CHEMICAL EVIDENCE FOR THE 3'-LINKAGE OF AMINO ACIDS TO S-RNA[+]

H.Feldmann and H.G.Zachau
Institut für Genetik der Universität Köln

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In an intermediate step of protein biosynthesis the amino acids are bound to s-RNA (Hoagland,Zamecnik and Stephenson, 1957); they are linked by an ester bond to the terminal adenosine (Zachau,Acs and Lipmann,1958; Hecht, Stephenson and Zamecnik,1959; Preiss,Berg et al.,1959). Model experiments (Wieland,Merz and Pfleiderer,1960; Nathans and Neidle,1963) and theoretical considerations (Zamecnik,1962) did not allow definite conclusions as to whether the linkage is to the 2'- or 3'-hydroxyl group of the adenosine. Chemical reactions with amino acyl adenosine isolated from amino acyl s-RNA with basic ion exchangers always yielded 1:1 to 1:2 mixtures of 2'-and 3'-derivatives (Rammler,Wolfenden and Lipmann,1963; McLaughlin and Ingram,1963;Frank and Zachau,1963).- We have recently described (Sonnenbichler,Feldmann and Zachau,1963) a method for the isolation of amino acyl adenosine using the weakly acidic ion exchange resin Amberlite IR-C 50.According to n.m.r.spectra measured in DMS, at least 95% of the amino acyl adenosine were the 3'-ester; only after prolonged standing was some 2'-ester formed. Although this evidence is conclusive, it would be desirable to have an independent chemical proof for the position of the linkage.

[+]Abbreviations: s-RNA = soluble ribonucleic acid, DMS = dimethyl sulfoxide, tosyl = p-toluene sulfonic, n.m.r.= nuclear magnetic resonance, c.p.s. = cycles per second, dpm = disintegrations per minute.

In the previous chemical experiments an acyl migration had apparently taken place during the isolation of the amino acyl adenosine from s-RNA and/or during the subsequent reactions. Acyl migrations are quite common to this type of compound (e.g.Rammler and Khorana,1963). We have now avoided acyl migration during the isolation of the amino acyl adenosine by using the chromatography on Amberlite IR-C 5o. Subsequently the substance was carried through the series of reactions (Fig.1), which was slightly modified from Frank and Zachau(1963).

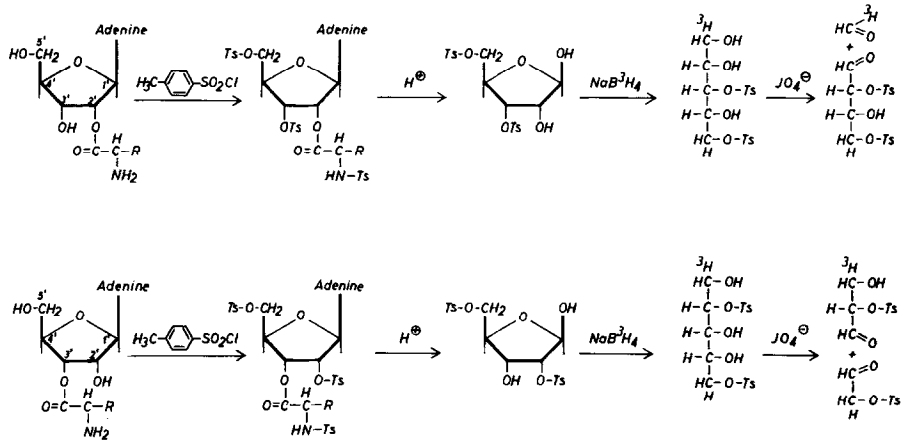

Fig.1: Reaction sequence for the localisation of the ester bond in amino acyl adenosine (Ts= p-toluene sulfonyl)

