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Aminoacyl Position in Aminoacyl sRNA

Abstract. *Chromatographic systems capable of resolving the 2′- and 3′-isomers of valyladenosine and* N*-acetylvalyladenosine are used to show that valyladenosine isolated from valyl-sRNA is the equilibrium mixture of 2′- and 3′-acyl isomers by the time of chromatography. Acyl migration in* N*-acetylvalyladenosine is shown to be a rapid, base-catalyzed reaction with a rate 40,000 times the rate of hydrolysis under equivalent conditions. Calculations indicate that the pool of free aminoacyl sRNA exists in vivo as the equilibrium mixture of the 2′- and 3′-aminoacyl isomers.*

The discovery that the aminoacyl sRNA is bound in an ester linkage (*1*) to the 2′- or 3′-hydroxyl group of the terminal adenosine residue leads to the problem of determining whether the ester linkage is 2′- or 3′- in the aminoacyl sRNA pool in vivo. The position of the aminoacyl group has been the subject of some speculation (*2*), and three chemical methods (*3–6*) have been devised to determine the acyl position in aminoacyl adenosine released from aminoacyl sRNA by pancreatic ribonuclease. McLaughlin and Ingram (*4*, *7*) and Wolfenden, Rammler, and Lipmann (*6*) found that the distribution of the 2′- and 3′-isomers was the same for the aminoacyl adenosines isolated from sRNA and for those prepared synthetically. In the absence of suitable standard compounds (such as a pure 2′- or a pure 3′-aminoacyl adenosine) it is difficult to tell whether acyl migration to the equilibrium distribution occurred before or during the chemical determination of the aminoacyl position, or at both times. Furthermore, if acyl migration between the 2′- and the 3′- hydroxyl groups is rapid under the conditions of the chemical determination of the acyl position, then the blocking reagent itself, by preferential reaction with either the 2′- or 3′-hydroxyl, may result in an apparent shift in the equilibrium distribution in favor of the aminoacyl ester of the hydroxyl group which is less reactive towards the blocking agent.

To avoid these difficulties in the chemical methods of determining acyl position, we developed a series of chromatographic systems based on buffered ammonium sulfate solutions capable of separating the 2′- and 3′-isomers of several 2′ (and 3′-)-*O*-valyladenosine derivatives. Figure 1 shows the separations observed with thin-layer cellulose (*8*) chromatography of 2′ (and 3′-)-*O*-valyladenosine and 2′ (and 3′-)-*O*-(*N*-acetylvalyl)-adenosine. The separation of the 2′- and 3′-valyl-isomers is not complete, and in all solvent systems tested the 2′-isomer streaks into the 3′-isomer. Elution of the material in the spots as marked in Fig. 1 indicates about 85 percent of the 3′-isomer and 15 percent of the 2′-isomer, but because of streaking this may not be the true equilibrium value. These numbers are to be compared with our results by the phosphorylation method (*4*) which gave a value of 67 percent for the 3′-isomer of valyl-adenosine. On the other hand, the chromatographic separation of the 2′- (and 3′-)-*O*-(N-acetylvalyl)-adenosine is complete; the equilibrium distribution is 75 percent of the 3′-isomer. Furthermore, studies on 2′ (and 3′-)-*O*-valyladenosine-5′-phosphate and 2′ (and 3′-)-*O*-(*N*-acetylvalyl)-adenosine-5′-phosphate suggest that the normal equilibrium distribution and aminoacyl groups in sRNA under aqueous conditions around neutrality is likewise about 3 to 1 in favor of the 3′-isomer (*7*).

