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On the Free-Energy Changes in the Synthesis and Degradation of Nucleic Acids†

Leonard Peller

ABSTRACT: Standard free-energy changes for reactions involving single- and double-stranded nucleic acids have been related to that for polynucleotide synthesis from ribonucleoside diphosphates for which $\Delta G^{\circ\prime} \approx 0$. For polynucleotide formation from triphosphates this quantity is about -1 kcal. In the replication reaction the base pairing interactions are quantitatively of comparable importance. Production of a hydrolytic break in a double strand is substantially less favorable than in a single strand. The resealing of breaks utilizing ATP and NAD^+ have similar free-energy changes and are entropy driven processes. The highly exergonic hydrolysis of pyrophosphate is maintained to be of significance for both in vivo and in vitro polymerizations.

The free-energy changes accompanying enzyme-catalyzed synthetic and degradative reactions of nucleic acids have received little systematic experimental investigation. Nevertheless, the limited available data allow estimates to be made of these thermodynamic quantities for a number of important reactions. While the thermodynamics is of intrinsic interest, it is our view that considerations of this nature may be of greater significance in understanding these processes than has generally been assumed.

The results and analysis presented here are conveniently divided into two categories. Information in the first category concerns the thermodynamics of reactions involving single-stranded nucleic acids. These results when combined with thermodynamic data on the coil to helix transition provide estimates of companion quantities for reactions in the second category, double-stranded nucleic acids. Finally, the reactions involved in nucleic acid synthesis are examined in relation to the hydrolysis of the pyrophosphate by-product.

The reactions to be considered all involve phosphoric acids, esters, and anhydrides. It has long been recognized that such equilibria exhibit a marked dependence on pH and divalent metal ion concentration, e.g. Mg^{2+} (Podolsky and Sturtevant, 1955; Podolsky and Morales, 1956). We have adhered to the common practice of choosing a standard state of 1 *M* concentration of the total equilibrium distribution of ionic forms of each reactant species present at pH 7.0 with Mg^{2+} equal to 10^{-3} *M* and ionic strength of 0.15 at 25°C. As data are not always at hand for this state, we have occasionally been compelled to combine results obtained under somewhat different conditions. From this difficulty as well as from inherent experimental variations an uncertainty of ±1 kcal must attend all the primary results quoted below. However, even a wider incertitude would not seriously impair the main conclusions of this report as to the relative importance of the various contributions to the driving forces for the reactions of double-stranded nucleic acids.

Single-Stranded Nucleic Acids

Polynucleotide Synthesis from Nucleoside Diphosphates. The enzyme polynucleotide phosphorylase catalyzes the readily reversible reaction (a) depicted in Figure 1. The equilibrium constant for the addition of one nucleotide can be obtained in the manner first employed for the analogous primer initiated polysaccharide synthesis from glucose 1-phosphate with orthophosphate also the product (Trevelyan et al., 1952). With a given ribonucleoside diphosphate (rNDP) as reactant this quantity can be computed from the ratio of the equilibrium concentrations of orthophosphate to the unreacted monomer, i.e.

$$K_{1a} \approx [P]_{eq}/[rNDP]_{eq} = [P]_{eq}/\{[rNDP]_0 - [P]_{eq}\} \quad (1)$$

where $[rNDP]_0$ is the initial concentration of this latter species.

Some years ago this was shown to be tantamount to requiring that the probability (p) of propagation for the equilibrium most probable distribution where

$$p = ([rNDP]_{eq}/[P]_{eq})\, K_{1a} \quad (2)$$

approach unity (Peller, 1961; Peller and Barnett, 1962). This is the condition for achievement of high degrees of polymerization. Obviously the choice of values of p somewhat

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(a) $(pN)_{i-1} + rNDP \rightleftharpoons (pN)_i + P$

(b) $(pN)_{i-1} + dNTP \rightleftharpoons (pN)_i + PP$

(c) $(pN)_i + H_2O \rightleftharpoons (pN)_{i-1} + NMP$

FIGURE 1: Reactions of polynucleotide chains. The length of the arrow for the reverse reaction roughly conveys the degree of reversibility.

less than one will not greatly reduce the estimated equilibrium constant.

