EFFECT OF tRNA AMINOACYLATION ON ITS INTERACTION WITH RIBOSOMES IN THE ABSENCE OF MESSENGER.

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Summary

The adsorption of tRNA on the P-site of the 50S subunit was measured. Aminoacylated tRNA proved to bind much weaker than the deacylated one. At 5.10^{-3} M Mg^{2+} the ratio of corresponding equilibrium constants is 2.6, and at 0.015 M Mg^{2+} it increases till 7.

The influence of tRNA aminoacylation on its binding in the P-site of the ribosome is not clear up to now. The data are confusing. In papers (1,2) no difference was found but in (3) some influence is assumed based on indirect evidence.

In this work we showed that a significant difference does exist. Aminoacylation decreases the adsorption of tRNA on the P-site of a 50S subunit in the absence of messenger.

Materials and Methods

Adsorption of isotopically labeled tRNA on 50S subunits was measured by means of ultracentrifugation as described earlier(4) A mixture of labeled tRNA and ribosomes was centrifuged without dilution. The ribosomes sedimented to the bottom of the cell and the free tRNA remained in the supernatant. A part of the supernatant was extracted for the measurement of free tRNA by its radioactivity. The bottom layer was investigated for the amount of ribosomes and tRNA.Therefrom the adsorbed part of tRNA was computed, assuming that the concentration of free tRNA was equal to that in the supernatant

In the paper (4) the procedure of preparation and purificatio

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of ribosome subunits from the strain E.coli MRE 600 was described. $tRNA^A$ uniformly labeled with ^{32}P (^{32}P-tRNA) was prepared according to the same paper.

^{14}C-aatRNA was prepared by means of aminoacylation of unlabeled tRNA with a mixture of 10 aminoacids from Chlorella hydrolyzate uniformly labeled with ^{14}C. The specific radioactivity of the aminoacids was about 180 cpm per pmole. ^{14}C-aatRNA was extracted with phenol, precipitated with ethanol and preserved at -196°.

The idea of the experiment was to use a mixture of ^{14}C-aatRNA and deacylated ^{32}P-tRNA for the measurement of the ratio of dissociation constants of deacylated and acylated tRNA K_d/K_{ac}. If the adsorption of both types of tRNA is in accord with Langmuir's isotherm

$$A_{ac}=RM\frac{C_{ac}/K_{ac}}{1+C_{ac}/K_{ac}+C_d/K_d};\quad A_d=RM\frac{C_d/K_d}{1+C_{ac}/K_{ac}+C_d/K_d} \qquad (5)$$

where A_{ac} and A_d - are the adsorbed amounts of acylated and deacylated tRNA per mole of subunits(assuming that each subunit contains M specific sites common for both types of tRNA), C_{ac} and C_d- are the concentrations of free aminoacylated and deacylated tRNA respectively, R - the concentration of ribosomes. Hence we obtain

$$K_d/K_{ac}=\frac{A_{ac}/C_{ac}}{A_d/C_d}$$

Only relative radioactivities are needed to compute the right part of this equation and therefore it is superfluous to know the specific radioactivities of our preparations.This is very important since ^{32}P-tRNA is always diluted by some unknown amount of unlabeled deacylated tRNA contained in the preparation of ^{14}C-aatRNA,

We must take into account that some part of our ^{32}P-tRNA is unable to bind to ribosomes and introduce a correction. If the limiting percentage of bound ^{32}P-tRNA (at high ribosome concentration) is α, then $\frac{A_d}{C_d \cdot V} = \left(\frac{I_{ad}}{\alpha I_o - I_{ad}}\right)_{^{32}P}$, where I_o-is the total amount of ^{32}P-radioactivity in the sample, I_{ad}- the amount of adsorbed ^{32}P-radioactivity found on the ribosomes, V- the volume of the sample. The value of α depends on the quality of the preparation of ^{32}P-tRNA, it was approximately equal to 70%(4). In case of aminoacylated tRNA we can write respectively

$$\frac{A_{ac}}{C_{ac} \cdot V} = \left(\frac{I_{ad}}{I_o - I_{ad}}\right)_{^{14}C}$$

