Transfer of Methyl Groups from Methionine to Soluble RNA from *E. coli*

Ingvar Svensson, Hans G. Boman, Kerstin G. Eriksson and Kerstin Kjellin

Institute of Biochemistry, University of Uppsala, Uppsala, Sweden

We have for some time been studying the formation of methionyl RNA using *E. coli* sRNA and synthetase preparations from coli as well as yeast. A detailed examination of our time curves for the reaction with a partly purified yeast methionyl RNA synthetase [1], revealed a slow second phase of incorporation. The phenomenon could only be recorded with methionine labelled in the methyl group and not with C-1, C-2 or sulphur label and the second reaction was therefore tentatively believed to be a methylation of sRNA. After adjustment of assay conditions, a methyl group incorporation could be obtained, about 15 times exceeding the maximum formation of methionyl RNA. Borek's group [2,3] has briefly reported *in vivo* and *in vitro* experiments indicating that the methylated bases in sRNA derive their methyl groups from methionine and that this formation occurs on a polynucleotide level. Their work was done with *E. coli* $K_{12}W6$, a strain which has a methionine requirement and a deficient control of the RNA synthesis, which makes it continue RNA synthesis even under methionine starvation. The strain was kindly donated by Dr. Borek and has been used in most of the experiments to be reported. In addition to the yeast enzyme we have used a partly purified enzyme preparation obtained from *E. coli* $30S_0A5$ by streptomycin and ammonium sulphate precipitations of a French press extract. The pH of the reaction mixture used for assay was 8.0 and the concentrations of the reagents were the following: 2 mM ATP, 10 mM $MgCl_2$, 20 mM NH_4Cl, 2 mM GSH, 2 mM PEP, 100 mM Tris-HCl, PEP kinase, varying amounts of labelled methionine, RNA and enzyme, other details of the assay being as previously described [4]. Total RNA was prepared [5] and separated into ribosomal RNA (rRNA) and soluble RNA (sRNA) using gel chromatography on Sephadex G-200 [6]. Each peak was assayed for ability to accept methyl groups from ^{14}C-methyl labelled methionine, using the *E. coli* and the yeast enzyme (see Table 1). Larger batches of sRNA were prepared as outlined [4] using log phase W6 and cells starved 2 h for methionine. Part of each sample was oxidized with periodate [7]. For each type of RNA, the maximum level of methylation was determined with each of the enzymes and the

Table 1. Saturation values for methylation of RNA. All values represent plateaus in time curves and are expressed as mμmoles per optical unit RNA. The arginine incorporation was performed with a partly purified *E. coli* enzyme [4].

Type of RNA from *E. coli* $K_{12}W6$		Arginine incorporation	Methylation with		
			E. coli enzyme	yeast enzyme	*E. coli* + yeast enzymes
Sephadex peaks:					
Methionine starved cells	sRNA	—	0.36	1.01	—
	rRNA	—	0.01	0.01	—
Log-phase cells	sRNA	—	0.01	0.60	—
	rRNA	—	<0.01	0.01	—
sRNA preparations:					
Methionine starved cells	sRNA	0.10	0.21	0.42	0.50
	ox-sRNA	0.00	0.27	0.56	—
Log-phase cells	sRNA	0.12	0.00	0.69	0.67
	ox-sRNA	—	0.01	0.76	—

results are summarized in Table 1. The time curves gave plateaus and the reaction showed an absolute RNA dependence and was RNase sensitive. The reaction product is stable up to 30 min at 37° in 0.1 M triethyl amine-acetic acid buffer, pH 11, while methionyl-RNA during this condition has a half life of about 30 sec.

In vivo incorporations of methyl labelled methionine into both log phase and methionine starved W6, have shown an extensive labelling of the sRNA peak and a smaller but significant incorporation also into rRNA.

Counter-current distribution of sRNA from strain $30S_0A5$ gives a fractionation with respect to ability to accept methyl groups. Phase systems D and F[8] were used in 19-transfer experiments. When assayed with the yeast enzyme, the curve for the methylation was almost the same as the one for the distribution of total sRNA in the bottom phase. — Together our results indicate that the yeast and the *E. coli* enzymes have different specificities. They show also that the methylation reaction is highly specific and that the periodate oxidation and the methionine starvation independently make more sites available for methylation. A full account of this work is in preparation and will be published elsewhere.

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Two Fluorescent Amino Acids which Function as Cross-linkages between the Peptide Chains in Resilin, a Rubber-like Protein

Svend Olav Andersen

Zoophysiological Laboratory B, Juliane Mariesvej 36, University of Copenhagen, Denmark

Resilin is a structural protein which occurs in insects. Its most characteristic property is that it behaves as a perfect rubber when swollen with water, and that it is completely insoluble in all solvents which do not break peptide linkages. Its properties are best explained by the assumption that the protein contains some sort of very stable linkages between the peptide chains connecting these to a huge threedimensional network [1-3]. These linkages must be of a hitherto unknown nature.

During our investigations of resilin obtained from the desert locust *Schistocerca gregaria (Forsskål)* we have isolated two fluorescent compounds [4] which apparently do not occur in other proteins. Both compounds are phenolic amino acids, and one of them is a diamino dicarboxylic acid and the other is a triamino tricarboxylic acid. If both the amino groups and the carboxylic groups are connected to the other amino acids in the protein by means of normal peptide linkages these compounds can function as cross-linking agents. It has been shown that none of the amino groups are free to react with dinitrofluorobenzene in the native protein, indicating that they are involved in some sort of chemical linkage, but it has not been possible to show whether the carboxylic groups are free or not.

From the amounts of these compounds present in resilin it is possible to calculate the average molecular weight of the peptide chain between two neighbouring cross-linkages, when it is assumed that one of these compounds connects two peptide chains and the other one connects three peptide chains together in one junction point. The average molecular chain weight is then found to be 3000 g. This result is in good agreement with the results obtained by physical measurements made on resilin from dragonflies (*Aeshna* species) [3].

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