Valyl-C^{14}-sRNA (specific activity 205 mc/mmole) was prepared enzymatically and the valyl-C^{14}-adenosine fragment produced by ribonuclease treatment was isolated by electrophoresis. The initial incubation to form aminoacyl sRNA had been carried out at *p*H 8.45 at 37°C for 15 minutes. A Sephadex column (G-50, *p*H 5.5, 2°C, 45 minutes) was used to remove the excess valine-C^{14}; the aminoacyl sRNA was then incubated with pancreatic ribonuclease at *p*H 5.5 for 10 minutes at 37°C. The solution was chilled and adjusted to *p*H 3.0 with acetic acid; the precipitate was removed, and the supernatant was submitted to paper electrophoresis at *p*H 3.0 for 3 hours at 10°C with synthetic valyladenosine as a standard. The band for synthetic valyladenosine was located on the damp paper by ultraviolet light; the band was cut out, and the material was eluted with 0.01*M* formic acid at 2°C. The eluate was concentrated by rotary evaporation and applied to a thin-layer cellulose plate for chromatography. The result, 86 percent of the valyladenosine as the 3′-isomer and 14 percent as the 2′-isomer, indicated that valyladenosine as isolated had the equilibrium distribution of aminoacyl groups. Calculations indicate that for base-catalyzed migration our isolation system should cause over 100-fold less acyl migration than does the isolation system which Zachau and co-workers (*5*, *9*) had developed to prevent acyl migration. For acid- or water-catalyzed migration the situation is much the same. However, the calculations also indicate that most of the base-catalyzed acyl migration occurs during the initial incubation rather than

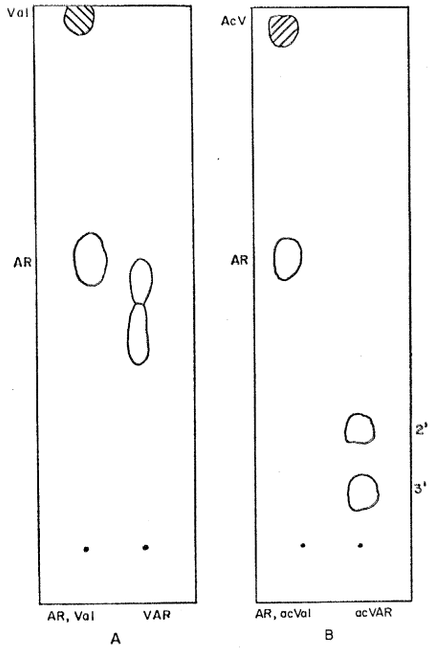

Fig. 1. Thin-layer cellulose chromatogram of 2′ (and 3′-)-*O*-valyladenosine (VAR) and 2′ (and 3′-)-*O*-(*N*-acetylvalyl)-adenosine (acVAR). Chromatographic solvent *A*: Saturated ammonium sulfate adjusted to *p*H 1.0 with concentrated sulfuric acid. Chromatographic solvent *B*: A mixture of saturated ammonium sulfate and 1*M* ammonium formate *p*H 3.2 (80 : 20). Abbreviations: AR, adenosine; val, valine; acVAL, *N*-acetylvaline. The 2′- and 3′-isomers are assigned on the basis the equilibrium distribution observed chromatographically and by phosphorylation.

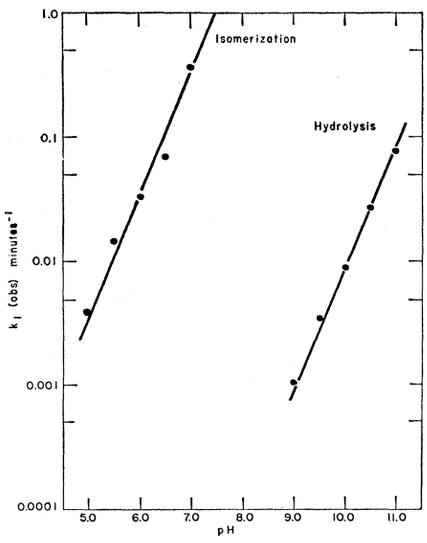

Fig. 2. Rate of isomerization and hydrolysis of 2′-*O*-(*N*-acetylvalyl)-adenosine as a function of *p*H at 15°C. The solid lines are calculated from the second-order rate constants given in the text.

during the isolation of the aminoacyl adenosine. If it is assumed that aminoacyl migration will show the same *p*H dependence as does the hydrolysis of aminoacyl sRNA (*10*), then we feel that so far as base-catalyzed migration is concerned, the initial incubation conditions for the present system and for that developed by Zachau and coworkers are very similar.

