Hydrolysis of tRNA^{Phe} on Suspensions of Amino Acids

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Dedicated to Professor *Edgar Heilbronner* on the occasion of his 80th birthday

RNA is adsorbed strongly on suspensions of many moderately soluble organic solids. In some cases, the hydrolysis of tRNA^{Phe} is greatly accelerated by adsorption, and the major sites of hydrolysis are changed from those that are important in homogeneous solution. Here we show that the hydrolysis is greatly accelerated by suspensions of aspartic acid and β-glutamic acid but not by suspensions of α-glutamic acid, asparagine, or glutamine.

Introduction. – The non-enzymatic hydrolysis of RNA has been studied extensively, especially because of its relevance to the mechanisms of action of ribozymes [1–4] and to biotechnology and therapy [5–22]. Many ribonucleases, ribozymes, and non-biological catalysts function *via* acid-base catalysis of an intramolecular transesterification mechanism in which the 2′-OH group attacks the adjacent phosphate group [14][23]. The pentacoordinated phosphorane intermediate may collapse back to starting material, or yield isomerized or cleaved products [12][14][16][18][24].

It is well-known that metal ions, for example, Pb^{II}, Mg^{II}, Mn^{II}, Zn^{II}, Co^{II}, Ni^{II}, Cu^{II}, and Eu^{II} catalyze the hydrolysis of RNA [25–40]. The specific and highly efficient cleavage of the D-Loop of tRNA^{Phe} by Pb(II) ions has been investigated by crystal-structure analysis [28][29]. The potential to prepare artificial RNases and the need to elucidate the catalytic mechanism of protein enzymes has led to a number of studies of novel cleavage reagents [5][8][9][13][17]. Most of these compounds are based on metal ions, but organic multifunctional catalysts, such as di- and polyamines, imidazole-based cleaving agents, bis(guanidinium)-based cleaving agents, and bis(carboxylate)-based agents, also show some promise [14][41]. Studies on the hydrolysis of RNA by imidazole and related buffers have been carried out in an attempt to elucidate enzymatic mechanisms [42–45]. Catalysis by imidazole and morpholine buffers has been proposed as a model of RNase action, although catalysis is modest even in concentrated imidazole or morpholine solutions.

Two factors have been shown to influence the efficiency of catalysis. The first is the availability of groups that deprotonate the 2′-OH group of ribose or protonate the phosphate oxygen atom or the 5′-oxyanion leaving group. The second is the maintenance of the proper orientation of the 2′-OH group relative to the leaving group. Buffer catalysts are required in very high concentrations because they do not stabilize the productive conformation.

We have found that a variety of oligonucleotides and nucleic acids are strongly adsorbed from aqueous solution onto the surfaces of crystals of many modestly soluble

organic compounds. Adsorption seems usually to be due to the hydrogen bonding, but stacking may be important in some cases [46]. Here we describe the hydrolysis of $tRNA^{Phe}$ on the surfaces of crystals of several polar amino acids.

Results. – In our preliminary experiments, we found that $tRNA^{Phe}$ is adsorbed on suspensions of α-aspartic acid (α-Asp), α-glutamic acid (α-Glu), β-glutamic acid (β-Glu), histidine (His), glutamine (Gln), asparagine (Asn), and tyrosine (Tyr) with very high efficiency (more than 95%) under our experimental conditions. The tRNA is eluted only slightly by pyrophosphate ions, suggesting that non-electrostatic interactions, for instance, hydrogen bonding interactions, could play an important role in binding. Presumably, the adsorption of RNA on the solid surface increases the concentration of potentially catalytic groups in the neighborhood of the cleavable phosphodiester bonds.

Fig. 1,a, illustrates the cleavage patterns obtained in the presence of the β-glutamic acid and the enantiomers of α-Asp and α-Glu in the absence of $MgCl_2$. Comparison with the control lane (*Fig. 1,a*, *Lane 8*) shows that each amino acid, whether in homogeneous aqueous solution or in suspension, catalyzes the hydrolysis of $tRNA^{Phe}$. In other experiments, we showed $tRNA^{Phe}$ is not significantly hydrolyzed in simple buffers adjusted to pHs 3–4. The catalytic hydrolysis of RNA by dicarboxylic acid in solution has been reported [41], while hydrolysis by carboxylate buffers follows second-order kinetic [44][47][48]. It is not surprising, therefore, that amino acids such as Asp and Glu in solution induce some hydrolysis (*Fig. 1,a*, *Lanes 2*, *4*, *6*, *10*, and *12*). Interestingly, the presence of solid leads to accelerated hydrolysis in some (*Fig. 1,a*, *Lanes 5*, *9*, and *11*) but not all cases (*Fig. 1,a*, *Lanes 1* and *3*).

