CONFORMATIONAL CHANGES IN RIBOSOMES INDUCED BY ALCOHOLS

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1. Introduction

It is well known that some organic solvents can affect the function of the ribosomes stimulating some of their activities that normally require the presence of other components. Peptide bond formation has been found [1,2] catalyzed by the larger ribosomal subunit alone, provided that alcohols are present. EF-G-dependent GTPase can be strongly stimulated by methanol [3] even in the absence of some ribosomal proteins [4–6] and the hydrolysis of GTP can be also uncoupled from the EF-Tu-dependent binding of aminoacyl-tRNA in the presence of methanol [7,8]. Similarly methanol can affect several steps of the protein synthesis termination reaction [9].

These functional effects on the ribosome are presumably mediated by conformational modifications of the ribosomal structure due to changes in the polarity of the medium in which the particles are dissolved [3,8,10]. However no direct evidence of such conformational changes in the presence of alcohols has been reported.

Chemical modification of ribosomal components is a technique extensively used to detect conformational changes in the ribosome. We have tried, therefore, to detect structural alterations by labeling the ribosomes using reductive methylation in the presence and in the absence of methanol. We have centered our attention on the larger ribosomal subunit, which, from a functional point of view, appears to be more sensitive to alcohols; the results obtained are reported here.

2. Materials and methods

Ribosomes and ribosomal subunit were obtained from *Escherichia coli* D-10 as in [5]. When radioactive ribosomes were required the cells were grown in minimal medium in the presence of [^{3}H]leucine.

To label ribosomes by reductive methylation, the particles, at ~2 mg/ml, were treated with [^{14}C]formaldehyde (7.5 mM final conc.) at 0°C for 40 s in 100 mM sodium borate, 10 mM $MgCl_2$, 20 mM KCl and 6 mM β-mercaptoethanol adjusted to pH 8.5. Borohydride (10 mg/ml) at 70 μl/ml reaction mixture was immediately added and after 10 min the sample was dialyzed against 20 mM Tris–HCl (pH 7.8), 10 mM $MgCl_2$, 80 mM NH_4Cl. When required 20% methanol was present in the reaction mixture. Free ribosomal proteins were labeled in similar conditions but in the presence of 6 M urea.

Ribosomal proteins were extracted with 67% acetic acid and precipitated with 5 vol. acetone at −20°C. Two dimensional gel electrophoresis was carried out following the standard system [11]. The gels were stained with 0.04 mg/ml of Coomasie blue G-250 in 3.5% perchloric acid [12]. The radioactivity in the gel was estimated after extraction of stained spots with 70% acetic acid as in [13].

[^{14}C]Formaldehyde (14 mCi/mmol) and [^{3}H]leucine (54 Ci/mmol) were purchased from The Radiochemical Center, Amersham.

3. Results

3.1. *Effect of methanol in labeling of total ribosomal proteins by reductive methylation*

In order to detect changes of conformation of the

Table 1
Effect of methanol on the extent of ribosomal protein methylation

Substrate	Total methyl groups incorporated	
	+Methanol (pmol)	–Methanol (pmol)
50 S subunits	24.4	28.9
Free ribosomal proteins (TP 50)	21.0	18.5

38 pmol 50 S subunits and 10 μg TP 50 were treated as in section 2 and the radioactivity incorporated was measured by precipitation with 5% trichloroacetic acid and filtration on glass fiber filters

ribosome by chemical modification of its components it is important to show that the compound or condition that supposedly causes the effect does not affect the chemical reaction itself. Table 1 shows that the labeling of total free and ribosome bound proteins is not drastically affected by the presence of 20% methanol. We have used a concentration of alcohol that is optimal to stimulate several ribosomal functions such as EF-G-dependent GTPase [3] and uncoupled EF-Tu-dependent GTP hydrolysis [7]. Other activities required even higher alcohol concentrations [1,2].

In these conditions of treatment, about 30 lysine residues are modified per ribosome as an average, that is, 1 per ribosomal protein. With this low labeling, changes in the modification of the individual proteins are thought to be more easily detected than in conditions where extensive methylation is achieved.

