THE INHIBITION OF ELONGATION FACTOR 1 ACTIVITY BY HEPARIN

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Received August 11,1976

SUMMARY: The effect of the mucopolysaccharide heparin on elongation factor 1 (EF-1) from embryos of the brine shrimp Artemia salina was investigated. Heparin was found to be a potent inhibitor of the purified enzyme in binding aminoacyl-tRNA to ribosomes and had a comparable effect on polyuridylic acid dependent polyphenylanine synthesis. However, no effect on the binding of GTP to EF-1 or the ability of the factor to form a ternary complex with GTP and aminoacyl-tRNA was observed, suggesting that heparin interferes with the ribosome-attachment site on the ternary complex. In addition EF-1 bound to heparin-Sepharose gels and such gels could be used to partially purify the factor from post-ribosomal supernatant fractions.

Introduction

Recent studies have established that heparin is a potent inhibitor of initiation of mammalian protein synthesis in cell free systems (1,2). Moreover, Waldman et al. have reported that rabbit reticulocyte initiation factors may be purified by affinity chromatography using columns of heparin bound to Sepharose (3). To establish whether the action of heparin on protein synthesis is limited to the initiation step, the effect of heparin of elongation factor 1 (EF-1) activity was investigated. This communication presents evidence that heparin is also a potent inhibitor of EF-1 activity. In addition heparin covalently bound to Sepharose can be employed to partially purify EF-1 from crude cell extracts.

Materials and Methods

The sodium salt of heparin was purchased from Serva. Purified phenylalanine specific tRNA (Boehringer) was charged with [^{3}H] phenylalanine (Amersham) using a partially purified mixture of

aminoacyl-tRNA synthetases from E.Coli (4). The product had a specific activity of 1200 cpm/pmol at 30 per cent counting efficiency [γ ^{32}P] GTP (14.6 Ci/mmole) and [^{3}H] GTP (10.5 Ci/mmole) were obtained from Amersham.

Preparation of EF-1, Ribosomes and Post-ribosomal Supernatant

Cysts of the brine shrimp Artemia salina (Metaframe, San Fransisco, California) were used for the purification of EF-1, as reported elsewhere (5,6). Salt-washed 80S ribosomes and post-ribosomal supernatants from Artemia were prepared as described (6).

Assay of EF-1 Activity and Polyphenylalanine Synthesis

EF-1 activity was determined by the binding aminoacyl-tRNA to 80S ribosomes as described previously (6). Briefly, assay mixtures (50µl) contained the following components: 20mM Tris-HCl, pH 7.5, 100 mM KCl, 10 mM 2-mercaptoethanol, 5 mM Mg $(OAc)_2$, 1.0 A_{260} units of salt washed ribosomes, 025 mM GTP, 10 µg of polyuridylic acid and 10-15 pmol of [^{3}H] phenylalanine-tRNA. The amount of EF-1 added is indicated in the legends to the tables and figures. Assay mixtures were incubated for 20 minutes at 30° and processed for radioactivity measurements as described (6). Polyphenylalanine synthesis was measured in a similar assay mixture except that 0.3 A_{260} units of ribosomes were used and the amount of [^{3}H] Phe-tRNA was increased to about 25 pmol. After incubation for 30 minutes at 30° 1 ml of 5% (v/v) of trichloracetic acid was added to each reaction mixture and the mixtures were incubated for an additional 20 minutes at 90°. The acid precipitates were brought to 0°, filtered through Whatman (GF/c) glass fiber filters, washed with three 3 ml portions of ice-cold 5% trichloracetic acid, dried and counted at 25 percent counting efficiency in a Packard Model 2450 liquid scintillation spectrometer.

Preparation of Heparin-Sepharose 4B Gel

Heparin was covalently bound to CNBr-activated Sepharose 4B (Pharmacia) as described by Waldman et al.(3). A control gel containing no heparin was prepared by reacting CNBr-activated Sepharose 4B with ethanolamine.

Chromatography of Post-ribosomal Supernatant and on heparin-Sepharose

A heparin-Sepharose column (18.5 x 2.0 cm) was equilibrated with a buffer containing 20 mM Tris-HCl, pH 7.5, 25 mM KCl, 10 mM 2-mercaptoethanol and 0.1 mM EDTA (Buffer A). Post-ribosomal supernatant (50 ml containing 1g protein) was dialyzed against Buffer A and applied to the heparin-Sepharose column. The column was washed with Buffer A until no more A_{280} absorbing material appeared in the eluate. EF-1 activity was then eluted at approximately 75 mM KCl after application of a linear gradient(250x250 ml) between 25 mM and 250 mM KCl in Buffer A. Five milliliter fractions were collected.

