Acta Biochimica Polonica

Vol. 42 No. 3/1995

347–350

QUARTERLY

EF-1α Is a target site for an inhibitory effect of quercetin in the peptide elongation process

Cezary Marcinkiewicz, Władysław Gałasiński and Andrzej Gindzieński

Department of General and Organic Chemistry, Institute of Chemistry, Medical Academy, 15–230 Białystok, Poland

Received: 24 June, 1995

Key words: elongation factor 1, EF-1α, quercetin, specific inhibitor

The effect of quercetin (3,3',4',5,7-pentahydroxyflavone) on the polypeptide elongation system isolated from rat liver cells, was investigated. Quercetin inhibited [^{14}C]leucine incorporation into proteins *in vitro* and the inhibitory effect is being directed towards the elongation factor eEF-1, but not to eEF-2 and ribosomes. Quercetin was found to form a complex with EF-1α, which was inactive in GTP-dependent binding to ribosomes. It can be suggested that quercetin can block the total or the part of the domain of EF-1α structure that is responsible for formation of the ternary complex EF-1α-GTP-[^{14}C]Phe-tRNA and therefore preventing formation of the quaternary complex with ribosomes.

In all organisms protein biosynthesis is the fundamental process which can be regulated, stimulated or inhibited by various substances. The eEF-1 is the eukaryotic elongation factor that catalyses the circular binding of aminoacyl-tRNAs to ribosomes; its subunit form EF-1α is responsible for the binding step. In earlier publications we have described some features of the elongation factors isolated from rat liver and Guerin epithelioma cells [1–10]. The demonstrated differences in the organization of subunit forms in eEF-1 of tumour and normal cells might be relevance in the search for specific inhibitors of protein biosynthesis.

Some plants have been used for a long time as antitumour remedies in folk medicine but there is little information on their effect on protein biosynthesis in the course of tumour development.

Quercertin (3,3',4',5,7-pentahydroxyflavone), the compound of rutine (Quercetin 3β-D-rutinoside) exhibits pharmacological properties, but at the same time it is a mutagenic agent and inhibitor of hyaluronidase, phosphodiesterase and prostaglandin synthesis. Quercetin arrests human leukemic T-cells in late G1 phase of the cell cycle [11].

The effect of quercetin on the peptide elongation system isolated from rat liver cells, is the subject of this work.

MATERIALS AND METHODS

Male Wistar rats, 150–200 g in weight were used for the experiment. The cell-free system (ribosomes, elongation factors eEF-1 and eEF-2, [^{14}C]phenylalanyl-tRNAs) were isolated from rat liver and EF-1α was purified as described earlier [2, 5, 12, 13]. The binding of [^{14}C]phenylalanyl-tRNA to ribosomes has been described [5].

Quercetin solution. Quercetin (5 mg) was dissolved in 1 ml of ethanol, then the ethanolic solution was diluted with 10 vols. of 50 mM Tris/HCl buffer, pH 8.0, and immediately added into the reaction mixture of the tests on

[^{14}C]leucine incorporation into proteins or [^{14}C]phenylalanyl-tRNA binding to ribosomes.

Ternary EF-1α-GTP-[^{14}C]Phe-tRNA complex formation. First, a binary complex EF-1α- GTP was formed by incubation at 37°C of the mixture containing in 0.5 ml: 50 mM Tris/HCl, pH 7.6, 15 mM $MgCl_2$, 60 mM KCl, 15% glycerol, 5 mM DTT, 0.2 mM GTP and adequate amounts of EF-1α untreated or treated with quercetin. After 5 min [^{14}C]Phe-tRNA (approx. 20 000 c.p.m.) was added and incubation was continued for another 2 min. The reaction was stopped by adding 3 ml of buffered solution containing: 33 mM Tris/HCl, pH 7.6, 67 mM KCl and 67 mM $MgCl_2$. Samples were immediately filtered through cellulose filters (0.45 μm, Millipore) and washed three times with the use of the same buffered solution, 3 ml for each washing. The radioactivity retained on the dried filters was measured in the PPO-POPOP-toluene scintillation fluid on Isocap 300, Nuclear Chicago counter.