After 2 days at 22° in presence of suspended solid, *ca.* 80% of $tRNA^{Phe}$ was hydrolyzed by D-Asp (*Fig. 1, a*, *Lane 11*), *ca.* 40% by L-Asp (*Fig. 1, a*, *Lane 9*), and *ca.* 50% by β-Glu (*Fig. 1, a*, *Lane 5*). Hydrolysis by β-Glu (*Fig. 1, a*, *Lane 5*) gives a unique pattern of products with major cleavages after C13, C60 and C61, and minor cleavages are after other pyrimidine bases and after Y37. Apart from the cleavage after Y37, which occurs also in solution, the pattern of products is that anticipated from the primary sequence specificity of hydrolysis, cleavage always occurs after a pyrimidine ribonucleotide. A similar pattern ($pyrimidine_pA$) of sequence specificity had been shown as a spontaneous hydrolysis of tRNA in solution [49][50]. Major products found in the presence of a suspension of D-Asp (*Fig. 1, a*, *Lane 11*), the most effective cleavage agent, correspond to cleavage after residue U8 (50%) and residue C13 (20%). Minor products correspond to cleavage after residues C28, C61, C63, and Y37. Hydrolysis in the presence of a suspension of L-Asp (*Fig. 1, a*, *Lane 9*) is much less effective than with the D-Asp. The most striking effect of suspended L-Asp is to bring about extensive cleavage after Y37.

The hydrolysis reactions discussed above occur in unbuffered solutions at acidic pHs (3.3–3.6) and in the absence of Mg^{2+} or monovalent cations. Under these conditions, the $tRNA^{Phe}$ must be denatured, which probably explains why cleavage occurred almost entirely after pyrimidine residues. Hydrolysis is specifically catalyzed by the amino acids both in solution and on the solid surface, since no hydrolysis is observed at pH 3.5 in a simple buffered solution.

Fig. 1, b, illustrates the effect of 10 mM $MgCl_2$ on the cleavage of $tRNA^{Phe}$ by the various dicarboxylic acids. Strikingly, the specific hydrolysis induced by the suspended