3.2. *Changes in relative methylation of individual proteins*

Table 2 summarizes the results obtained when the radioactivity of individual proteins in two dimensional gel from electrophoresis of ribosomes labeled in the presence and in the absence of 20% (v/v) methanol was measured. As an internal standard, ^{3}H-labeled proteins were added to the samples, and the data presented have been normalized taking the $^{14}C/^{3}H$ ratio of the total mixture of proteins in each sample as the unit. The ratio of normalized values of $^{14}C/^{3}H$ in samples treated in the absence and in the presence of methanol will allow us to identify the proteins whose labeling is different in the two conditions. If we establish 20% as the limit for variability of the data based on the standard deviation of our results, there are several proteins that are definitely less labeled when methanol is present: L2, L14, L15, L17, L19, L20 and L28. Proteins L21 and L25, on th other hand, appear to be more labeled in the presence of the alcohol.

When similar types of experiments were carried out using total proteins extracted from the 50 S subunits (TP50), no significant difference could be detected between proteins labeled in the two conditions, except for proteins L2 and L33 (table 2). It has to be mentioned that the data in the case of proteins L33 have to be taken with caution because they showed a considerable high variability.

4. Discussion

The results presented here clearly indicate a change of reactivity of several proteins when incorporated into the ribosomal structure due to the presence of 20% (v/v) methanol. This concentration of methanol is able to stimulate strongly the EF-G-dependent GTPase on 50 S subunits and on L7/12 deprived core particles [3,5,6] and also to uncouple the hydrolysis of GTP from the EF-Tu-dependent binding to aminoacyl-tRNA to the ribosome [7]. The simplest explanation for these results is the induction of a conformational change in the ribosomal structure by the alcohol, which alters the accessibility of some components to the reagent. This interpretation is confirmed by analysis of the proteins whose reactivity is affected. It turned out that 5 of the 9 proteins are primary RNA-binding proteins, namely L2, L17, L18 and L20 which binds 23 S RNA and L25 which binds 5 S RNA.

If the RNA-binding sites in the proteins are lysine rich regions of the molecule, as has been shown in protein S4 [14], it is conceivable that changes in the conformation of the tertiary structure of the rRNA might change the accessibility of these lysine residues.

These results are compatible with the idea that alcohols affect mainly the hydrophobic interactions which are important for the tertiary structure of the rRNA [10]. A direct effect on the protein–RNA interaction is less probable. However it is clearly

Table 2
Labeling of 50 S ribosomal subunits and free ribosomal proteins by reductive methylation – Effect of methanol

Protein	50 S subunits			Free proteins		
	$-CH_3OH$	$+CH_3OH$	$+CH_3OH$ / $-CH_3OH$	$-CH_3OH$	$+CH_3OH$	$+CH_3OH$ / $-CH_3OH$
	($^{14}C/^{3}H$)	($^{14}C/^{3}H$)		($^{14}C/^{3}H$)	($^{14}C/^{3}H$)	
L 1	1.48	1.47	0.99	1.34	1.40	1.05
L 2	0.62	0.51	0.83	1.09	0.82	0.75
L 3	1.01	1.00	1.00	1.48	1.44	0.97
L 4	0.54	0.52	0.96	0.70	0.76	1.10
L 5	1.06	1.14	1.07	0.92	0.85	0.92
L 6	0.65	0.68	1.04	0.82	0.72	0.86
L 7	2.40	2.17	0.90	1.34	1.29	0.96
L 9	1.04	1.11	1.07	0.89	0.81	0.91
L10	0.74	0.73	0.99	0.77	0.80	1.03
L11	1.79	1.79	1.00	0.88	0.80	0.90
L12	1.50	1.54	1.03	0.68	0.78	1.14
L13	0.35	0.40	1.15	1.15	1.09	0.95
L14	1.09	0.63	0.58	0.91	0.83	0.91
L15	0.16	0.07	0.45	0.95	0.92	0.98
L16	0.83	0.98	1.18	1.31	1.27	0.97
L17	0.16	0.12	0.73	0.64	0.59	0.91
L18	0.62	0.66	1.06	0.88	0.87	1.00
L19	1.71	1.23	0.72	1.40	1.48	1.06
L20	0.21	0.12	0.59	0.62	0.62	1.01
L21	1.67	2.06	1.24	1.22	1.16	0.95
L22	0.99	1.02	1.03	1.22	1.20	0.98
L23	0.71	0.63	0.89	1.15	1.08	0.94
L24	0.59	0.53	0.91	1.77	1.51	0.85
L25	2.29	3.08	1.34	1.31	1.40	1.07
L27	1.63	1.64	1.01	0.91	0.77	0.85
L28	0.27	0.20	0.76	0.89	0.92	1.04
L29	0.78	0.82	1.06	0.34	0.38	1.14
L30	0.85	0.94	1.10	0.53	0.60	1.14
L32	1.16	1.10	0.95	1.04	1.15	1.10
L33	5.93	5.87	0.99	1.67	3.06	1.83

