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Native transfer RNA catalyzes Diels–Alder reaction

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Abstract

In this paper we show that transfer ribonucleic acids (tRNAs) catalyze the Diels–Alder cycloaddition reaction. A new DNA oxidative damage product, 6-furfuryladenine (kinetin) or its riboside (diene), was transformed with dimethyl acetylenedicarboxylate or maleic anhydride (dienophile). The reaction proceeds in the presence of tRNA at high pressure but not at ambient condition. If so tRNA in prebiotic conditions (RNA world) had at least two functions: catalytic and a carrier of genetic information. It means that tRNA at high pressure shows catalytic properties and is a true Diels-Alderase.

Keywords: RNA world; Diels–Alder reaction; RNA catalysis; Transfer RNA; High pressure; Kinetin

The RNA world is defined as a hypothetical ancestral era in which ribonucleic acid (RNA) carried out the earliest chemistry for various metabolic pathways. That idea has got strong support after the first catalytic RNAs (ribozymes) were discovered and later on many naturally occurring ribozymes have been characterized. They efficiently catalyze a synthesis and cleavage of phosphodiester bonds, stimulate an aminoacylation of RNA, promote glycosidic and amide bond formation, hydroxyl phosphorylation, alkylation, and acyl transfer [1]. Ribozymes have a large potential for treating various diseases ranking from cystic fibrosis to muscular dystrophy and sickle cell anemia [2]. All those activities strongly support a view that in an early life catalysis was performed by RNA rather than by proteins [1]. The prebiotic world can be traced with in vitro selection (SELEX) approach by searching for RNA with new binding properties or catalytic activities [3–7]. Various RNAs have been identified, which catalyze a wide range of chemical reactions including a reduction of carbon–carbon double bonds via alkylation and synthesis of six-membered carbon ring via Diels–Alder reaction [8]. RNAs were an essential prerequisite for a complex ancestral metabolism reaction, although they have been selected at ambient conditions, different from those in prebiotic world [7]. It is known that high temperature and pressure strongly affect structure, properties, and interactions of RNA. It suggests a difference between a higher-order structure of RNA evolved with SELEX and that existed in early steps of evolution. To go deeply into RNA world, we designed Diels–Alder reaction of 6-furfuryladenine (kinetin), a well-known DNA damage constituent (diene) [9] with two dienophiles: maleimide anhydride or dimethyl acetylenedicarboxylate in the presence of native transfer RNA. It is the main component of the protein biosynthesis machinery. The first native RNA for which the crystal structure has been solved and many functional data were collected [10,11]. tRNA does not show catalytic properties by itself, although yeast $tRNA^{Phe}$ is the first known leadzyme acting in cis [12]. In this paper we showed that 3-billion-year-old naturally evolved tRNA molecule has ribozymic properties in addition to those involved in translation of the genetic code. These unusual capabilities of tRNA can be explained with its unique structure acquired at extreme conditions. tRNA forms a scaffold which hold together substrates.

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Materials and methods

Kinetin (6-furfuryladenine) or its riboside (diene) was used for reaction with maleic anhydride or dimethyl acetylenedicarboxylate (dienophile). Equimolar (0.1 mM) amounts of the both reagents were dissolved in water and mixed up with mung bean tRNA 250 μg (6 OD) [13]. Pressuring was done in 1 ml Teflon tubes, specially designed for high-pressure experiments [14], using High Pressure U101 apparatus (Unipres Warsaw) at 10 kbar for 10–12 h.

Reaction products were analyzed by low-resolution mass spectra. They were recorded on AMD 402 two-sector mass spectrometer (AMD Intectra, Germany) of B/E geometry. High-resolution data were obtained with the same instrument using a peak-matching technique. Molecular mass of the observed ions was determined with an error of less than 10 ppm in relation to Perfluorokerosene (Fluka, Switzerland) at a resolving power of 10,000. A sample of reaction products was injected directly into the mass spectrometer working in EI mode (70 eV; 0.5 mA total emission current) with an accelerating voltage of 8 kV, at source temperature of 300 °C and inlet temperature of 70 °C.