Analysis of the kinetics of polymer synthesis on a fixed number of initiator or primer sites has shown that this ratio can reach its limiting equilibrium value far in advance of the time of attainment of the broad chain size distribution characteristic of the complete equilibrium (Miyake and Stockmayer, 1965). Put another way, a relatively narrow Poisson distribution of chain sizes can persist for some time with an equilibrium number average degree of polymerization before the onset of the redistribution of chains brought about by the reverse reaction. Such kinetic questions merit further examination for these systems.

Under the usual conditions of primer initiated polymerization, the polynucleotide phosphorylase reaction plateaus in time with the ratio in eq 1 lying between one and four (Grunberg-Manago, 1963). The magnitude of this ratio appears to increase with the anticipated degree of stacking of the nucleotide bases. With UDP as the substrate this quantity is close to unity, consistent with the apparent lack of secondary structure of poly(uridylic acid) (Felsenfeld and Miles, 1967). We therefore take $K_{1a} \approx 1$ and $\Delta G^\circ{}_{1a}' \approx 0$ as characteristic of the reaction uncomplicated by base–base interactions.

Polynucleotide Synthesis from Nucleoside Triphosphates. Reaction (b) indicated in Figure 1 with deoxyribonucleoside triphosphates as the monomeric sources and pyrophosphate as the product is catalyzed by terminal deoxynucleotidyltransferase and appears to be less reversible than (a). By recourse to the argument cited above a value of K_{1b} where

$$K_{1b} = [PP]_{eq}/\{[dNTP]_0 - [PP]_{eq}\} \quad (3)$$

of approximately 100 has been reported with a reaction mixture containing deoxyadenosine triphosphate (Kato et al., 1967).

A less direct method of determining this quantity exploits the fact that reaction (b) of Figure 1 is the sum of reaction (a) of that figure and the *difference* between the two reactions written in eq I and II. (Here as elsewhere in this report differences between ribo- and deoxyribonucleotides are ignored.) Hence, the free-energy change of this reaction can be written as:

$$ATP + H_2O \rightleftharpoons AMP + PP \quad (I)$$

$$ADP + H_2O \rightleftharpoons AMP + P \quad (II)$$

$$\Delta G^\circ{}_{1b}' = \Delta G^\circ{}_{1a}' + \Delta G^\circ{}_{I}' - \Delta G^\circ{}_{II}' \approx \Delta G^\circ{}_{I}' - \Delta G^\circ{}_{II}' \quad (4)$$

This reaction emphasizes that the differences between the polymerization free energies of nucleoside triphosphates and diphosphates must be reflected in an equivalent difference between the free energies of hydrolysis of ATP and ADP. We might note that the adenylate kinase catalyzed disproportionation reaction with an equilibrium constant of the order of unity ensures that the free energy of hydrolysis of ATP to yield ADP and orthophosphate must also be

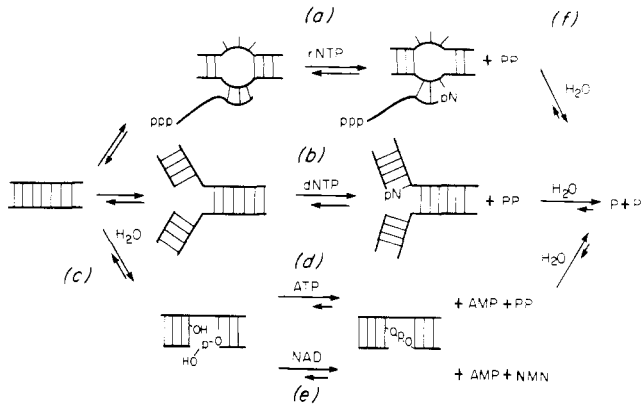


FIGURE 2: Synthetic and degradative reactions of dobule-stranded polynucleotides with coupled hydrolysis and repair steps. The same convention as in Figure 1 applies for the degrees of reversibility.

closely equivalent to that of reaction II. Consequently, the difference between the free-energy changes of polymerization utilizing nucleoside triphosphates vs. diphosphates comes down to a difference between the free energies of hydrolytic rupture of the α,β and the β,γ phosphoanhydride linkages of ATP.