Quercetin-EF-1α complex formation. EF-1α (1.7–2.0 mg) was incubated at 0°C or 37°C for 5 min with 800 μg of quercetin in 0.5 ml of the buffered solution containing: 50 mM Tris/HCl, pH 8.0, 25% glycerol and 10 mM 2-mercaptoethanol. Then the incubation mixture was filtered through Sephadex G-25 column (0.8 cm × 30 cm), equilibrated with the same buffered solution, at a flow rate of 20 ml/h and absorbance of the fractions was measured at A_{280} (for protein) and A_{355} (for quercetin).

Reagents. Guanosine-5'-triphosphate (GTP) — Calbiochem; dithiothreitol (DTT) — Loba Chemie, Vien-Fischamend, Austria; 2,5-diphenyloxazole (PPO) — E. Merck; *p*-bis [2-(5-phenyloxazolyl)benzene] (POPOP), scintillation grade — New England Nucl. Corp. Chem. Div.; L-[^{14}C]leucine (60 mCi/mmol) and L-[^{14}C]phenylalanine (475 mCi/mmol) — Amersham, U.K.; poly(U) — Sigma; quercetin — Serva; Millipore filters HA (0.45 μm) — Millipore Corp., Bedford, MA, U.S.A.; glass fibre filters GF/A — Whatman Biochemicals Ltd, U.K. All the other chemicals used were of reagent grade.

RESULTS AND DISCUSSION

Quercetin was found to inhibit [^{14}C]leucine incorporation into proteins *in vitro*; the inhibitory effect, investigated in peptide elongation system, being directed towards elongation factor eEF-1 (Fig. 1), but not to eEF-2 and ribosomes. This result was confirmed by the action of quercetin on the eEF-1-dependent binding of [^{14}C]phenylalanyl-tRNA to ribosomes (Table 1). The subsequent experiments demonstrated that direct action of quercetin on the ribosomes did not affect their activity (not shown). Some substances (e.g. tetracyclines) can block the binding site A on the ribosome with eEF-1 still being active [14, 15]. Thus, the inhibitory target site for quercetin is different than that of tetracyclines.

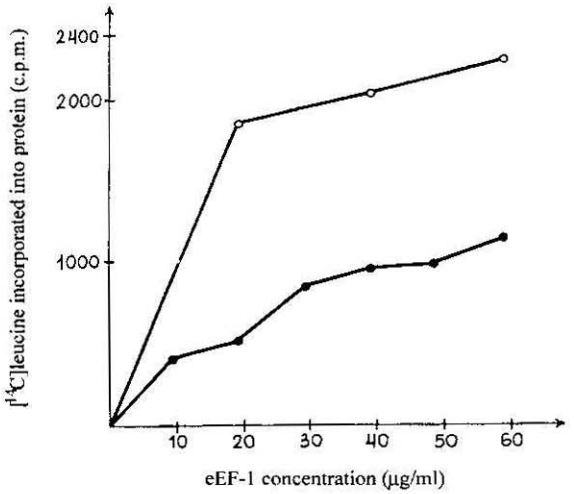

Fig. 1. The effect of quercetin on the EF-1 activity. The eEF-1 activity was assayed by [^{14}C]leucine incorporation into proteins as described previously [3]. (○), Without quercetin; (●), with quercetin (150 μg/ml).