Yeast $tRNA^{Phe}$ from Behringer was labeled at 3′-end with [^{32}P]Cp and T4 RNA ligase (2 U) (Pharmacia) [13]. Labeled tRNA was purified by electrophoresis on 15% polyacrylamide gel with 7 M urea. A cleavage reaction of yeast $tRNA^{Phe}$ with Pb^{2+} was carried out in total volume of 50 μl of 10 mM Tris–HCl, pH 7.5, buffer containing 40 mM NaCl. Reactions were done at ambient and high pressure (10 kbar) for 2–4 h in room temperature. The reaction was stopped with 0.1 M EDTA and analyzed with electrophoresis on 15% polyacrylamide gel with 7 M urea. [^{32}P]$tRNA^{Phe}$ was partially cleaved with (0.1 U) RNase T1 (Pharmacia) in 20 mM sodium citrate pH 5.0 buffer containing 7 M urea and 1 mM EDTA at 55 °C for 20 min. tRNA Ladder was obtained by formamide hydrolysis with 0.1% EDTA at 100 °C for 15 min [13].

Results and discussion

Diels–Alder is a reaction of diene and dienophile, promoted by heat, pressure, acids, antibodies, micelles, medium, template effects, and reverse encapsulation [15]. There is a simultaneous movement of six electrons with breaking three bonds and forming three new linkages [4]. The reaction can proceed in organic solvents as well as in water, which activates a dienophile and form of a transition state [3]. Diels–Alder reaction is facilitated by electron donating and withdrawing groups on the diene and dienophile, respectively. Recently some of the in vitro selected RNAs show abilities to catalyze the Diels–Alder reaction [6–8]. It has been shown that synthesis of biotin maleimide with RNA–PEG–anthracene [6] is accelerated about 18,500-fold by a 49-nucleotide long RNA molecule isolated from the combinatorial RNA library [16]. That RNA has catalytic properties. Generally ribozymes have different lengths, variable nucleotide sequences as well as a various three-dimensional structure formed at ambient conditions. Because RNA does not contain titriable functional groups at physiological pH, its catalytic properties are due to metal ions located in its catalytic center. That observation got strong support by finding that ribosome and splicosome are ribozymes [17,18].

We put forward question whether contemporary native transfer RNA is able to work beyond RNA world and catalyze the Diels–Alder reaction. As a diene, we used 6-furfuryladenine, a modified base of DNA showing cytokinin properties [9]. We showed that kinetin and its riboside react with maleic anhydride (II) as well as dimethyl acetylenedicarboxylate (III) (Fig. 1). These reactions are catalyzed with tRNA at high pressure of 10 kbar. Reaction of kinetin (I) with 215 m/z and maleic anhydride yields an adduct (III) with 313 m/z (Fig. 1A), but with dienophile (IV), produces compound (V) of 357 m/z (Fig. 1B). The structure of V was proved with a high-resolution mass spectrum, which shows mass signal of 357.10649 (calculated 357.10733, permissible error—10.0 ppm, recorder error 2.4 ppm). A reaction of kinetin riboside with dimethyl acetylenedicarboxylate gives the product of 489 m/z with higher yield than for maleic anhydride. These products are not formed (no corresponding mass signals) at high pressure without high pressure (not shown). It seems that a conformation of tRNA induced at high pressure meets structural requirements of a transition state of Diels–Alder reaction. It provides complementarity of size and shape of both substrates and is a driving force in molecular recognition. tRNA shows stable three-dimensional structure at ambient condition but changes significantly under pressure [19]. Recently conformational changes of RNA as well as DNA at high pressure have been observed [19,20]. They were induced by new water structure [21] as well as by lowering pH. To trace formed effects of high pressure on tRNA, we applied specific cleavage of yeast $tRNA^{Phe}$ with lead. That reaction takes place only when Pb ion is co-ordinated in ribothymidine loop in a close distance to sensible bond of the residue 17 of D loop. A detailed mechanism of Pb-cleavage at ambient conditions involves $(PbOH)^+$ co-ordinated to U59 and C60, which abstracts proton from the 2′OH groups of D17 to facilitate a phosphodiester bond hydrolysis with cyclic phosphodiester intermediate formation [12]. However D loop of yeast $tRNA^{Phe}$ is not hydrolyzed by Pb^{2+} ions at high pressure (Fig. 2, lanes 4–6), probably because a compact structure of $tRNA^{Phe}$ stabilized by hydrogen bonds between D and T loops is disrupted. This was also identified by CD spectra [20]. We think that tRNA at high pressure forms a kind of scaffold to which kinetin is docked by stacking interaction with backbone residues of RNA. This allows dienophile to be aligned directly on top of diene and give endo or exo products when diene and dienophile staggered with respect to each other [4]. Analogous situation one can find within the ribosome, where 23S rRNA surrounded by ribosomal proteins acquires unique conformation which is able to catalyze a synthesis of peptide bond [17,18]. It is also possible that an intermediate RNA conformation in tRNA catalysis involves cation–π interaction between diene and dienophile [22–24]. The crucial role of water structure in Diels–Alder reaction catalyzed by tRNA at high pressure is suggested by lack of reaction product of