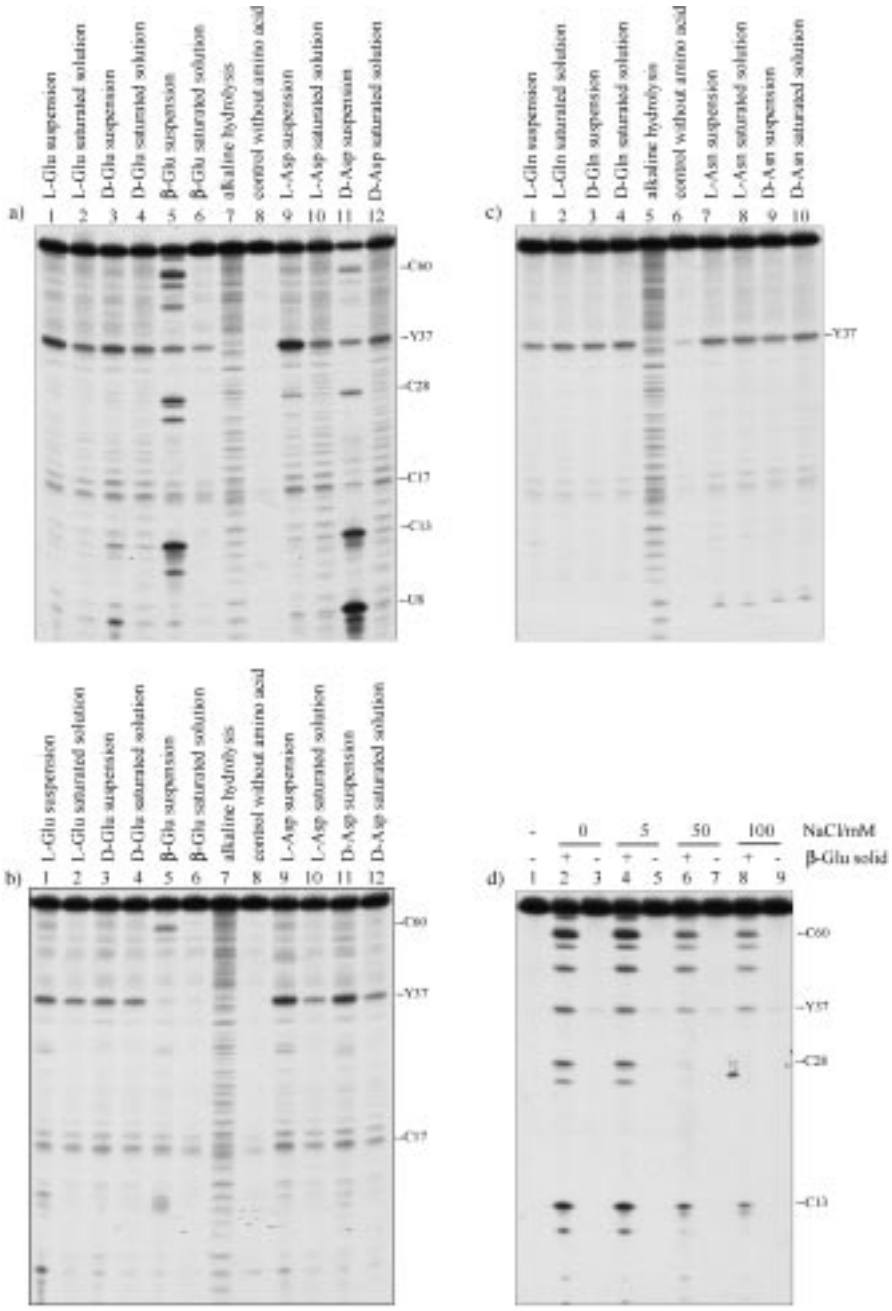
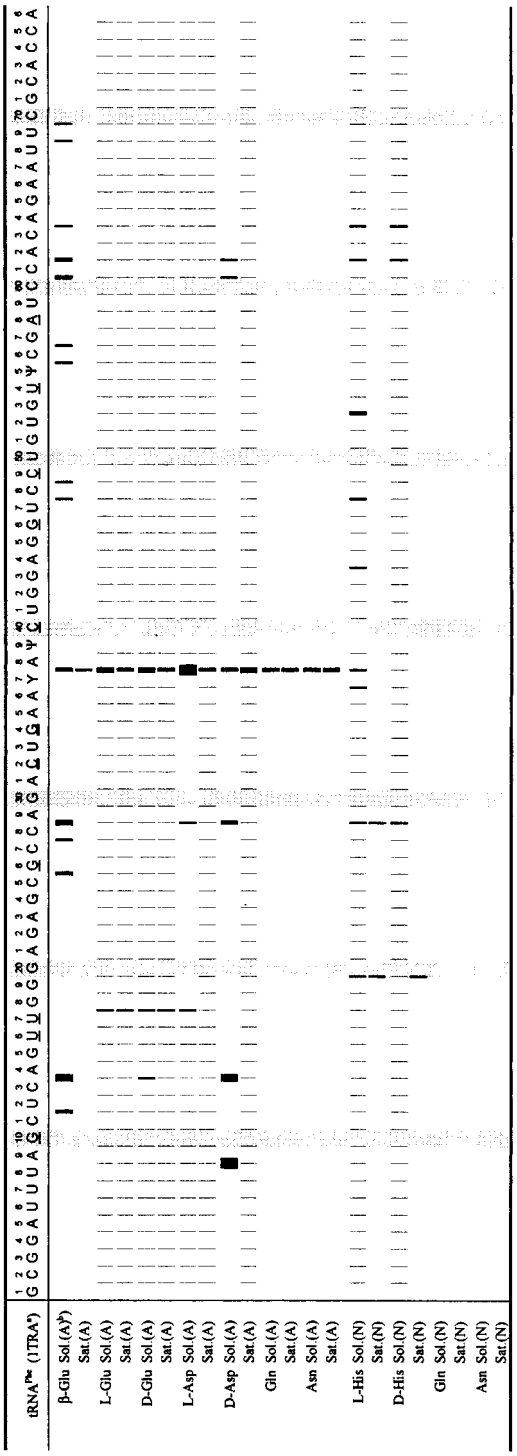


Fig. 1. *Autoradiographs of 12% polyacrylomide gels of the hydrolysis products of tRNA^{Phe} glutamic and aspartic acids, and their derivatives under acidic conditions. a*) Hydrolysis by glutamic and aspartic acids under acidic conditions. *b*) Hydrolysis by Glu and Asp under acidic conditions in the presence of 10 mM $MgCl_2$. *c*) Hydrolysis by Gln and Asn under acidic conditions (pH 3.5). *d*) Hydrolysis of tRNA^{Phe} by β-Glu under acidic conditions in the presence of varying concentration of NaCl. *Lane 1:* starting material; *Lanes 2, 4, 6,* and *8:* β-Glu suspension and 0 mM, 5 mM, 50 mM, and 100 mM NaCl, respectively; *Lanes 3, 5, 7,* and *9:* NaCl concentrations as in *Lanes 2, 4, 6*, and *8*, but with β-Glu-saturated solution.

