gradually plateaued with further increase in T_2 concentration.

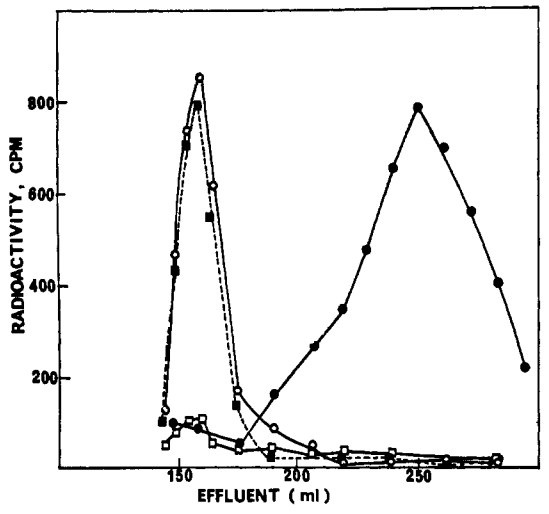

FIGURE 3: Gel filtration pattern of Novikoff tumor supernatant enzymes. The fraction obtained from hydroxylapatite (210 mg of protein) was applied to the same column used for the separation of Ehrlich ascites supernatant enzymes (Figure 1). Conditions used were the same as specified in the legend to Figure 1. Polymerization activity of each individual fraction alone (10 μl) (□). Polymerization activity in the presence of 5 μl of the fraction eluted between 155 and 160 ml: (●). Polymerization activity in the presence of 20 μl of the fraction eluted between 255 and 260 ml (○). Binding activity of the fractions assayed (5 μl) (■).

TABLE III: Complementary Effect of T_1 and T_2 Prepared from Various Sources in Phenylalanine Polymerization.

Supernatant Enzymes			[^{3}H]Phe-tRNA Incorporated (cpm) Source of Ribosomes	
Novikoff	Ehrlich	Mouse Liver	Mouse Liver	Ehrlich Ascites
T_1			50	46
T_2			0	0
$T_1 + T_2$			670	690
	T_1		52	49
	T_2		0	0
	$T_1 + T_2$		680	690
		T_1	45	50
		T_2	0	0
		$T_1 + T_2$	650	680
T_1	T_2		645	666
T_1		T_2	680	680
T_2	T_1		650	670
T_2		T_1	670	680
	T_2	T_1	660	650
	T_1	T_2	665	660

[a] The reactions were conducted according to the standard polymerization assay procedure described in Methods except that the supernatant enzymes and ribosomes used were varied as indicated. The amount of T_1 and T_2 used was: 10 and 25 μg for Ehrlich tumor, 9 and 25 μg for Novikoff tumor, and 10 and 28 μg for mouse liver, respectively.

In addition to the ribosomes and supernatant enzymes, the peptide elongation reactions required Mg^{2+}, NH_4^+, K^+, GTP, and dithiothreitol. The optimal concentrations of the cofactors in millimolar are the following: Mg^{2+}, 8; NH_4^+, 80; K^+, 100; and GTP 0.8. The stimulatory effect of dithiothreitol approaches a plateau at about 4 mM.

Resolution of the Supernatant Enzymes of Novikoff Ascites Cells. As shown in Figure 3, the supernatant enzymes of Novikoff ascites cells were resolved into two complementary fractions. The resolution pattern from G-200 Sephadex filtration was essentially the same as that of Ehrlich ascites enzymes (Figure 1).

Molecular Weights of the Supernatant Enzymes. The molecular weight of T_2 from Ehrlich, Novikoff ascites cells, and mouse liver was found to be 76,000 by sucrose gradient centrifugation. The molecular weight of T_1 of Ehrlich ascites is slightly higher (190,000) than that of mouse liver and Novikoff ascites (170,000). For both tumor cells, two T_1 peaks were located in the gradient fractions: one was well defined from which the above value was calculated and the other value calculated from the second peak was 260,000 for both tumor cells. Multiple forms of T_1 were obtained from rat liver (Schneir and Moldave, 1966) and yeast (Albrecht *et al.*, 1970).

Interchangeability of the Corresponding Enzymes of Tumors and Mouse Liver. Table III shows the polymerization of phenylalanine catalyzed by various combinations of T_1 and T_2

obtained from Ehrlich and Novikoff ascites cells and mouse liver. The data indicate that the T_1 and T_2 enzymes from these three sources are freely interchangeable, *i.e.*, any combination of T_1 and T_2 from homologous or heterologous sources are equally effective in catalyzing the polymerization of phenylalanine.