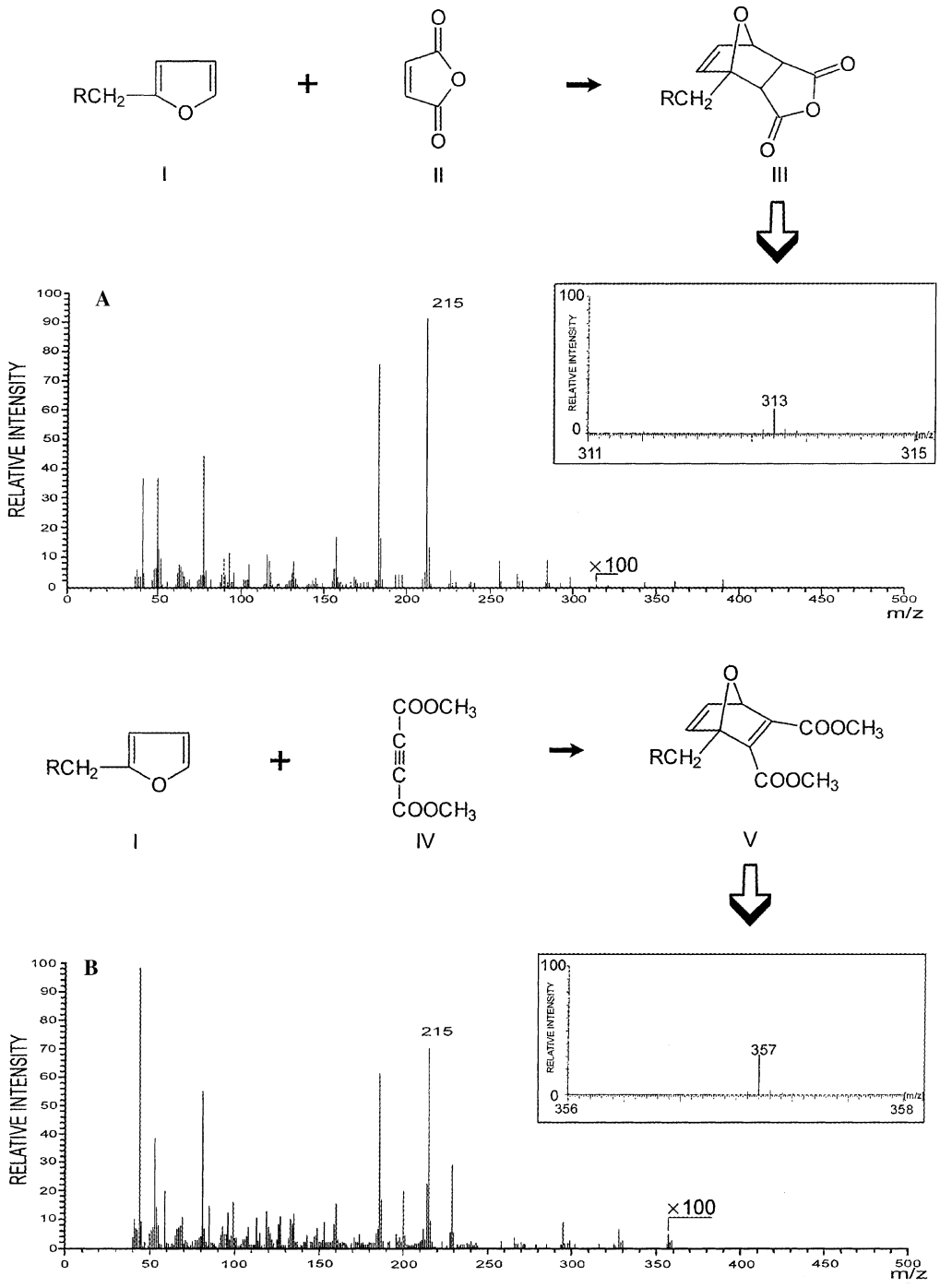

Fig. 1. Cycloaddition (Diels–Alder) reaction of 6-furfuryladenine (kinetin I) with maleic anhydride (II) or dimethyl acetylenedicarboxylate (IV) at high pressure (HP) in the presence of tRNA, R—adenine; Electron impact mass spectrometry analysis of kinetin reactions with maleic anhydride shows signal 313 m/z corresponds to adduct III (upper spectrum) (A) and with diemethyl acetylenedicarboxylate shows that signal 357 m/z, identified as the adduct V (B).

furan with dimethyl maleic anhydride at high pressure (unpublished). Two methyl groups of anhydride not only decrease its dienophilicity but also increase hydrophobic as well as steric effects, which prevent synthesis of cantharidin [25] because these methyl groups disturbed the formation of a new water structure.

Mechanism of tRNA catalysis at high pressure is very similar to that shown by catalytic antibodies. The immune system generates various de novo antibodies, which also function as Diels-Alderase [26,27]. A complementarity between enzyme and transition state is the key issue of biological catalysis. The X-ray structure of the complex shows how antibodies bind to the diene and

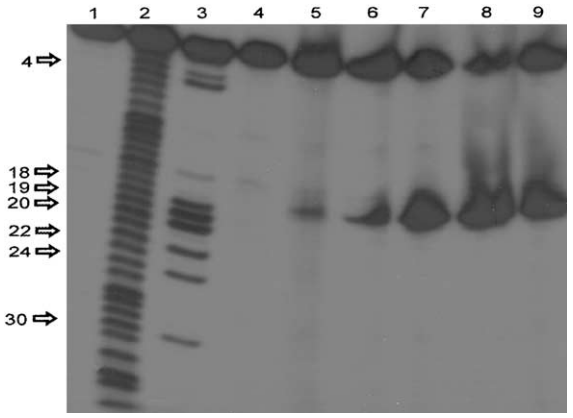