From a recent comprehensive analysis of the thermodynamic parameters involving adenosine phosphates (Alberty, 1969), we can interpolate the values $\Delta G^\circ{}_{I}' = -9.7$ kcal and $\Delta G^\circ{}_{II}' = -8.7$ kcal. Thus, $\Delta G^\circ{}_{1b}' \approx -1$ kcal. This is a somewhat smaller value for this quantity than was obtained previously using the same argument (Peller, 1966).

An earlier completed survey yields free-energy changes for reaction I lying between −7.7 and −10.3 kcal (Jencks, 1970). From the adenylate kinase near isoergonic equilibrium, this same source would suggest free-energy changes for reaction II varying from −7.3 to −9.6 kcal. These comparable ranges of values also argue for a small difference between $\Delta G^\circ{}_{I}'$ and $\Delta G^\circ{}_{II}'$.

Lastly, one widely read text quotes a free-energy change for this polymerization of *plus* 0.5 kcal of undisclosed provenance (Watson, 1970). More perplexingly still this reference suggests values of −8 and −6 kcal for reactions I and II which by the above argument would require $\Delta G^\circ{}_{1b}' \simeq -2$ kcal.

Our tentative conclusion is that the synthesis of polynucleotide chains utilizing nucleoside triphosphates has only a modestly favorable standard free-energy change which we will take to be −1 kcal.

Hydrolytic Scission of a Polynucleotide Chain. Exonucleolytic cleavage of a nucleotide unit is depicted in reaction (c) of Figure 1. This hydrolysis is clearly seen to be the difference between reaction II and reaction (a) of this figure. Consequently:

$$\Delta G^\circ{}_{1c}' = \Delta G^\circ{}_{II}' - \Delta G^\circ{}_{1a}' \approx \Delta G^\circ{}_{II}' \approx -9 \text{ kcal} \quad (5)$$

We note further that the free-energy change accompanying endonuclease action must be close to that for exonuclease attack. Any contribution due to the interruption of base stacking interactions should be virtually the same for both processes. Only differences in long-range electrostatic effects of the phosphate backbone could alter this equality.

Thus, hydrolysis of a phosphodiester bridge is approximately equivalent energetically to that of a "high energy" phosphoanhydride linkage. In marked distinction to this is the better than 15 kcal more negative free energy of hydrolysis of carboxylic acid anhydrides than carboxylic acid es-

ters (Jencks, 1970).

Double-Stranded Nucleic Acids

RNA Synthesis from Ribonucleoside Triphosphates. For reaction (a) depicted in Figure 2, the RNA transcript is only transiently bonded to one strand of the DNA template. There is no stabilization of the product through the establishment of persistent base pairs. Consequently, the *net* free-energy change for a step in the transcription process must be the same as that for the synthesis of single-stranded polynucleotides discussed in the previous section, i.e. $\Delta G°_{2a}' = \Delta G°_{1b}' = -1$ kcal.

This synthetic process requires no special initiator or primer species (Chamberlin, 1974). Of necessity then, high degrees of polymerization cannot be procured by raising the ratio of monomeric source to primer molecules (Peller and Barnett, 1962). The presence of high molecular weight chains in this system (Dunn and Studier, 1973) is clearly a kinetic phenomenon arising from the fact that the establishment of the first few phosphodiester bonds occurs more slowly than the subsequent bond synthesis steps (Peller, 1975).

