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Short Communication

Interference in the binding of ribosomes to reticular membranes by tannic acid

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Abstract

The effect of tannic acid on the binding of polysomes to reticular membranes has been studied *in vitro*. Treatment of chemically degranulated rough endoplasmic reticulum by tannic acid damages the binding sites both for free and bound polysomes on light membrane fraction, but those on heavy membrane fraction remain unaffected. Also, heavy fraction binds very poorly to bound polysomes both before and after the treatment with tannic acid. The damaging effect of tannic acid on ribosome–membrane interactions may be due to change in the conformation of proteins involved in the binding of these ribosomes to reticular membranes.

Keywords: Tannic acid, ribosome-membrane interactions, free polysomes, bound polysomes, rough endoplasmic reticulum.

1. Introduction

Tannic acid, a hepatocarcinogen, is an important component of a number of foods[1]. Tannins have been reported to cause tumours at the site of application[2]. Tannic acid disaggregates polyribosomes[3] and degranulates microsomal membrane both *in vivo*[4] and *in vitro*[5]. The activation of tannic acid to potential carcinogens and its mode of action in detachment of ribosomes from the reticular membranes has already been reported[6]. The present investigation aims to study the effect of tannic acid on binding of polysomes to reticular membranes after reconstitution of rough endoplasmic reticulum (RER).

2. Materials and methods

Rough endoplasmic reticulum preparations from the livers of female albino Wistar rats (weighing approx. 150 g each) fed *ad libitum* were made in STKM buffer according to the method of Fielder *et al*[7]. The free and membrane-bound polysomes were isolated from the livers of female albino rats by the method of Takiguchi *et al*[8]. The RER membranes were chemically degranulated by citrate and pyrophosphate treatment[9] and then further treated by tannic acid (40 μg/ml) in an incubation mixture containing 1 μmol NADPH).

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These membranes were incubated with free and bound polysomes at 25°C for 1 h. Centrifugation at $1,05,000 \times g$ for 4 h resulted in the separation of light and heavy membrane fractions. The binding of ribosomes to reticular membranes has been determined on the basis of RNA/protein ratio. RNA[10] and protein[11] in various preparations were estimated by standard procedures.

3. Results and discussion

The data presented in Table I show the effect of tannic acid on the reconstitution of recticular membranes from polysomes and RER. Citrate and pyrophosphate treatment caused about 84% degranulation of RER. Chemically degranulated RER was further treated with tannic acid and bound to both free and membrane-bound polysomes. It is interesting to note that the reconstituted microsomes appear in two layers, a light membrane fraction (0.25 M/1.35 M interface) and a heavy membrane fraction (1.35 M/2.0 M interface). Both the light and heavy membrane fractions bind free polysomes but the light fraction loses the capacity of binding free polysomes to about 26% when it is pretreated with tannic acid. On the other hand, heavy membrane fraction (tannic-acid-treated) binds free polysomes quite efficiently. The increase in RNA/protein

Table I Interference in the binding of polysomes to chemically degranulated reticular membranes further treated with tannic acid

Sample	RNA (mg/ml)	Protein (mg/ml)	RNA/Protein
Rough endoplasmic reticulum (RER)	3.264	19.250	0.170
Chemically degranulated RER (DRER)	0.266	9.313	0.028 (83.53%)[a]
Light membrane fraction (0.25 M/1.35 M STKM interface) after recombination of DRER with free polysomes	0.374	1.920	0.195
Heavy membrane fraction (1.35 M/2.0 M STKM interface) after recombination of DRER with free polysomes	0.523	2.040	0.256
Light membrane fraction after recombination of tannic-acid-treated DRER with free polysomes	0.245	1.680	0.145 (25.64%)[b]
Heavy membrane fraction after recombination of tannic-acid-treated DRER with free polysomes	0.805	1.920	0.419 (63.64%)[c]
Light membrane fraction after recombination of DRER with bound polysomes	0.324	1.680	0.193
Heavy membrane fraction after recombination of DRER with bound polysomes	0.116	2.040	0.054
Light membrane fraction after recombination of tannic-acid-treated DRER with bound polysomes	0.252	2.160	0.117 (39.38%)[b]
Heavy membrane fraction after recombination of tannic-acid-treated DRER with bound polysomes	0.177	3.360	0.052 (3.70%)[b]

The values given are means of two observations.
[a]Percentage degranulation with respect to control.
[b]Percentage decrease in ribosomal binding in comparison to respective control.
[c]Percentage increase in ribosomal binding in comparison to respective control.

ratio in this case might be due to some spurious binding between ribosomes and heavy membrane fraction[12]. As far as reconstitution of RER using membrane-bound polysomes and chemically degranulated membrane is concerned, the light membrane layer binds quite efficiently whereas the heavy membrane fails to do so. Even the light membrane fraction loses the capacity to reattach bound polysomes up to about 40% when it is pretreated with tannic acid. However, tannic-acid-treated heavy membrane fraction reattaches very poorly to the bound polysomes. The above data show that tannic acid has a damaging effect on the binding of ribosomes to reticular membranes.

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