Proteins from the same amount of ribosomes were labeled with [^{14}C]formaldehyde in the presence and in the absence of methanol and mixed with a fixed amount of ^{3}H-labeled proteins. After two dimensional gel electrophoresis the $^{14}C/^{3}H$ ratio was determined for every spot and the results were normalized considering one the $^{14}C/^{3}H$ ratio in the total protein mixture. This ratio was 0.277 and 0.18 as an average for samples treated in the absence and in the presence of methanol, respectively

shown, in agreement with [16], that free proteins can also be affected by the alcohol; for instance, protein L2, and since this is a RNA-binding protein, it cannot be excluded a direct participation of ribosomal proteins in the alcohol induced alterations of the ribosome.

It may be a waste of effort to try to correlate the proteins with altered reactivity and the ribosomal functions affected by the alcohols since conformational changes are probably long ranging. It might be significant, however, that two of the proteins affected, L2 and L15, have been related to peptidyl transferase by other techniques [15].

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References

[1] Monro, R. E. and Marcker, K. A. (1967) J. Mol. Biol. 24, 347–350.
[2] Maden, B. E. H. and Monro, R. E. (1968) Eur. J. Biochem. 6, 309–316.
[3] Ballesta, J. P. G. and Vázquez, D. (1973) Biochemistry 12, 5063–5068.
[4] Ballesta, J. P. G., Montejo, V. and Vázquez, D. (1971) FEBS Lett. 19, 79–82.
[5] Ballesta, J. P. G. and Vázquez, D. (1972) FEBS Lett. 28, 337–342.
[6] Hamel, E. and Nakamoto, T. (1972) J. Biol. Chem. 247, 6810–6817.
[7] Ballesta, J. P. G. and Vázquez, D. (1972) Proc. Natl. Acad. Sci. USA 69, 3058–3062.
[8] Hamel, E. and Nakamoto, T. (1972) Biochemistry 11, 3933–3938.
[9] Tompkins, R. K., Scolnick, E. M. and Caskey, C. T. (1970) Proc. Natl. Acad. Sci. USA 65, 702–708.
[10] Fox, J. W., Owens, D. P. and Wong, K. P. (1978) Biochemistry 17, 1359–1364.
[11] Kaltschmidt, E. and Wittmann, G. H. (1970) Anal. Biochem. 36, 401–412.
[12] Reisner, A. H., Nemes, P. and Bucholtz, C. (1975) Anal. Biochem. 64, 509–516.
[13] Hardy, S. J. S. (1975) Mol. Gen. Genet. 140, 235–274.
[14] Ehresmann, B., Reinbolt, J. and Ebel, J. P. (1975) FEBS Lett. 58, 106–111.
[15] Cantor, C. R., Pellegrini, M. and Oen, H. (1964) in: Ribosomes (Nomura, M. et al. eds) pp. 573–586, Cold Spring Harbor Lab, New York.
[16] Bull, H. B. and Breese, K. (1978) Biopolymers 17, 2121–2131.