DNA Synthesis from Deoxyribonucleoside Triphosphates. The replication reaction shown as reaction (b) in Figure 2 involves the formation of two product double strands from a single parental bihelix. There is thus a net increase of one base pair for every two phosphodiester bonds formed. The free-energy change for the extension by one nucleotide of a *single* progeny chain can be written as the sum of two contributions.

$$\Delta G°_{2b}' = \Delta G°_{1b}' + \Delta G°_{BI}/2 = \Delta G°_{1b}' - (RT/2) \ln s \quad (6)$$

Here $\Delta G°_{BI}$ is the free energy change derived from the interaction of neighboring base pairs and s is the corresponding equilibrium constant for the addition of a base pair to a growing bihelical structure (Zimm, 1960). Recent estimates of this free-energy change corresponding to the propagation step in the coil to helix transformation derived from studies of oligoribonucleotide association range from -1 to -5 kcal depending on the base pairs involved and their nearest neighbors in the double helix (Borer et al., 1974). A reasonable average value would be $\Delta G°_{BI} = -3$ kcal.

Combining the previous result of $\Delta G°_{1b}' = -1$ kcal with this average free-energy change for double helix formation, we find that $\Delta G°_{2b}' = -2.5$ kcal. Noting again that this parameter will show considerable variation due to the differences in base interaction, we can conclude two things from this simple argument. Firstly, the portion of this free-energy change derived from the base stacking interactions is at least of comparable importance to that arising from covalent bond formation. Secondly, as this contribution is always negative where the double helix is stable, one can state that irrespective of the magnitude of $\Delta G°_{1b}'$ (within the limits discussed above) the replication process must occur with a decrease in free energy.

Hydrolytic Scission of a Double-Stranded Polynucleotide. The introduction of a hydrolytic break in one strand of a double helical polynucleotide must certainly be less favorable thermodynamically than such a cleavage in a single strand. This difference arises because reaction (c) of Figure 2 does not lead to physically separated chains as does an endonucleolytic reaction in a single polynucleotide chain.

The free-energy change for this process can also be represented as the sum of two contributions:

$$\Delta G°_{2c}' = \Delta G°_{1c}' - RT \ln \sigma_0 \quad (7)$$

The first term in eq 7 is the free energy of hydrolysis of a single strand given previously. The second term accounts for the continued state of proximity of the two chains after hydrolytic rupture. It involves the equilibrium constant (here designated σ_0) for the establishment of the *first* base pair in the association of two polynucleotide chains.

A simple argument can be constructed to justify the above quantitative form for this term. The effective equilibrium constant for the association of two complementary homopolymeric strands leading to an intact double helix of N base pairs is $\sigma_0 s^{N-1}$. The corresponding equilibrium constant for the association of *two* strands with a third strand whose degree of polymerization is the sum of its two complements is $\sigma_0^2 s^{N-1}$. The free energy difference between these two association processes is then

$$-RT \ln \sigma_0^2 s^{N-1}/\sigma_0 s^{N-1} = -RT \ln \sigma_0$$

which is the second term of eq 7. If the chains are sufficiently long, one anticipates that s^{N-1} can be replaced by the appropriate partition function for the double helix so that the above result should apply even within the helix to coil transition region. This estimate does presume that the base–base interaction remains effectively unaltered at the hydrolytic rupture point. If this were not the case, there would be a reduction of the magnitude of σ_0.

Determinations of σ_0 have rested on studies of the influence of concentration on the association of complementary *oligomeric* strands. A value of σ_0 of the order of 10^{-3} to 10^{-2} with a one molar standard state for the reactant chains has been reported for the acid combination of oligomers of riboadenylic acid (Applequist and Damle, 1965). More germane to the analysis here are recent results for the reaction of complementary oligomeric ribonucleotides from which values of σ_0 for the two base pairs of 2.5×10^{-4} (G·C) and 4×10^{-5} (A·U) are reported (Borer et al., 1974). If Mg^{2+} were present in these systems, these association constants would be expected to be larger. This association involves primarily an entropy decrease mitigated by a small enthalpy decrease due to hydrogen bond formation. Taking as a representative value $\sigma_0 = 10^{-4}$, we find that $\Delta G°_{2c}' = -3$ kcal.