GTP Binding and Ternary Complex Formation

The binding of [γ^{32}P] GTP to EF-1 was determined by the conventional nitrocellulose filter method as previously described (6). Each assay mixture (50 µl) contained 2 µg of purified $EF\text{-}1_L$ and 1.6 x 10^{-5}M [γ^{32}P] GTP at a specific activity of 280 CPM/pmole. The formation of the aminoacyl-tRNA.EF-1.GTP ternary complex was measured

by the nitrocellulose filter assay as described by Jerez et al.(7). Each assay mixture (25 µl) contained 2 µg of EF-1, 40 pmol of phe-tRNA^{phe} and 8 µM [^{3}H] GTP. The amount of ternary complex was calculated by substracting the amount of [^{3}H] GTP retained on a nitrocellulose filter in the presence of phe-tRNA from the amount bound to the filter in its absence. Protein was determined by the Lowry method (8) using bovine serum albumin as a standard.

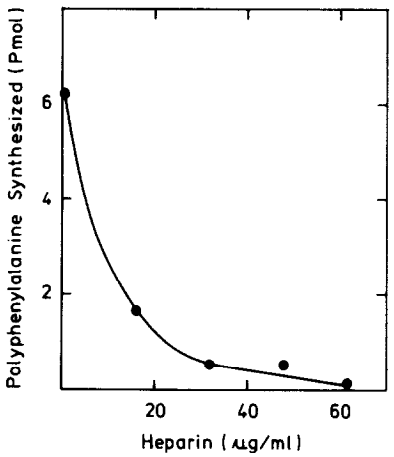

Fig. 1. The effect of heparin on polyphenylalanine synthesis. Each reaction mixture contained 43 µg of the post-ribosomal supernatant from Artemia cysts. For additional details see Materials and Methods.

Results

Post-ribosomal supernatants from dehydrated gastrulae (cysts) of the brine shrimp Artemia salina contain both EF-1 and elongation factor 2 (EF-2) and are capable of catalyzing the synthesis of polyphenylalanine when supplemented with ribosomes, polyuridylic acid and energy source (9). Addition of heparin at low concentration to such a system resulted in complete inhibition of polyphenylalanine synthesis (fig.1) . These results suggested that one of the elongation factors was directly effected by the presence of heparin. To examine this possibility I tested the effect of heparin on EF-1

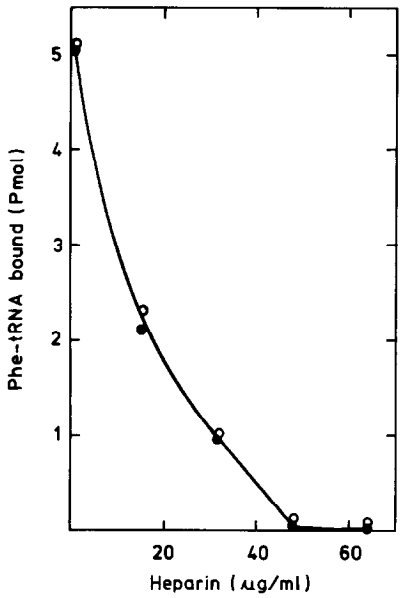

Fig. 2. The effect of heparin on the EF-1 dependent binding[^{3}H] Phe-tRNA to ribosomes. Each 50 µl assay mixture contained either 0.67 µg of purified EF-1_H (0) or 044 µg of purified ef-1_L (0). For addition details see Materials and Methods.

activity. Artemia embryos contain two forms of EF-1: (1) a high molecular weight complex (EF-1_H,Mr≈200,000) containing at least two different polypeptide subunits and present exclusively in cysts; (2) a basic low molecular weight polypeptide (Mr=53,000) which is found in the embryo after it developes into a free-swimming nauplius (5,6). Both forms of the factor have been highly purified and characterized as will be documented elsewhere (5,10). As seen fig.2 heparin completely inhibited both forms of EF-1 in their capacity to bind aminoacyl-tRNA to ribosomes. Identical heparin concentrations had similar effects on both aminoacyl-tRNA binding to ribosomes and polyphenylalanine synthesis, suggesting that the primary action of the polysaccharide was on EF-1.

The low molecular weight form of EF-1 from Artemia strongly resembles EF-1_L from mammalian sources (11,12) in that it binds

Table I

Effect of heparin on the binding of GTP to EF-1_L. A value of 0.7 pmol of [^{32}P] GTP bound in the absence of EF-1_L was substracted from each determination.

Heparin (µg/ml)	[^{32}P] GTP bound (pmol)
0	5.0
16	5.1
32	4.9
48	5.9
64	5.9

Table 2

The effect of heparin on the formation of a GTP - aminoacyl-tRNA - EF-1_L complex.

