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A mechanism for the RNA-catalyzed formation of 5′-phosphates

The origin of nucleases

Robert Cedergren, B. Franz Lang and Denis Gravel+

Département de biochemie and +*Département de chimie, Université de Montréal, Montréal, Québec H3C 3J7, Canada*

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Processes involved in RNA metabolism can be distinguished by the nature of the sugar phosphate substitution (5′ or 3′) in intermediates or products. Although it is known that 3′-phosphates are produced via a 2′,3′-cyclic phosphate intermediate, formed by nucleophilic attack on the phosphodiester bond by the adjacent 2′-OH, little is known about the production of 5′-phosphate products. We attribute 5′-phosphate intermediates and products to a preferred configuration of the pentavalent phosphorus intermediate resulting from the attack of a distant nucleophile. This intermediate is favored, since its formation is possible without major conformational changes in the molecule. Based on the two products of nucleic acid hydrolysis we define: the conjunct and disjunct nucleophile mechanisms, each of which would have independent origins. Indeed, the products of an overwhelming number of nucleases and RNases are consistent with one of these mechanistic models demontrating that the origin of these enzymes are deeply rooted in the intrinsic chemistry of phosphate esters.

Splicing; Reaction mechanism; Nuclease evolution; Phosphate; RNA

1. INTRODUCTION

The totally unexpected finding that some RNAs have catalytic functions has shaken some of the fundamental concepts in biochemistry [1]. In particular, how can the 5′-phosphate substitution in some RNA splicing intermediates [2] and RNA processing products [3] be rationalized in view of the fact that 3′-,2′- and some 2′,3′-cyclic phosphates are the products of ribonuclease and base hydrolyses of oligoribonucleotides? Many data show that these latter hydrolyses rely on a nucleophilic attack at a phosphodiester bond by an adjacent 2′-OH. Because of the geometrical restrictions imposed by the ribose ring and the requirement for apical attack [4], the 2′-hydroxyl must approach the phosphorus opposite to the 5′-P-O bond to give the trigonal-bipyramid intermediate **1** (scheme 1). The apical C5′ fragment is then ejected as shown in a model compound by Westheimer and associates [5] and verified recently by Kluger and Thatcher [6]. This mechanism predicts the observed 2′,3′-cyclic phosphate and the 2′- or 3′-phosphate products [7]. Crystallographic analysis of the lead (II) hydrolysis of tRNA, which gives 2′,3′-phosphate products as well, shows that the reaction proceeds by nucleophilic attack on phosphorus by an adjacent 2′-OH, which is activated by lead ions [8].

Contrary to this scheme, RNA splicing and processing, which are transesterification/substitution reactions, involve the selective formation of 5′-phosphate by ejection of the 3′-fragment. In

Correspondence address: R. Cedergren, Dept of Biochemistry, or D. Gravel, Dept of Chemistry, University of Montreal, Montreal, Quebec H3C 3J7, Canada

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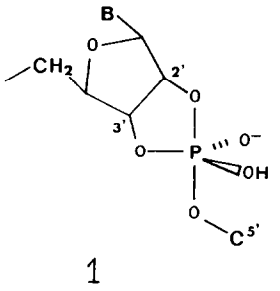

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Scheme 1. The trigonal-bipyramid intermediate.

trying to rationalize this fact, we have first considered steric and electronic effects. Although the 5′-hydroxy, being a primary alcohol, would normally be a better leaving group than the 3′-hydroxyl due to its greater acidity, the acidity and thus the leaving group ability of the 3′-OH is augmented because of hydrogen bonding possibilities with the adjacent 2′-OH. The 3′-OH would generally be a better leaving group because of the greater steric hindrance in a secondary alcohol. However in a polymer, steric hindrance is likely to be very similar for both leaving groups. Thus, steric and electronic considerations do not allow for an unequivocal choice between the possible products.