We have determined the rate of acyl migration in 2′-*O*-(*N*-acetylvalyl)-adenosine under various conditions. The band from a thin-layer chromatogram corresponding to 2′-*O*-(*N*-acetylvalyl)-adenosine (containing C^{14} in either the acetyl or the valyl group) was scraped off and eluted with 0.01*M* formic acid. The sample was divided into a number of small tubes, lyophilized to remove the volatile (formic acid) buffer, resuspended in appropriate buffer, and incubated at 15°C. Portions were removed at intervals, the volume of each was reduced, carrier *N*-acetylvalyladenosine was added, and the sample was again chromatographed. The first-order rate constants for migration k_1(obs) were obtained from a graph where log [percent 2′ (obs) − percent 2′ (eq)] was plotted against time for each *p*H value. Figure 2 shows a graph of the k_1(obs) values plotted as a function of *p*H. The rate dependence demonstrates that, at least over the range of *p*H 5 to to *p*H 7, migration is base-catalyzed and exhibits a strict dependence on hydroxide-ion concentration. Also plotted in Fig. 2 are the k_1(obs) values for the base-catalyzed hydrolysis of 2′ (or 3′-)-*O*-(*N*-acetylvalyl)-adenosine at 15°C (*7*). The second-order constants for the two rate equations at 15°C are:

rate = k_2 [2′ (or 3′-)-*O*-(*N*-acetylvalyl)-adenosine] [OH]

k_2(obs) migration = 3,600,000 M^{-1}min^{-1}

k_2(obs) hydrolysis = 89 M^{-1}min^{-1}

The ratio of the k_2(obs) for migration to the k_2 for hydrolysis is 40,000. This ratio is considerably higher than the ratio of the base-catalyzed migration to the base-catalyzed hydrolysis of β-glycerol acetate (*6*). Presumably this high ratio reflects the more favorable steric arrangement of the *cis* hydroxyl groups of adenosine for acyl migration (*6, 11*).

The similarity of the mechanism of migration (intramolecular alcoholysis) to alcoholysis and to hydrolysis (*12*) suggests that the aforementioned ratio of the rate of migration to the rate of hydrolysis may be used to obtain the $t_{\frac{1}{2}}$ (half-life) of migration for aminoacyl sRNA from the $t_{\frac{1}{2}}$ of hydrolysis of various aminoacyl sRNA molecules (*13*). In this way the values for $t_{\frac{1}{2}}$(obs) of migration at *p*H 7.25 and 37°C are estimated to range from 0.01 second for glycyl sRNA to 0.1 second for valyl sRNA. This very rapid migration to the equilibrium mixtures of 2′- and 3′-acyl isomers suggests that in the cell the pool of free aminoacyl sRNA as an intermediate in protein synthesis is present as an equilibrium mixture of 2′- and 3′-acyl isomers, as has also been suggested by Wolfenden *et al.* (*6*). The acyl migration lifetimes are less than, or on the same order as, the estimated lifetimes of aminoacyl sRNA during protein synthesis (*14*); indicating that migration to equilibrium must take place in vivo. At present there is no experimental evidence concerning the initial site acylation of sRNA, while studies on puromycin analogs (*15*) indicate that the 3′-isomer is used at the ribosomal level.

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Rubella Antibodies in Human Serum: Detection by the Indirect Fluorescent-Antibody Technique

Abstract. *Antibodies for rubella virus were detected in human serum and titrated by the indirect method of immunofluorescence; a chronically infected, continuous line of monkey kidney cells was used as antigen. Positive reactions were obtained with serums from convalescent patients or persons who had been exposed to the virus while serums from patients in the acute stage of the disease and those from unexposed individuals were negative.*

The demonstration and titration of antibodies for rubella virus has been dependent upon the neutralization technique which relies upon either the interference of the rubella agent grown in tissue culture with another virus, ECHO-11 (*1*), or the inhibition of specific cytopathogenic activity in primary