Heparin (µg/ml)	Ternary complex formed (pmol)
0	2.1
16	1.9
32	1.7
48	1.8
64	1.9

GTP effectively and can form a stabile ternary complex with GTP and aminoacyl-tRNA (10). However heparin had no observable effect on either the binding of GTP to the factor (Table I) or ternary complex formation (Table II). The lack of interference by heparin in ternary complex formation could also be demonstrated by gel filtration experiments (results not shown). The inhibition of EF-1 activity by heparin suggests a direct interaction between these two

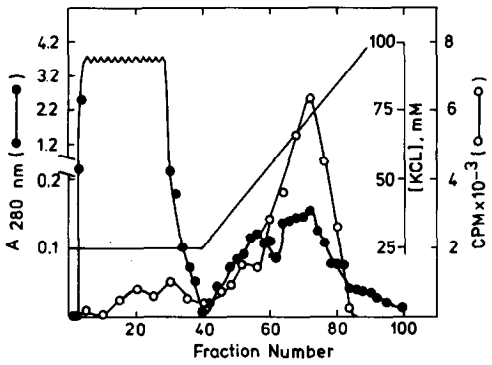

Fig. 3. Chromatography of post-ribosomal supernatant from Artemia cysts on heparin-Sepharose. Aliquots (5 µl) of every fourth fraction were assayed for EF-1 activity as described in Materials and Methods.

substances. To confirm this interaction the technique of affinity chromatography was employed. When the post-ribosomal supernatant of Artemia cysts was passed through a column of heparin-Sepharose equilibrated with Buffer A essentially all the EF-1 activity was retained while 95% of the starting protein was eluted (fig.3). The EF-1 activity could be recovered by increasing the KCl concentration of the elution buffer. As judged by specific activity measurements as well as electrophoresis in the presence of sodium dodecylsulfate, EF-1 was found to constitute about 20 to 30 percent of the protein eluted at 75 mM KCl (results not shown). Thus the single step of heparin-Sepharose chromatography resulted in 20 to 25-fold purification of EF-1 activity from the post-ribosomal supernatant. Chromatography of EF-1 eluted from heparin-Sepharose on hydroxylapatite as previously described (6) was sufficient to purify the enzyme to homogeneity. Heparin-Sepharose also proved effective in purifying the low molecular weight form of EF-1. Neither form of EF-1 was retained by a gel of CNBr-activated Sepharose treated with ethanolamine.

Discussion

The data presented in this communication show that heparin is a potent inhibitor of elongation factor 1 activity. At a concentration of approximately 10^{-6}M (based on Mr=14,600 for heparin; see ref.18) heparin inhibited the EF-1 dependent binding of aminoacyl-tRNA to ribosomes by more than 50 per cent (fig.1). The same heparin concentration inhibited polyphenylalanine synthesis to a similar extent (fig. 2). The binding of heparin to EF-1 was confirmed by affinity chromatography of the enzyme on heparin-Sepharose gels (fig.3). The concentration range at which heparin exerted its effect on EF-1 was essentially identical to the one which inhibited initiation of protein synthesis in a cell free system derived from rabbit reticulocytes (3). Waldman et al.(3) reported that protein synthesis in assay mixtures containing reticulocyte S-30 passed through a heparin-Sepharose affinity column, although considerably reduced compared with mixtures containing control S-30 was unaffected by the presence of heparin. These authors suggested that only initiation ability was lost by passage of the S-30 through heparin-Sepharose. However, the amount of protein synthesis supported by the reticulocyte S-30 after heparin-Sepharose chromatography leaves some doubt as to whether chain elongation was actually measured.

It is possible that the effect of heparin on polypeptide chain elongation is limited to invertebrate systems such as Artemia. I do not favour this view for several reasons. It is well documented that many of the components of protein synthesis are completely interchangeable between Artemia and rabbit reticulocytes (13,14). Furthermore, the structural and functional properties of purified $EF\text{-}1_L$ from Artemia are indistinguishable from a lower molecular form of EF-1 purified from pig liver (12). Thus it seems likely that heparin will

also inhibit polypeptide chain elongation in mammalian systems.

The mode of action of heparin in inhibiting EF-1 activity is not yet clear. No effect of heparin on the ability of EF-1 to bind GTP or to form a GTP·aminoacyl-tRNA·EF-1 ternary complex was observed (Table I and Table II) suggesting that inhibition of EF-1 activity is due to the binding of heparin to the ribosome attachment site of the factor. Work in several laboratories has shown that heparin interferes with proteins which bind to nucleic acids such as ribonucleases (15,16) and bacterial and eucaryotic DNA-dependent RNA polymerases (17,18). Perhaps the binding of the EF-1·aminoacyl-tRNA·GTP ternary complex to ribosomes involves recognition of the complex by a segment of rRNA. In this regard Lanzani et al. (21) have reported recently that the heavy form of wheat embryo EF-1 binds to poly (U), a result which I have confirmed for the Artemia factor.

Finally, affinity chromatography on heparin-Sepharose gels has already proven useful in the purification of initiation factors(3), lipoprotein lipase (19) and RNA polymerase (20). The work presented in this investigation indicates that the purification of EF-1 may also be aided by the use of heparin-Sepharose.

ACKNOWLEDGEMENT

This work was supported in part by grants from the National Science Foundation (U.S.A.) and the Netherlands Foundation for Chemical Research (S.O.N.).
I wish to thank Mrs. H. Reinalda-Poot for exellent technical assistance.

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