Table. *Cleavage of tRNA^{Phe} by the Solid Surfaces of Some Amino Acids* (the thickness of the lines corresponds to the efficiency of the hydrolysis)



[a]) The sequence of tRNA^{Phe} is from PDB databank (1TRA). The thin underline indicates a modified base, and the thick underline indicates a methylated ribose [51] [52].
[b]) Sol. stands for the hydrolysis pattern at presence of solid, and Sat. stands for the hydrolysis pattern in amino acid-saturated solution. A: acidic condition; N: neutral condition.

solids is completely suppressed. *Fig. 1, d*, shows that increasing concentrations of NaCl bring about increasing protection from hydrolysis of $tRNA^{Phe}$ by suspension of β-Glu (*Fig. 1, d*, *Lanes 2*, *4*, *6*, and *8*), but the effect is relatively weak.

The effects of Mg^{2+} or NaCl are not easily explained. In every case, we have confirmed that the RNA is almost completely adsorbed and that the presence of $MgCl_2$ or NaCl has not changed the pH of the solution. The most plausible function of the Mg^{2+} is to associate with carboxylate groups on the solid surfaces, thus suppressing catalysis by undissociated carboxylic acid groups. It is surprising that this mechanism is so efficient at low pH, since the association of isolated carboxylate groups with Mg^{2+} is weak at low pH. Perhaps the carboxylate groups in the surface are oriented in such a way as to permit chelation. A second mechanism for protection of RNA would be the stabilization of some form of secondary or tertiary structure. The protection against hydrolysis by 100 mM NaCl (*Fig. 1, d*) suggests that this mechanism may be relevant, although the stabilized structure at pH 3–4 is unlikely to be the biologically active structure.

Fig. 1, c, illustrates the products of hydrolysis in the presence of the enantiomers of Asn and Gln at pH 3.5. The only significant cleavage site with these amino acids, whether in suspension or in saturated solution is after residue Y37. A comparison of *Fig. 1, a* and *c*, shows that hydrolysis by the free acids must be dependent on the presence of carboxylate groups, while the data in *Fig. 1, a*, shows that the β-carboxylate groups are probably responsible for the accelerated hydrolysis.

The hydrolysis of RNA on the surface of dicarboxylic acids is necessarily studied at low pH. It seemed interesting to work with a neutral or basic amino acid, so that the behavior of undenatured $tRNA^{Phe}$ could be observed. We chose His on account of the wide range of reactions catalyzed by imidazole groups. The results of experiments performed with saturated solution or suspensions of D- and L-His at pH 7.5 are illustrated in *Fig. 2*. As anticipated, His in saturated solution accelerates the hydrolysis of $tRNA^{Phe}$ (*Fig. 2*, *Lanes 4* and *6*) and solid His brings about a much greater acceleration (*Fig. 2*, *Lanes 3* and *5*). Under neutral conditions, Gln and Asn have almost no impact on the hydrolysis of $tRNA^{Phe}$, even in the presence of solid suspensions (data not shown).

Discussion. – The results described above indicate that the ability of the functional groups on a surface to bring about the hydrolysis of adsorbed $tRNA^{Phe}$ depends on the detailed match between the surface structure and the RNA structure. This is seen most clearly in the differences between the efficiencies of hydrolysis by suspended D- and L-Asp. Since only the chirality of the solid changes, only the orientations of the polymer with respect to functional groups on the surface can be responsible for the differences. It is also interesting that suspensions of the β-dicarboxylic acids (L-aspartic, D-aspartic and β-glutamic) are effective catalysts, while suspensions of γ-dicarboxylic acids (D- and L-Glu) are no more effective than their saturated solutions. It would be interesting to see whether this generalization holds true for a larger sample of dicarboxylic acids.