Synthetic valyl adenosine (Zachau,196o) was used as a model substance. Former tosylation experiments in pyridine as a solvent indicated that an approximately 1:1 mixture of 2'-and 3'-ester was present (Frank and Zachau,1963). By n.m.r.measurements in DMS on the other hand ,it was shown to be an approximately 1:2 mixture (Sonnenbichler et al.,1963). To clarify this discrepancy, it was checked, if an initially present 1:2 mixture of the isomers is converted to a 1:1 mixture by acyl migration in pyridine, which had to be used as a solvent

in the tosylation reaction. N.m.r.spectra of valyl adenosine were taken in pentadeutero pyridine (E.Merck,Darmstadt) using the techniques described by Sonnenbichler-et al.(1963). From the relative intensities of the shifted 1'-proton resonance signals (403 and 412 c.p.s.) it was concluded that also in this solvent the ratio of 2'-and 3'-ester is about 1:2 to 1:3; this ratio remains constant for at least 58 hours. We therefore reinvestigated the reaction sequence following the tosylation. The same preparation of valyl adenosine was taken as had been used for the n.m.r.spectra in DMS and pyridine. Oxidation of the starting material to remove a possible contamination of adenosine, tosylation, reduction with non-radioactive sodium borohydride, acid hydrolysis, reduction with labeled sodium borohydride and the periodate oxidation were done as before. The mono-and ditosyl ribitols were more extensively purified (2 ether extractions of the aqueous solution, 3 rechromatographies instead of one) in order to remove quenching material before scintillation counting. To ensure complete transfer of the oxidized material into the scintillation vial, water and subsequently dioxane were used as solvents. As can be seen in Table 1, the non-volatile fraction of the oxidized ditosyl ribitol[++)] corresponding to the 3'-amino acid ester represents 70 %, the average of two separate experiments. For the isolation of amino acyl adenosine the same yeast

++)Only the oxidation of the ditosyl ribitol gives reliable results. The volatile fraction of the oxidized monotosyl ribitol is found to be too high;model experiments with adenosine and 5'-trityl adenosine show that this is probably due to an incomplete initial tosylation.- Also, too high a volatile fraction is obtained, if prior to tosylation the 5'-hydroxyl group is protected (an alternative method in former experiments), probably because this reaction is not complete.Therefore in the experiments reported here, direct tosylation was preferred.

Table 1: Periodate oxidation of ditosyl ribitol derived from adenosine esters.

Substance	Experiment	Unoxidized sample+ (dpm)	Oxidized sample+ (dpm)	Non-volatile fraction (%)
Synthetic valyl adenosine	1	9144	6561	72
	2	9907	6728	68
Amino acyl adenosine from s-RNA	1	5204	4834	93
	2	4280	3930	92

+The oxidized and unoxidized aliquots of each experiment were flash evaporated (to remove $^{3}HCHO$) and prepared for counting in an identical manner. The counting efficiency (27-30 %) in the Tricarb 314 EX (Packard) was determined each time by internal standardization with ^{3}H-toluene. The accuracy of counting was ± 1.3 %.

s-RNA fraction (5g) and the same procedure were used as previously described (Sonnenbichler et al.,1963). In addition to ^{14}C-leucine, ^{12}C-glycine,-alanine,-valine,-isoleucine, -phenylalanine and -methionine,which had been used in the before mentioned paper, the following ^{12}C-amino acids were incorporated: serine, proline, lysine, arginine, histidine, threonine and tyrosine. The remaining amino acids do not give high enough incorporation rates in our system to contribute significantly to the amino acyl adenosine. The amino acyl s-RNA was processed as described in the quoted paper to yield 14 µmoles amino acyl adenosine. The sequence of reactions starting with tosylation up to the analysis of ditosyl ribitol was carried through in a manner completely analogous to the procedure described above for valyl adenosine. As shown in Table 1, the non-volatile fraction corresponding to the 3'-amino acyl adenosine represents 92.5 %, the average of two separate experiments.

In conclusion it can be said: chemical reactions show the amino acyl adenosine of s-RNA to be 92.5 % 3'-ester.According to n.m.r.spectra it is at least 95 % 3'-ester. Since after prolonged standing some 2'-ester is formed, as can be seen from n.m.r.spectra, it was concluded that the 3'-ester is the primary product. Similar considerations may be applicable to the results of the chemical reaction sequence.- The amino acyl s-RNA in the n.m.r. experiments was charged with 7, the amino acyl s-RNA for the chemical studies with 14 amino acids. The results, however, may perhaps be generalized for all 2o amino acids. Since the amino acyl s-RNA investigated here is biologically active in the back reaction and in the ribosomal system, it is concluded that in protein biosynthesis the amino acids are linked to the 3'-hydroxyl group of the terminal adenosine of s-RNA.

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