2. LEAST MOTION MECHANISM

Since the above reasoning did not seem completely satisfactory, we pursued the matter further by reconstructing different stages of the reaction with molecular models, bearing in mind that trigonal-bipyramid intermediates of phosphorus are formed by apical attack and decompose by apical loss [4]. These models illustrate that during attack, conversion of the tetrahedral phosphate to a corresponding trigonal-bipyramid intermediate as represented in fig.1 requires rather large

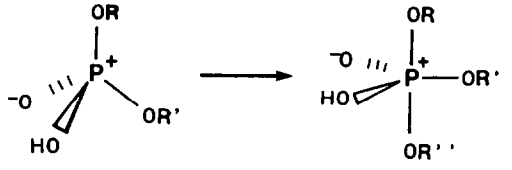

Fig.1. Atomic displacements involved in the orbital rehybridization in going from a tetrahedral phosphate to a trigonal bipyramid.

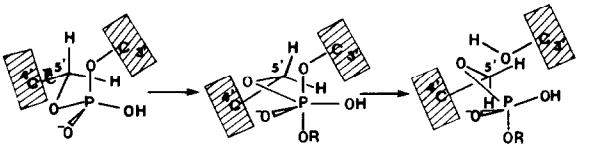

Fig.2. Proposed mechanism leading to C3′ displacement. A nucleophilic attack on a typical phosphate link leads to the trigonal-bipyramid intermediate. As indicated in the text, the C4′, C5′ and P nuclei do not undergo any translation in space during formation of the trigonal-bipyramid intermediate as would be the case in an attack opposite the O5′. The second step in the mechanism involves expulsion of the C3′ fragment, which can also be accomplished with 'least motion' by continuation of the movement involved in the first step of the mechanism (the swivel motion). The product is consequently the 5′-phosphate observed in the splicing intermediate and in RNA processing.

displacements by some atoms in order to accommodate the angle change of approx. 20° (109°–90°) at the phosphorus atom. However, when attack takes place opposite the 3′-P-O bond, this angle change is greatly facilitated by the C4′-C5′ link of the ribose moiety, which allows orbital rehybridization at phosphorus without affecting the spatial positions of the ribose, the phosphorus or the C5′ substituent. Only the oxygen and the two C5′ hydrogen atoms are required to partially rotate around the C5′-C4′ bond in a swivel-type motion (fig.2).

In contrast, should attack take place opposite the 5′-P-O bond, then electronic reorganization at phosphorus would require concomitant nuclear motion of the furanose ring atoms (except C3′ and C4′) along with their appendages. This pathway would thus need appreciably more energy to form the trigonal bipyramid, and proceeding on to product would demand major conformational changes in the molecule. The 'least motion principle' [9–11] would strongly favor an attack opposite the 3′-P-O bond, since only this geometry would lead most easily to the intermediate and product with the least conformational change. The fact that the substrate is polymeric and less able to undergo major displacements in space than simple molecules would reinforce the preference for attack opposite the 3′-P-O bond. This same argument implies that pseudorotation in the

trigonal-bipyramid intermediate, which is possible in the case of simple phosphate esters, is most unlikely in the polymer [4]. Although consideration of potential stereoelectronic effects [12–16] in this reaction partly supports the mechanism suggested here (fig.2), this effect cannot be responsible for the observed specificity of the products.

A recent experiment from Cech's laboratory [17] strongly supports our mechanism. In a nucleotidyl transfer reaction catalyzed by L-19 IVS RNA, a sequence of 5 deoxycytidylic acid residues followed by a ribocytidylic acid (dC_5-rC) is cleaved at the internal phosphate attached to a deoxyribose. If the adjacent 2′-OH played a role in the mechanism of hydrolysis as required by arguments related to the leaving group ability, its absence should eliminate the reaction. However, the reaction proceeds normally as our mechanism would predict.

