EFFECT OF LIPIDS ON AMINOACYL-tRNA SYNTHESIS IN *ESCHERICHIA COLI*

J.HRADEC and Z.DUŠEK
Department of Biochemistry, Oncological Institute, Prague 8-Bulovka, Czechoslovakia

Received 4 November 1969

1. Introduction

Lipids were demonstrated to stimulate protein synthesis in cell-free systems of rat liver *in vitro* [1]. Evidence has been presented that CMH* is the lipid responsible for this effect [2]. This cholesteryl ester apparently plays an important role in the incorporation of labelled amino acids into tRNA. After extraction with organic solvents, purified mammalian aminoacyl-tRNA synthetases lost their activity. The original activity was recovered by the addition of pure CMH in quantities corresponding to those removed by the extraction [3]. Only few structurally similar cholesteryl esters [4], but no other lipid fractions were effective in this respect [5].

Since cholesteryl esters are characteristic for mammalian tissues and no similar compounds are known to be present in microorganisms it seemed of interest to investigate whether lipids also affect early steps of protein synthesis in bacteria. Results presented in this paper indicate that lipids do have an effect on the labelling of tRNA with amino acids in *E. coli* and, moreover, that a cross-reactivity exists between these lipids and CMH.

2. Material and methods

Crude aminoacyl-tRNA synthetases of *E. coli B* were precipitated with $(NH_4)_2SO_4$ form the S-100 fraction as described by Avital and Elson [6]. The isolation of rat liver pH 5 enzymes is described earlier [7]. Both enzyme preparations were freeze-dried and extracted with ethylether as described elsewhere [5]. One unit of the lipid extract is defined as the quantity of lipids extracted from 1 mg of aminoacyl-tRNA synthetase protein with ethylether during 4 hr in a Soxhlet apparatus. The isolation of rat liver tRNA and the synthesis of CMH is also described earlier [2,3]. *E. coli* tRNA was kindly donated by Dr. I.Rychlik, Institute of Organic Chemistry and Biochemistry, Prague. Incubation mixtures for the assay of aminoacyl-tRNA synthesis in *E. coli* contained Tris-HCl, pH 7.40,100 mM; KCl, 6 mM; magnesium acetate, 20 mM; 2-mercaptoethanol, 1 mM; ATP (sodium salt), 5 mM; tRNA, 50 μg; enzyme protein, 0.18–0.25 mg and *E. coli* lipid extract or CMH (or polyethylene glycol, mol. wt. 600 in control mixtures), 0.025 ml; and L-^{14}C-leucine, 0.55 nmoles; in a total volume of 0.2 ml. Incubation mixtures with rat liver pH 5 enzymes consisted of Tris-HCl, pH 7.50, 100 mM; $MgCl_2$, 10 mM; 2-mercaptoethanol, 2 mM; ATP (sodium salt), 5 mM; tRNA, 0.1 mg; enzyme protein, 0.8-1.2 mg; lipid extract of *E. coli* or CMH (or polyethylene glycol in control mixtures), 0.15 ml; and L-^{14}C-leucine, 0.55 nmoles; in total volume of 0.5 ml. Both mixtures were incubated for 10 min. The reaction was terminated by the addition of 5% trichloroacetic acid, precipitates were collected on membrane filters, washed 3 times with the above acid, dried and counted with a low-background gas flow counter.

3. Results and discussion

The incorporation of labelled L-leucine into tRNA

* Abreviation: CMH, cholesteryl-14-methylhexadecanoate.

North-Holland Publishing Company – Amsterdam

in the presence of non-extracted *E. coli* aminoacyl-tRNA synthetases was significantly enhanced by the addition of lipids extracted from *E. coli* aminoacyl-tRNA synthetases. A similar result was found when CMH was added instead of the lipid extract. In both cases, the stimulation was roughly proportional to the quantity of lipids added. However, high amounts of CMH induced a partial inhibition of aminoacyl-tRNA synthesis (fig. 1). Similar results were also found with

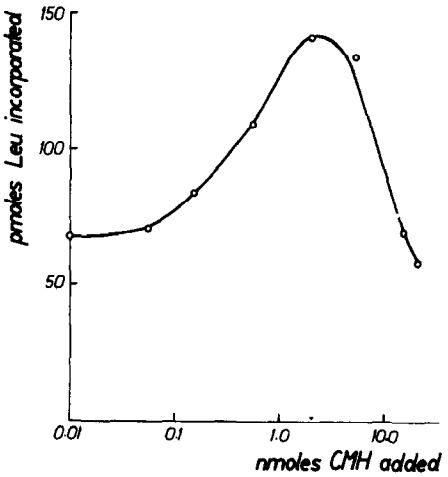

Fig. 1. Effect of CMH on the incorporation of ^{14}C-leucine into tRNA in the presence of non-extracted *E. coli* aminoacyl-tRNA synthetases.

the lipid extract. Quantities of CMH, in the range of 5–10 nmoles of CMH added per mg enzyme protein showed the most pronounced stimulating effect. This amount is 100–1000 fold higher than the most effective dose of CMH found for rat liver enzymes [2]; such high quantities of CMH usually bring about a partial inhibition of aminoacyl-tRNA synthesis with mammalian systems [2,5].