Fig. 2. The autoradiogram of 15% polyacrylamide gel with 7 M urea showing Pb^{2+} induced hydrolysis products of [3′ $-^{32}$ P]$tRNA^{Phe}$: lane 1: control $tRNA^{Phe}$ in T1 buffer at 55 °C for 20 min; lane 2: formamide ladder; lane 3: limited hydrolysis of $tRNA^{Phe}$ with RNase T1 at 55 °C for 20 min; lanes 4–6: hydrolysis reaction products of $tRNA^{Phe}$ at high pressure (10 kbar), respectively, in the presence of 2 nM Pb^{2+} (lane 7), 5 nM Pb^{2+} (lane 8) for 2 h, and 2 nM Pb^{2+} (lane 9) for 4 h; lanes 7–9: hydrolysis reaction products of $tRNA^{Phe}$ at ambient pressure, respectively, in the presence of 2 nM Pb^{2+} (lane 4), 5 nM Pb^{2+} (lane 5) for 2 h and 2 nM Pb^{2+} (lane 6) for 4 h.

dienophile [28]. The last one is positioned by hydrogen bonding and π-stacking interactions with the maleimide ring [24]. The diene is bound in a hydrophobic pocket in close proximity to the dienophile with the position of the carbamate substituent fixed by a water-mediated hydrogen bond to Trp [28]. Evolution has permitted enzymes to attain their catalytic efficiency. In the case of tRNA catalysis we suggest that water at high pressure is responsible for changes in the structure of tRNA, which forms a transition complex with substrates. It is clear that native transfer RNA at high pressure plays a role of Diels-Alderase.

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