So far no selective inhibitor of eEF-1 activity has been discovered. We have demonstrated that EF-1A (eEF-1α), a subunit form of the heterogeneous elongation factor eEF-1, is responsible for the binding of aminoacyl-tRNA to

Table 1

The effect of quercetin on the eEF-1 activity in binding of [^{14}C]Phe-tRNA to ribosomes

Experiment	[^{14}C]Phe-tRNA binding to ribosomes (c.p.m.)		Inhibition (%)
	without quercetin	with quercetin	
1	418	114	72.5
2	503	48	90.4

The experiment was carried out with the use of 30 μg of eEF-1 and without or with 150 μg of quercetin.

ribosomes [5]. It has been also shown that quercetin (10 μg), added to the EF-1α (28.5 μg), inhibits the activity of this factor by about 50% (Fig. 2). It could be supposed that quercetin, to be bound with EF-α prevents the formation of EF-1α-GTP complex. Therefore quercetin was incubated with EF-1α and the mixture was passed through Sephadex G-25. The results have shown (Fig. 3) that quercetin can form a complex with EF-1α, which is inactive in GTP-dependent binding to ribosomes. The absorbance at 280 nm was much higher in the peak fractions of the EF-1α incubated with quercetin than that of EF-1α incubated without quercetin (Fig. 3A). The absorbance, that was parallelly measured at maximum for quercetin (355 nm), has shown univocally the presence of quercetin in the same fraction (Fig. 3B). These results support the suggestion on formation of the EF-1α-quercetin complex.

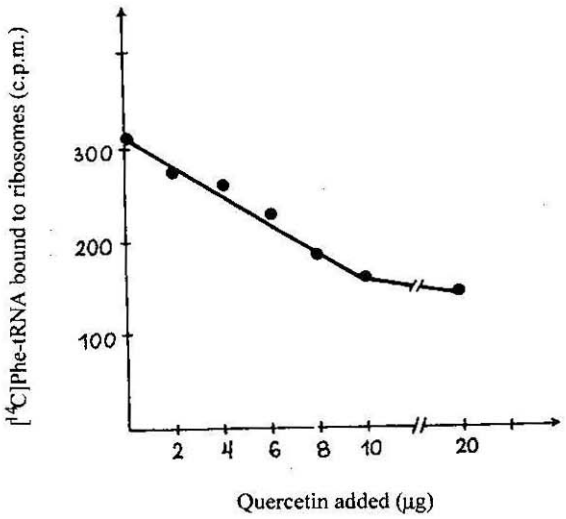

Fig. 2. The effect of quercetin on the activity of EF-1α.
The activity of EF-1α was assayed by [^{14}C]Phe-tRNA binding to ribosomes as described previously [10].

The EF-1α-dependent binding of aminoacyl-tRNA to ribosomes is a three-step process. In order to produce a ternary complex of EF-1α-GTP-aa-tRNA, a binary complex of EF-1α-GTP must first be formed. The ternary complex binds to the ribosomes forming a quaternary complex. On the graph of Fig. 4, the results of the EF-1α-dependent ternary complex formation are presented. The amount of [^{14}C]phenylalanyl-tRNA bound with the binary complex of EF-1α-GTP was by 40%–50% smaller on incubation in the presence of quercetin. It was noted simultaneously that quercetin did not form di-