Taking the ratio of both we derive our final expression: $\left(\frac{I_o}{I_{ad}}\right)_{^{32}P} = \frac{1}{\alpha} \frac{K_d}{K_{ac}} \left(\frac{I_o}{I_{ad}} - 1\right)_{^{14}C} - \frac{1}{\alpha}$

A plot of $\left(\frac{I_o}{I_{ad}}\right)_{^{32}P}$ versus $\left(\frac{I_o}{I_{ad}} - 1\right)_{^{14}C}$ must yield a straight line. Its intersection with the axis of ordinate gives $1/\alpha$ and its slope $- 1/\alpha \cdot K_d/K_{ac}$. All runs were performed at 2°C in a standard buffer(0.01 M tris-HCl, pH 7.4, 0.05 M NH_4Cl), containing in most cases 0.015 M $MgCl_2$. In contrast to (4) the subunits were not annealed before use.

Radioactivities were measured by means of a scintillation counter.

RESULTS

The adsorption of ^{14}C-aatRNA on 50S subunits is totally prevented by an excess of deacylated tRNA (table I). We conclude therefore that both are adsorbed on the same single site on the 50S subunit and it must be the P-site(6).

On fig.I data for the adsorption are presented in a broad range

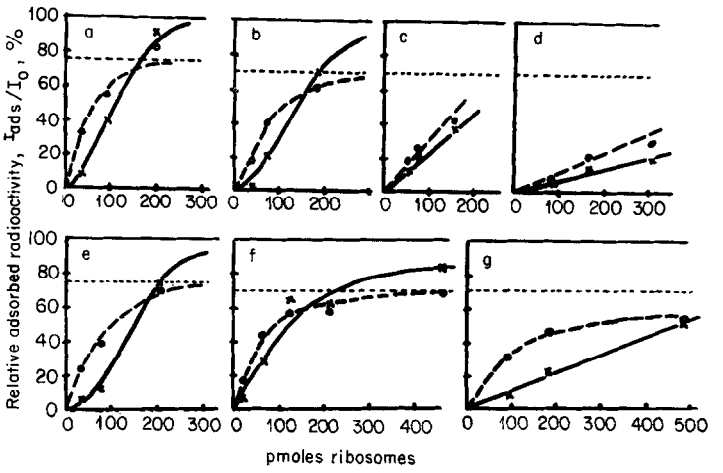

Fig.I Competitive adsorption of ^{14}C-aatRNA and ^{32}P-tRNA on 50S subunits at 2° in standard buffer.
Volumes of the probes (in ml): a,b,c - 0.07; c - 2.0; d - 0.15; e,f,g - 0.3.
Concentrations of Mg^{2+} (in M): a,b,c - 0.015; d,e - 0.027; f - 0.0075; g - 0.005.
Amount of ^{14}C-aatRNA in each sample: a,d - 210 pmoles (5800 cpm); b,c,d,e,f,g - 120 pmoles (7000 cpm).
Amount of ^{32}P-tRNA in each sample: a - 7.0 (5800 cpm); b - 74 (4300 cpm); c - 56 (7000 cpm); d - 7.0 (5000 cpm); e- 93 (4800 cpm); f - 93 (4000 cpm); g - 93 (3800 cpm).
To each probe the amount of subunits shown on the curves was added. The free and adsorbed tRNA were found as described in Methods. Relative amount of adsorbed ^{14}C-aatRNA: -x-x-x- ; the same for ^{32}P-tRNA: -o-o-o-. The horizontal broken line shows the limiting level of ^{32}P-tRNA binding measured directly (4).