A second hydrolytic break in the phosphodiester linkage in the complementary chain opposite to the first leads to the separation of the two bihelical products. This process must be accompanied by a free-energy change equal to $\Delta G°_{1c}' + RT \ln s = -5.5$ kcal. This is also less favorable than a single-strand cleavage. There is then a thermodynamic predisposition to remove hydrolytically bases that are not stabilized by pairing interactions. This may be the origin of the enzymatic propensity for excising such elements (Kelly et al., 1969).

Repair of Phosphodiester Bond Scissions in a Double-Stranded Polynucleotide. In recent years enzymes have been isolated from a variety of sources which catalyze the re-formation of phosphodiester linkages between neighboring 3′-hydroxyl groups and 5′-phosphate moieties. The restitution reaction occurs at the expense of phosphoanhydride bonds in ATP (Weiss and Richardson, 1967) or NAD^+ (Gellert, 1967; Gefter et al., 1967). The former, reaction (d) of Figure 2, yields AMP and PP as split products while the latter, (e) of Figure 2, yields AMP and NMN as the immediate product species.

The ligase reaction utilizing ATP is simply the difference between reaction I and reaction (c) of Figure 2. The free-energy change for the overall reaction is then given by:

$$\Delta G°_{2d}' = \Delta G°_{I}' - \Delta G°_{2c}' = \Delta G°_{I}' - \Delta G°_{1c}' + RT \ln \sigma_0$$
$$= \Delta G°_{I}' - \Delta G°_{II}' + RT \ln \sigma_0 = -6.5 \text{ kcal} \quad (8)$$

This argument clearly shows that it is the presence of both reactive functional groups at the termini of the two chains bound to a *common* complementary strand which is the dominant factor in making this reaction thermodynamically favorable. The repair reaction is thus primarily driven by an increase in entropy ($-R \ln \sigma_0$).

With NAD^+ as the substrate in reaction (e) of Figure 2, the overall process can be viewed as the difference between the hydrolytic reaction:

$$NAD^+ + H_2O \rightleftharpoons NMN + AMP \quad (III)$$

and reaction (c) of that figure. Reaction III can in turn be related to reaction (I) by the known metabolic step (Kornberg, 1948) (eq IV), inasmuch as III = I − IV.

$$NMN + ATP \rightleftharpoons NAD^+ + PP \quad (IV)$$

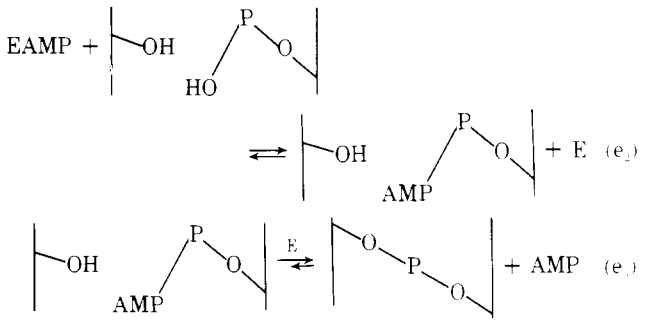
Consequently the free-energy change for ligation with NAD^+ as a reactant can be written as:

$$\Delta G°_{2e}' = \Delta G°_{III}' - \Delta G°_{2c}' = \Delta G°_{I}' - \Delta G°_{IV}' - \Delta G°_{2c}'$$
$$= \Delta G°_{2d}' - \Delta G°_{IV}' \quad (9)$$

Reaction IV is a very mobile process with a reported equilibrium constant of about unity (Imsande and Handler, 1961). Hence, $\Delta G°_{IV}' \approx 0$ and $\Delta G°_{2e}' \approx \Delta G°_{2d}'$. It is of course a not altogether unexpected result that two alternative mechanisms with the same metabolic objective should be characterized by nearly matching free-energy changes.