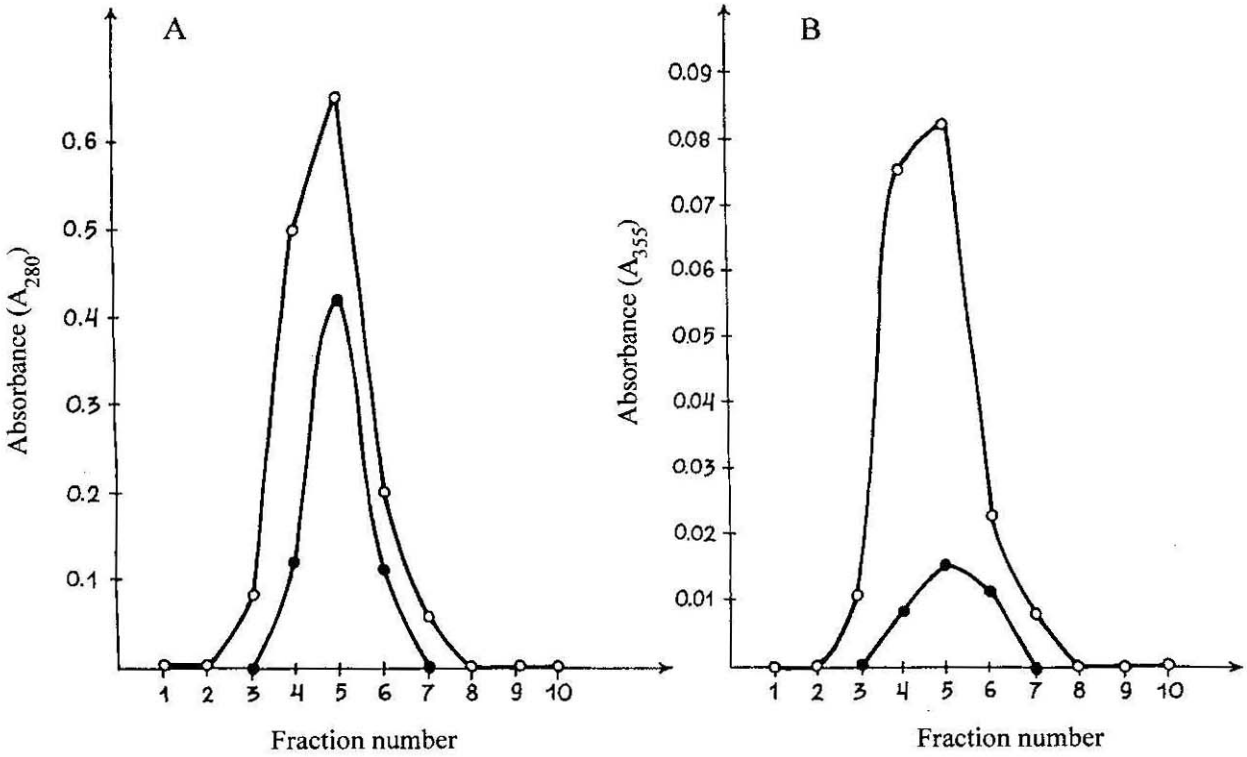

Fig. 3. The graphs of molecular filtration through Sephadex G-25 of the EF-1α preincubated for 5 min at 0°C with quercetin (○) or without quercetin (●), at absorbance maxima: A, of protein; B, for quercetin.

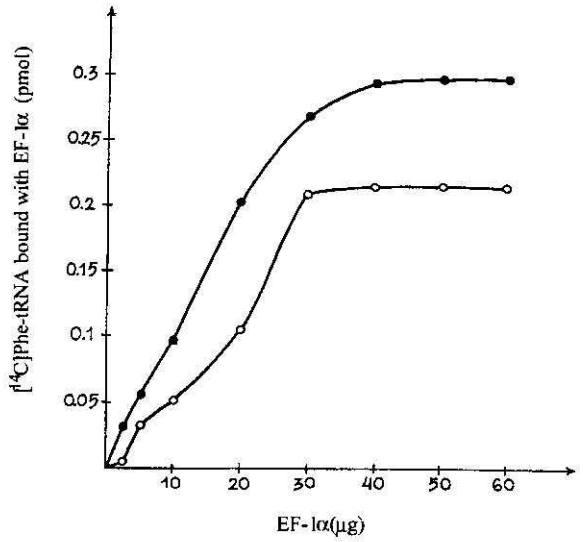

Fig. 4. The ternary complex EF-1α-GTP-[^{14}C]Phe-tRNA formation, dependent on the concentration EF-1α, preincubated with quercetin (○) or without quercetin (●).

rectly any bond with GTP. Our suggestion is that quercetin can block the total or part of the domain of EF-1α structure that is responsible for formation of a ternary complex, and thus the quaternary complex of EF-1α-GTP-[^{14}C]Phe-tRNA-ribosome can not be created.