Interchangeability of Ribosomes from Ehrlich Ascites Cells and Mouse Liver. In the experiments described in Table III, ribosomes prepared from Ehrlich ascites cells and from mouse liver were used. Comparison of data in Table III indicates that there is no difference between ribosomes obtained from mouse liver and Ehrlich ascites cells in supporting the polymerization of phenylalanine.

Discussion

Proteins synthesized by tumor cells could be quantitatively as well as qualitatively different from those synthesized by normal mammalian tissues (Campbell, 1958). It is not certain, however, whether the mechanism of protein synthesis in tumor cells differs from that in normal tissues. In their pioneering work on protein synthesis, Hoagland *et al.* (1958) and Hecht *et al.* (1959) showed that protein synthesis in cell homogenates of Ehrlich ascites tumor required tRNA, ribosomes, ATP, GTP, Mg^{2+}, and the 100,000*g* supernatant fraction. Using an *in vitro* system from mouse leukemia L-1210, Ochoa and Weinstein (1964) showed that addition of poly(U) could result in an approximately tenfold increase in phenylalanine incorporation into polypeptide. These results indicated that the overall process of protein synthesis in tumor cells may be similar to that in other systems. Studies on the Novikoff ascites tumor, on the other hand, showed that the supernatant enzymes for peptide elongation cannot be resolved into complementary fractions (Griffin and O'Neal, 1962; O'Neal and Griffin, 1963; Griffin, 1967). Furthermore, no requirement of exogenous GTP could be demonstrated in this system for peptide elongation.

In the present studies the supernatant peptide elongation enzymes of both the mouse Ehrlich and rat Novikoff ascites tumors were resolved into complementary fractions. The key problem appears to be the contamination of supernatant enzymes in the ribosomal preparation. As shown in Table I, the crude ribosomal fraction required no additional supernatant enzymes in the formation of polyphenylalanine. After washing the ribosome fraction with deoxycholate, addition of T_1 alone could stimulate product formation, indicating that at this stage of purification, the ribosomes still contained substantial amount of T_2 activity. After further washing of the ribosomes with NH_4Cl, there was still residual T_2 activity on the ribosomes. At this stage of purification, however, addition of T_2 could result in severalfold increase in the formation of polyphenylalanine, thus providing a valuable system for detecting T_2 activity. It is of interest to note that Krisko *et al.* (1969) postulated that ribosomes of Novikoff ascites tumor may be saturated with T_2.

Unless otherwise indicated, washed ribosomes of mouse Ehrlich ascites cells were used throughout the present experiments. Repeated attempts to wash ribosomes from rat Novikoff ascites tumor, however, resulted in the formation of inactive aggregates. The reason for this observation remains obscure at the present.

In the present studies, the T_1-catalyzed binding of [^{3}H]Phe-tRNA required GTP. Analogs of GTP, GDPCP, and GDP, were only slightly active. In other systems, it was shown that GDPCP and GDP were equally effective as GTP in promoting the binding of aa-tRNA to the ribosomes (Skoultchi *et al.*, 1969; Moldave *et al.*, 1969; Lengyel and Söll, 1969). A recent report on the reticulocyte system, however, agrees with our findings in the tumor cells (Lin *et al.*, 1969). It still remains to be answered, however, whether GTP hydrolysis occurs with the binding of aa-tRNA to the ribosomes in the tumor system.

In the Ehrlich ascites system, the T_1- and T_2-catalyzed peptide elongation reactions showed little differences from those catalyzed by enzymes prepared from normal mammalian tissues. The complementary effect of T_1 and T_2 prepared from heterologous tumors indicates that corresponding enzymes prepared from the two tumor strains could functionally substitute for each other. Furthermore, the tumor enzymes could replace the corresponding ones obtained from normal mouse liver in peptide elongation. Ribosomes prepared from Ehrlich ascites cells were equally effective in supporting the peptide elongation reactions as those prepared from mouse liver with any combination of T_1 and T_2 enzymes. This suggests that the ribosomal enzyme of this tumor, peptidyl transferase, is functionally interchangeable with that of mouse liver. It appears reasonable, therefore, to conclude that there is no evidence for any major differences in the peptide elongation enzymes between tumor cells and normal mammalian tissue.

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