The surfaces of organic crystals comprise a very large ensemble of arrays of ordered organic functional groups. In some ways, this ensemble resembles the family of protein surfaces, but with one essential difference – protein surfaces have evolved under natural selection, while organic crystals have surface structures determined by simple

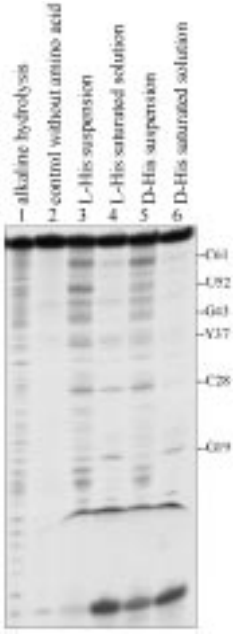


Fig. 2. *Autoradiographs of 12% PAGEs for the hydrolysis of tRNA^{Phe} by His, Gln, and Asn under neutral conditions*

physical interactions. It is interesting to ask how often a random surface approaches a selected enzyme in binding affinity and catalytic power. The approach that we have described might begin to answer these questions.

Experimental Part

All chemicals were purchased from commercial sources, unless otherwise noted. The amino acids, L-Glu, D-Glu, β-Glu, L-Asp, D-Asp, L-His, D-His, L-Gln, D-Gln, L-Asn, and D-Asn, were ground to fine powders before use. [γ-^{32}P]ATP was purchased from *Amersham Life Science*, calf intestinal alkaline phosphatase from *Boehringer Mannheim*, T4 polynucleotide kinase from *New England Biolabs*, and Nuclease T_1 from *USB*.

Denaturing gel electrophoresis (PAGE) was run on 10, 12, 15, or 20% polyacrylamide gels (8M urea) in TBE (tris-borate-EDTA) buffer and visualized by autoradiography on film (*Kodak*). Loading samples were prepared by combining equal volumes of the reaction mixture and 10M urea. Before loading, each sample was incubated at 95° for 5 min and chilled on ice. Bromophenol blue and xylene cyanol were used as markers.

Preparation of [5′-^{32}P]-Labeled tRNA^{Phe}. Phenylalanine-specific tRNA from yeast (*Sigma*) was dephosphorylated by alkaline phosphatase, and purified by 10% gel electrophoresis. The 5′-dephosphorylated tRNA^{Phe} was 5′-end-labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP. The [5′-^{32}P]-labeled tRNA^{Phe} was purified again by gel electrophoresis and precipitated by alcohol. A T_1 ladder was prepared by partial hydrolysis of [5′-^{32}P] labeled tRNA^{Phe} (1,000,000 cpm in 50 µl) with RNase T_1 (0.05 unit) at 37° for 5 min. A hydroxyl ladder was prepared by partial hydrolysis of [5′-^{32}P]-labeled tRNA^{Phe} (1,000,000 cpm in 50 µl) with 50 mM $NaCO_3$/$NaHCO_3$ (pH 9.2) at 90° for 5 min.

Cleavage of tRNA^{Phe} on Solid Surfaces of Amino Acids. Amino acid powder (1 mg; 2 mg in case of His and Gln) was suspended in 20 µl of a soln. containing 10 mM *Tris*-HCl (pH 7.4), and 5 mM NaCl or KCl. Additional NaCl or $MgCl_2$ was added as required. The pHs of the solns. in contact with solid Asp and Glu were in range of 3.3 – 3.6. The pH of the solns. in contact with His was *ca.* 7.5. The final pHs of the solns. in contact with Asn and Gln were adjusted to 3.5 or to 7.5. The solns. were vortexed and centrifuged. Then, aliquots of the supernatant (10 µl) were transferred to tubes containing dried [5′-^{32}P]-labeled tRNA^{Phe} (300,000 cpm) to provide control

experiments with sat. solns. of the amino acids, but without the suspended solids. The solid in the main reaction soln. was resuspended by vortexing, and transferred to a tube containing dried [5′-^{32}-P]-labeled $tRNA^{Phe}$ (300,000 cpm). The reaction and control solns. were vortexed and tumbled at 22° for 2 d and then quenched by the addition of 200 µl of buffer containing 10 mM EDTA, and 0.089M *Tris*-borate (pH 8). The mixtures were then vortexed until any solid was completely dissolved. An aliquot (10 µl) of the resulting soln. was mixed with 10 µl of 10M urea, and analyzed on 12% polyacrylamide sequencing gels. The positions of cleavage of the $tRNA^{Phe}$ were determined by comparison with the T_1 and hydroxyl ladders, with different percentage gels and different running times as necessary.

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