REFERENCES

1. Gajko, A., Średzińska, K., Marcinkiewicz, C. & Gałasiński, W. (1991) The effect of phosphorylation of the EF-2 isolated from rat liver cells on protein biosynthesis *in vitro*. *Acta Biochim. Polon.* **38**, 353–358.
2. Gałasinski, W. & Moldave, K. (1969) Purification of aminoacyl-transferase II (translocation factor) from rat liver. *J. Biol. Chem.* **244**, 6527–6532.
3. Jabłonowska, K., Telejko, E., Kopacz-Jodczyk, T. & Gałasiński, W. (1981) Heterogeneous forms of elongation factor 1 (EF-1) from Guerin tumour cells in rats. I. Purification of EF-1A, EF-1B and EF-1C from Guerin tumour cells. *Bull. Pol. Ac.: Biol.* **29**, 361–366.
4. Jabłonowska, K., Kopacz-Jodczyk, T., Niedźwiecka, J. & Gałasiński, W. (1983) Isolation and characterization of elongation factor EF-2 from Guerin tumour. *Acta Biochim. Polon.* **30**, 381–388.
5. Marcinkiewicz, C., Gajko, A. & Gałasiński, W. (1991) Purification and properties of the heterogeneous subunits of the elongation factor EF-1 from Guerin epithelioma cells. *Acta Biochim. Polon.* **38**, 129–134.
6. Marcinkiewicz, C., Gajko, A. & Gałasiński, W. (1992) The phosphorylation of elongation factor EF-1 isolated from Guerin epithelioma. *Acta Biochim. Polon.* **39**, 7–13.
7. Marcinkiewicz, C. & Gałasiński, W. (1993) Isolation and properties of subunit form EF-1C of elongation factor 1 from Guerin epithelioma cells. *Acta Biochim. Polon.* **40**, 225–230.
8. Średzińska, K., Gajko, A. & Gałasiński, W. (1991) Differences in the structures of the elongation factors (EF-2) isolated from Guerin epithelioma and rat liver. *Bull. Pol. Ac.: Biol.* **39**, 171–174.
9. Telejko, E., Niedźwiecka, J., Łopaczyński, W., Zwierz, J. & Gałasiński, W. (1977) *Characterization of the elongation factors EF-1 and EF-2 from the tumour cells of the experimental Guerin epithelioma.* (Nowak, H.F., ed.) pp. 46–50, Onkologia Doświadczalna, Medical Academy Białystok, (in Polish).
10. Telejko, E., Jabłonowska, K., Skowroński, J. & Gałasiński, W. (1981) Heterogeneous forms of elongation factor 1 (EF-1) from Guerin tumour cells in rats. II. The character of highly purified heterogeneous forms of EF-1 isolated from Guerin tumour cells. *Bull. Pol. Ac.: Biol.* **29**, 367–372.
11. Yoshida, M., Yamamoto, M. & Nikaido, T. (1992) Quercetin arrests human leukemic T-cells in late G_1 phase of the cell cycle. *Cancer Res.* **52**, 6676–6681.
12. Skogerson, L. & Moldave, K. (1969) The binding of aminoacyltransferase II to ribosomes. *Biochem. Biophys. Res. Commun.* **27**, 568–572.
13. Moldave, K. (1963) The preparation of C^{14}-aminoacyl *soluble*-tRNA. *Methods Enzymol.* **6**, 757–761.
14. Cundliffe, E. (1968) Polyribosomes and ribosomal sub-units of bacterial protoplasts. *Biochem. Biophys. Res. Commun.* **33**, 247–252.
15. Vazquez, D. & Monro, R.E. (1967) Effects of some inhibitors of protein synthesis on the binding of aminoacyl tRNA to ribosomal subunits. *Biochim. Biophys. Acta* **142**, 155–173.