We can proceed somewhat further with reaction (e) of Figure 2 as measurements have been made of the equilibrium concentration of the ligase adenylate. A more detailed reaction sequence (Lehman, 1974) depicts the sealing process occurring in the following stages (eq e_1–e_3).

$$E + NAD^+ \rightleftharpoons EAMP + NMN \quad (e_1)$$



The equilibrium constant for reaction e_1 has been reported as being equal to 28 (Modrich and Lehman, 1973) corresponding to a free-energy change $\Delta G°_{e_1}' = -2$ kcal. The last step, e_3, in which the ligase (E) plays a purely catalytic role is well approximated as the difference between reaction II and reaction (c) of Figure 2 so:

$$\Delta G°_{e_3}' = \Delta G°_{II}' - \Delta G°_{2c}' = \Delta G°_{II}' - \Delta G°_{1c}' + RT \ln \sigma_0$$
$$= RT \ln \sigma_0 = -5.5 \text{ kcal} \quad (10)$$

From the calculated overall free-energy change $\Delta G°_{2e}' = -6.5$ kcal, we estimate for the transfer of the adenylate moiety from the enzyme to the free 5′-phosphate at the bond rupture that:

$$\Delta G°_{e_2}' = \Delta G°_{2e}' - \Delta G°_{e_1}' - \Delta G°_{e_3}'$$
$$= \Delta G°_{I}' - \Delta G°_{II}' - \Delta G°_{e_1}' = +1.0 \text{ kcal} \quad (11)$$

For the NAD^+-ligase reaction, the last stage leading to the restoration of the phosphodiester bond makes by far the largest contribution to the negative free energy change for the total reaction. The reaction utilizing ATP as substrate differs from the above sequence only in the first step. The free-energy change for this partial reaction is $\Delta G°_{d_1}' = \Delta G°_{e_1}' + \Delta G°_{IV}' \approx \Delta G°_{e_1}'$ inasmuch as $\Delta G°_{IV}' \approx 0$. Consequently, for this pathway it is again the third step which provides the principal driving force.

The first two stages (e_1 and e_2 above) in both metabolic paths conserve the number of reaction species. For the last step resulting in the liberation of AMP there is an increase in the number of products over reactants. As previously stated, these repair reactions are largely entropy driven and this is seen in the standard free energy change of eq 8. At the high dilution where the reactions are studied in vitro, there is then a further addition of this nature to this entropically favored process.

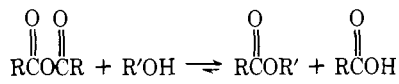
Pyrophosphate Hydrolysis Coupling

The two synthetic reactions discussed in the previous section lead to pyrophosphate formation which in vivo is immediately hydrolyzed to two orthophosphate ions by inorganic pyrophosphatase as in reaction (f) of Figure 2. The free-energy change for this reaction is $\Delta G°_{2f}' = -7.5$ kcal (Alberty, 1969) beside which $\Delta G°_{2a}'$ and $\Delta G°_{2b}'$ are insignificant. This is a feature shared with virtually all metabolic processes yielding pyrophosphate for which the author has found it possible to make such a comparison.

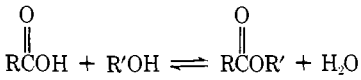
The repair reaction utilizing ATP also produces pyrophosphate directly. The ligation employing NAD^+ will yield pyrophosphate when the NMN primary product is recycled back to NAD^+ at the expense of ATP in reaction IV. Consequently both pathways benefit from the further driving force provided by the hydrolysis of pyrophosphate. That NAD^+ is a suitable substrate for the ligation reaction in contrast to NADH may be a consequence of reaction IV salvaging the NMN while no comparable path exists for a putative NMNH. Also, the requirement for ATP in some specially treated permeable bacterial cell replicating systems (Pisetsky et al., 1972) may reflect the role of reaction IV in regenerating NAD^+ which is the standard ligation substrate of these organisms.