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of concentrations of ^{14}C-aatRNA and ^{32}P-tRNA. Fig.2 shows that the predicted linear dependence $\left(I_0/I_{ad}\right)_{32P}$ on $\left(I_\alpha/I_{ad}-1\right)/c$ is confirmed. From this diagram we obtain α=0.65, which is in accord with direct measurements of ^{32}P-tRNA adsorption on ribosomes in excess(4).

Computed values of the ratio K_d/K_{ac} are presented on fig.3. We find that aminoacylated tRNA interacts with the P-site of 50S subunits weaker than deacylated, especially at Mg^{2+} concentrations higher than 0.01 M. With the decrease of Mg^{2+} concentration the differ-

Table I. Dilution of a ^{14}C-aatRNA bound to 50S subunits by a deacylated tRNA.

Number of probe	Amount of deacylated tRNA (pmoles)	^{14}C-radioactivity bound to the subunits (cpm)
I	0	I480
2	320	3I4
3	960	II7

Each sample contained in 0.3 ml: 85 pmoles of 50S subunits, 245 pmoles of ^{14}C-aatRNA (6500 cpm). To the probes 2 and 3 an amount of deacylated tRNA shown in the table was added.

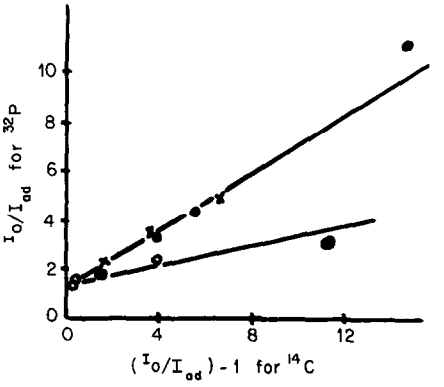

Fig.2 Determination of K_d/K_{ac} from the curves d-g of fig.I. -o-o-o- 0.027 M Mg^{2+} (d); -Θ-Θ-Θ- 0.027 M Mg^{2+} (e); -x-x-x- 0.0075 M Mg^{2+} (f); -●-●-●- 0.005 M Mg^{2+} (g).

rence in adsorption of aminoacylated and deacylated tRNA is leveled.

Selma et al(7)noticed that lincomycin is an inhibitor of adsorption of a N-acetylleucyl-tRNA-fragment on the P-site. We tried this antibiotic in our conditions and did not find any influence on the equilibrium of deacylated and aminoacylated tRNA. The explanation of this discrepancy may be in the difference of substrates and experimental conditions(lower tempera - ture, absence of ethanol in the medium).

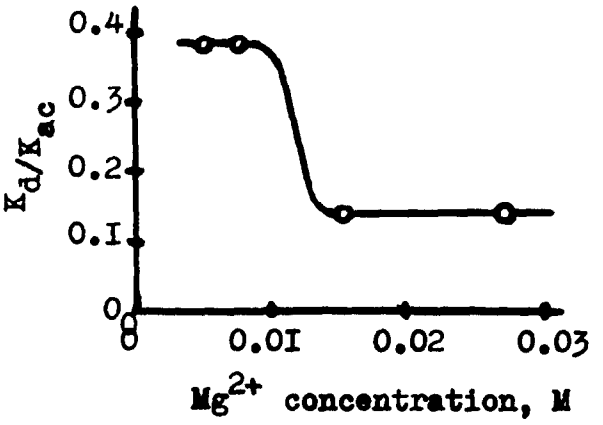

Fig.3 The ratio K_d/K_{ac} calculated from the data of fig.2.

DISCUSSION

H. Noll on the basis of his model of primitive initiation(3) made a prediction that aminoacylated tRNA would interact with the P-site much weaker than the deacylated. Our results, although obtained in the absence of messenger, are in accord with his expectations and can be regarded as an indirect confirmation of his idea.

The method used in these experiments allows to measure the equilibrium constants for the binding of individual peptidyl-tRNA's with ribosomes. This work is in progress in our laboratory.

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