Taken together the above arguments imply that there are two major mechanistic schemes by which phosphate may undergo hydrolysis/replacement: (i) the conjunct or adjacent nucleophile mechanism which is driven by the nucleophilic attack on phosphate by the adjacent 2′-hydroxyl group giving 2′,3′-cyclic phosphates and (ii) the disjunct or distant nucleophile mechanism based on nucleophilic attack by any nucleophile other than the 2′-OH (or 5′-OH, see below) and generally promoted by structural elements distant to the target phosphate. This latter mechanism produces 5′-phosphate products or intermediates.

3. PHOSPHORUS STEREOCHEMISTRY

The proposed disjunct mechanism predicts inversion of the tetrahedral phosphate configuration during nucleophilic displacement as in the nuclease S_1 hydrolysis of DNA which produces 5′-phosphates and involves phosphate inversion [18]. In the case of RNase P processing, the intermediate formed after nucleophilic attack would eject the C3′ fragment with inversion of the phosphorus configuration. However, if the nucleophile is other than a hydroxide ion (or activated water), a second step and inversion is needed to replace the nucleophile with a hydroxide ion. A two-step hydrolysis therefore leads necessarily to a 5′-phosphate product with overall retention of configuration, similar to the cases of T_4 ligase and snake venom phosphodiesterase reactions [19,20]. Retention in venom phosphodiesterase hydrolysis is particularly relevant, since, as in the case of RNase P hydrolysis, no intermediate had been suspected previously. Consequently, the configuration of the 5′-phosphate product of processing would provide a valuable clue to the nature of the nucleophile. An inverted phosphate product would favor a mechanism based on a direct attack by a hydroxide ion, even though this event is unlikely based on the pH dependence of the reaction [21].

4. THE ORIGIN OF PROTEONUCLEASES

Clearly one of the major attractions of RNA catalysis has been concerned with a possible relationship between present-day examples and possible primordial functions. Projections into the past have crystallized around the theme of an all 'RNA world' where informational and catalytic properties are found in the same RNA molecule. Although this primordial world may never be proven or disproven formally, predictions based on its existence can be evaluated. One such prediction deals with mechanisms of proteonucleolytic cleavage.

According to the 'RNA world' conjecture, proteins or at least encoded proteins would originate after RNA and as their diversity and sophistication increased they could eventually assist and, later, occasionally displace RNA molecules from their catalytic function. Since these 'new arrivals' would be integrated into existing processes, the fundamental atomic mechanism and stereochemistry of a given reaction would not change. Thus, modern nucleases should reflect these origins by demonstrating catalytic mechanisms rooted in the intrinsic chemistry of RNA of phosphate esters as shown above. In fact, the products derived from an overwhelming number of nucleases are consistent with one of the two mechanisms of phosphate hydrolysis above [22]. Most RNases would be classified as category I, conjunct nucleophile processes. Category II (disjunct nucleophile case) would regroup restriction enzymes, RNase H and many varied nucleases and phosphodiesterases.

Only nucleases which produce deoxyribonucleotide 3′-phosphates could not conform to either category, because this product would normally depend on the presence of an adjacent

2-OH. Such is the case of spleen phosphodiesterase, an exonuclease producing 3′-phosphates. Interestingly, this 'exception' may only be a special case of category I nucleases for, if the attacking nucleophile were the 5′-terminal hydroxyl, then intermediate **1** above would be obtained, except that the cyclic phosphate would be a six-membered 3′,5′- rather than the 'normal' five-membered 2′,3′-intermediate. This proposal is supported by the strict requirement for a terminal, free 5′-OH.

Other exceptions, such as the endonucleases producing deoxyribonucleoside-3-phosphates, have no obvious explanation. Nevertheless, in the discussion above relating the disjunct nucleophile mechanism, we have made an assumption that pseudorotation (translation of substituent between the apical and axial position) does not take place. As the stability of the transition state is increased, a common result of enzyme catalysis, pseudorotation could take place and account for these possibilities.

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