The repair reactions, unlike the synthetic reactions, have a free-energy change comparable in magnitude to that of pyrophosphate hydrolysis. However, in our view these processes must be considered with the hydrolytic cleavage reaction (c) of Figure 2 which is itself thermodynamically favorable. The added push to the resealing provided by pyrophosphate hydrolysis may represent an important safety margin against intracellular hydrolytic breakdown of the polynucleotide chains.

The nearly isoergonic synthesis of polynucleotides from nucleoside diphosphates (reaction (a) of Figure 1) had as one consequence the closely equivalent free energies of hydrolysis of a phosphoanhydride linkage (reaction II) and phosphodiester bonds (reaction (c) of Figure 1). This was noted above as contrasting strongly with the case of aliphatic acid anhydrides and esters. This observation suggests an examination of carboxylic acid ester formation from anhydrides and acids, i.e.



with



From the free energies of hydrolysis of these two species (Jencks, 1970), we conclude the free-energy change of these two esterification reactions are −14 and −0.8 kcal, respectively, where the standard state of the acid is the protonated form. The latter quantity is corrected to a standard state of 1 *M* for the water product rather than the customary pure water to facilitate the comparison. We note that the free-energy change of ester formation from the acid is the one more nearly comparable in magnitude to that of phosphodiester formation from the "activated" phosphoanhydrides.

It has long been realized that polyester formation from dihydric alcohols and dibasic acids is fundamentally understandable in terms of the second of these two reactions (Carothers, 1931). But in order to achieve high degrees of polymerization with such monomers the water product must be removed by distillation (Carothers and Hill, 1932). It should be added that the synthesis of polypeptides utilizing *N*-carboxyanhydrides—an activated monomeric source—in the Leuchs procedure (Roberts and Caserio, 1965) also gains by the liberation of a volatile side product, carbon dioxide.

In our view the intercession of inorganic pyrophosphatase to catalyze the hydrolysis of the pyrophosphate by-product of polynucleotide synthesis may be regarded as the enzymatic analogue of the removal of water by physical means in the preparation of polyesters.

Conclusion

That pyrophosphate release characterizes a vast spectrum of synthetic metabolic pathways is well documented (Kornberg, 1962). Furthermore, the coupling of these reactions to the catalyzed hydrolysis of the pyrophosphate product by inorganic pyrophosphatase is inscribed in text books widely (Lehninger, 1965) if not universally as providing an essential driving force for these reactions in vivo.

Nonetheless, both DNA and RNA synthetic investigations in vitro utilizing well-defined cell-free systems (Wells and Inman, 1973) are prosecuted without being linked to this reaction. This neglect arises despite occasional injunctions as to the thermodynamic significance of this reaction in the specific context of replication and transcription (Watson, 1970).

There is then an implicit proposition that this hydrolysis may be critical for in vivo processes but not for the corresponding ones in vitro. Such a view is expressed in assigning a role to inorganic pyrophosphatase as providing that "the integrity of DNA is assured against the vicissitudes of PP concentration" (Kornberg, 1962, 1974). Our interpretation of this function of the enzyme is that with a host of readily reversible synthetic reactions yielding pyrophosphate, its hydrolysis in vivo is mandatory if these processes of DNA replication inter alia are to occur free of an interdependence due to this common product. When these reactions are studied separately in vitro, the action of the pyrophosphatase would then be dispensable. Such is certainly the case for the repair process in the absence of concurrent hydrolysis.