The aminoacyl-tRNA synthesis was reduced to about 50% of the control value if extracted *E. coli* synthetases were tested. This value is very close to that obtained with rat liver pH 5 enzymes extracted in the same way [5]. A complete recovery of the enzymic activity was obtained when incubation mixtures were supplemented with lipids removed by the extraction. If the lipid extract was replaced by CMH, identical results, i.e. a complete recovery of the enzymic activity, was obtained with extracted *E. coli* aminoacyl-tRNA synthetases. A summary of these results is presented in table 1. The quantity of CMH that must be added for a complete reactivation of extracted synthetases is approximately 2–3 nmole of CMH/mg of enzyme protein. This amount is at least

Table 1

Effect of lipids extracted from *E. coli* aminoacyl-tRNA synthetases or CMH on the combination of ^{14}C-leucine with *E. coli* tRNA. Incubation mixtures contained 0.2 mg of crude aminoacyl-tRNA synthetases and 0.2 u. *E. coli* lipids or 0.48 nmole of CMH as indicated. All values are the net incorporation of ^{14}C-leucine (pmole) into the TCA-insoluble portion of the sample.

Lipid added	Enzymes	
	non-extracted	extracted
None	83.8	45.0
E. coli lipids	91.2	86.2
CMH	82.7	79.6

10 fold higher than that required by rat liver pH 5 enzymes extracted in the same way [5]. However, it is very similar to the requirement of extracted purified mammalian aminoacyl-tRNA synthetases [3]. There seem to be at least two possible explanations of these differences. It may be supposed that CMH is not able to replace bacterial lipids absolutely and that therefore higher quantities of this compound are needed if the same effect is to be reached. Alternatively, bacterial synthetases may have a higher lipid content than similar mammalian enzymes.

Results of experiments in which the effect of *E. coli* lipids was tested on extracted rat liver pH 5 enzymes indicate that both these possibilities may be correct. A complete reactivation of 1 mg of extracted rat liver pH 5 enzyme protein was induced by lipids extracted from 0.4–0.7 mg of bacterial aminoacyl-tRNA synthetases. The residual activity of this extracted enzyme was very similar to the bacterial synthetase treated in the same way. The cross-reactivity of lipids present in *E. coli* aminoacyl-tRNA synthetases with CMH was further demonstrated by the stimulation of aminoacyl-tRNA synthesis in the presence of non-extracted rat liver pH 5 enzymes after the addition of bacterial lipids. A summary of experiments with mammalian enzymes is given in table 2.

Table 2

Effect of lipids extracted from *E. coli* aminoacyl-tRNA synthetases or CMH on the incorporation of ^{14}C-leucine into rat liver tRNA. Incubation mixtures contained 1.0 mg of rat liver pH enzyme protein, 0.65 u. of lipids extracted from *E. coli* aminoacyl-synthetases or 0.11 nmole of CMH as indicated. All values represent the net incorporation of ^{14}C-leucine (pmole) into the TCA-insoluble portion of the sample.

Lipid added	Enzymes	
	non-extracted	extracted
None	25.8	14.9
E. coli lipids	42.2	31.7
CMH	27.3	26.6

Identical results on the deactivation of both bacterial and mammalian aminoacyl-tRNA synthetases by extraction with organic solvents as well as the possibility of replacing bacterial lipids by CMH and vice versa in both these systems indicate that similar mechanisms may be involved in protein synthesis in both these species. The role of CMH or other lipids, though an important one, is not as yet clear. It is also not clear whether bacterial lipids involved in aminoacyl-tRNA synthesis have a chemical structure similar to that of CMH. Attempts are being made in our laboratory to purify and further characterize the bacterial lipids active in protein synthesis.

Acknowledgements

We wish to thank Dr. I.Rychlík, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague, for his kind gift of tRNA from *E. coli* B. The careful technical assistance of Mrs. Z. Tuháčková and Miss N.Krejčová is also gratefully acknowledged.

References

[1] J.Hradec, Biochim. Biophys. Acta 47 (1961) 149.
[2] J.Hradec and L.Dolejš, Biochem. J. 107 (1968) 129.
[3] J.Hradec and Z.Dušek, Biochem. J., in press.
[4] J.Sommerau and J.Hradec, Abstracts 5th FEBS Meeting (Prague 1968), p. 67.
[5] J.Hradec and Z.Dušek, Biochem. J. 110 (1968) 1.
[6] S.Avital and D.Elson, Biochim. Biophys. Acta 179 (1969) 297.
[7] J.Hradec, Biochem. J. 105 (1967) 251.