EFFECT OF CHOLESTERYL ESTERS WITH DIFFERENT SATURATED FATTY ACIDS ON AMINOACYL-tRNA SYNTHESIS IN RAT LIVER

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1. Introduction

Cholesteryl 14-methylhexadecanoate (CMH) stimulates the formation of aminoacyl-tRNA complex in rat liver *in vitro* [1]. Moreover, the presence of this compound in enzyme molecules may be essential for the normal activity of mammalian aminoacyl-tRNA synthetase [2] as well as of peptide elongation factors [3].

Preliminary results indicated that other cholesteryl esters have no effect on enzymes required for protein synthesis [4]. Therefore it seemed desirable to investigate more systematically the possible relation between the chemical structure of cholesteryl esters and their activity in protein synthesis.

In this paper results are reported on the effect of cholesteryl esters with different saturated higher fatty acids on the charging of rat liver tRNA with amino acids.

2. Materials and methods

Cholesteryl esters were synthesized from purified cholesterol as described by Swell and Treadwell [5]. Myristic, palmitic, margaric and stearic acids were commercial products. Even-numbered iso-acids and odd-numbered anteiso-acids were isolated by preparative gas-liquid chromatography from wool fat hydrolysates. All other normal and branched-chain fatty acids were prepared synthetically [6]. All fatty acids were purified by preparative gas-liquid chromatography to about 98%. Isolation and extraction of rat liver pH 5 enzymes with ethylether was described earlier [7]. Incubation procedures used for the assay of charging of tRNA with 1-^{14}C-leucine were reported elsewhere [8].

3. Results and discussion

Esters of cholesterol with normal-chain saturated fatty acids have no stimulating effect on charging of rat liver tRNA with L-leucine nor are they able to reactive extracted pH 5 enzymes (table 1). The same is true for iso-acids of comparable chain length. Even the structural homologue of CMH, cholesteryl 15-methylhexadecanoate has no effect on aminoacyl-tRNA formation. From esters with anteiso-acids, only cholesteryl 13-methylpentadecanoate and 15-methylheptadecanoate, homologues of CMH differing only in one methylene group, are active (table 1). Furthermore, both these active cholesteryl esters showed the same typical dose-dependent reactivating effect on extracted pH 5 enzymes as demonstrated with CMH [1].

The data reported here show that the chemical structure of the fatty acid is of a critical importance for the activity of cholesteryl esters in protein synthesis. Presence of a methyl group in penultimate position in the saturated carbon chain of the fatty acid seems to be required for the activity of these esters. Further experiments are now in progress in this laboratory to investigate whether esterified fatty acids with branched methyl groups in positions nearer to the ester bond are also active. Moreover, a definite chain length of the fatty acid is apparently essential for the activity since only cholesteryl esters with anteiso-acids in the C_{16}—C_{18} region are active.

Results presented in this paper indicate a very close relation between the chemical structure of CMH

Table 1

Effect of cholesteryl esters with different saturated fatty acids on the charging of rat liver tRNA with ^{14}C leucine. The composition of the incubation mixtures has been described elsewhere [8]. All values represent the net incorporation of ^{14}C leucine (pmoles) into the TCA-insoluble portion of the sample.

Fatty acid in the cholesteryl ester			pH 5 Enzymes	
Carbon atoms	Branching	Name	non-extracted*	extracted**
C_{14}	–	Myristic	28.2	17.8
	anteiso-	11-Methyltridecanoic	28.4	17.7
C_{15}	–	Pentadecanoic	29.4	17.3
	anteiso-	12-Methyltetradecanoic	28.9	17.2
C_{16}	–	Pamitic	28.7	18.1
	iso-	14-Methylpentadecanoic	29.4	17.3
	anteiso-	13-Methylpentadecanoic	39.5	28.6
C_{17}	–	Margaric	28.6	17.8
	iso-	15-Methylhexadecanoic	27.3	18.1
	anteiso-	14-Methylhexadecanoic (CMH)	40.2	29.3
C_{18}	–	Stearic	29.1	17.7
	iso-	16-Methylheptadecanoic	28.7	17.5
	anteiso-	15-Methylheptadecanoic	40.3	29.1
C_{19}	anteiso-	16-Methyloctadecanoic	28.1	18.3
–	–	Control value	29.1	17.6

* 0.1 pmole of individual cholesteryl esters was added.

** The quantity of cholesteryl esters added corresponded exactly to the amount of CMH extracted from the enzyme preparation (80 pmoles/mg of pH 5 enzyme protein).

and its biological activity. Moreover, the only other two active cholesteryl esters of this whole series, cholesteryl 13-methylpentadecanoate and 15-methylheptadecanoate, cannot been regarded as natural products since anteiso-acids of even numbers are not present in biological materials [9]. Thus the activity of CMH in protein synthesis is apparently highly specific, as is expected for a compound participating physiologically in enzyme reactions.

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