Both in vitro RNA and DNA syntheses customarily yield high molecular weight chains without the hydrolysis of the pyrophosphate. In the former instance a quite straightforward argument has shown that with a very liberal assessment of the thermodynamic limits compared to the values of $\Delta G^{\circ}{}_{2a'}$ quoted here an extensive conversion of ribonucleoside triphosphates to chains of mRNA size cannot occur in such a closed system (Peller, 1975). An earlier analysis suggested that under the same conditions synthesis of the longer DNA chains involved in the replication of even moderate sized viral nucleic acids would be problematical (Peller, 1966). For both processes, coupling to pyrophosphatase action was predicted to lead to ceilings for the average degrees of polymerizations orders of magnitude larger.

There has been a developing thesis that the replication process must involve a panoply of protein factors which catalyze ribonucleic acid initiation of chains, and the removal of these initiation fragments as well as proteins which destabilize the double helix (Gefter, 1975). Microbial and viral mutants provide much of the evidence for the role of these essentially catalytic agents (Schekman et al., 1974). The widespread cellular importance of inorganic pyrophosphatase obviously precludes an elucidation by this means of its place in such a scheme. Only an in vitro examination of the influence of this coupling reaction on the average chain length, size distribution, and most importantly biological competence of the replicated and transcribed products could answer this question.

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Stereospecific Binding of Diastereomeric Peptides to Salmon Sperm DNA†

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ABSTRACT: Studies of the interaction specificities of L-lysyl-L-phenylalaninamide (**1**) and the diastereomeric dipeptide amide, L-lysyl-D-phenylalaninamide (**2**), with salmon sperm DNA reveal distinct differences in the binding site of the aromatic ring of the phenylalanine residue. The results of ^{1}H nuclear magnetic resonance (NMR), spin-lattice relaxation rates, viscometric, and flow dichroism studies indicate the aromatic ring of **1** is "partially" inserted between base pairs of DNA whereas the aromatic ring of **2** points outward toward the solution. The terminal L-lysyl residue presumably interacts stereospecifically with DNA helix thus dictating the positioning of the aromatic ring of the C-terminal phenylalanine residue. In the accompanying paper (E. J. Gabbay et al. (1976), *Biochemistry,* following paper in this issue), the interaction of several oligopeptide amides (containing the N-terminal L-Lys-L-Phe residue) with DNA is examined. The results are found to be consistent with stereospecific binding of the terminal L-lysyl residue, and in addition, the evidence suggests that oligopeptides may bind to DNA via a modified single-stranded β-sheet structure which is wrapped around the nucleic acid helix in a manner similar to that described by M. H. F. Wilkins ((1956), *Cold Spring Harbor Symp. Quant. Biol. 21,* 75).

The mechanism(s) by which proteins of defined amino acid sequence may recognize specific sequences of nucleic acid has been the subject of considerable interest in many laboratories. Our approach to this problem has been centered on model systems composed of small oligopeptide amides interacting with DNA of various AT/GC compositions (Gabbay et al., 1972, 1973). Although the relationship of these model studies to the overall problem of the recognition process between macromolecules (DNA and proteins) is not immediately obvious, nonetheless, the results obtained are valuable in elucidating the interaction specificities of nucleic acids with small oligopeptide systems. Preliminary study on the interaction specificities of L-lysyl-L-phenylalaninamide (**1**) and the diastereomeric peptide L-lysyl-D-phenylalaninamide (**2**) to DNA has been presented (Adawadkar et al., 1975). The results indicate that stereospecific peptide–DNA complexes are obtained whereby the aromatic rings of **1** and **2** point into and out of the helix, respectively. In this paper, further evidence is presented in support of the above mode of interaction of DNA with **1** and **2**. The results suggest that the ϵ- and α-amino groups of the N-terminal L-lysyl residue interact stereospecifically with the DNA helix thus dictating the positioning of the aromatic ring of the C-terminal phenylalanine residue. In the accompanying paper (Gabbay et al., 1976), the interaction of several oligopeptides, containing the N-terminal L-Lys-L-Phe residue, with DNA is examined. The results are found to be consistent with stereospecific binding of the ter-

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