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Pyridoxal Phosphate as a Probe of Reovirus Transcriptase†

Exeen M. Morgan and David W. Kingsbury*

ABSTRACT: The ribonucleoprotein core of reovirus is a multienzyme complex that transcribes messenger ribonucleic acid (mRNA) from double-stranded RNA templates. So far, the core has resisted attempts to disassemble it and identify the polypeptide species responsible for RNA polymerase activity. As an alternative approach, we tested pyridoxal 5-phosphate (PLP) as a potential affinity labeling reagent for reovirus transcriptase in vitro; PLP has been used as an affinity reagent for cellular and viral nucleic acid polymerases. We found that PLP inhibited reovirus transcriptase reversibly (apparent K_i = 0.2 mM), but the inhibition was noncompetitive with respect to each of the four ribonucleoside triphosphates. This interaction required both the aldehyde and phosphate moieties in PLP, since pyridoxamine and pyridoxal were relatively inactive. To identify the polypeptides involved, we labeled the PLP–core complex by reductive alkylation with [^{3}H]borohydride. At PLP concentrations close to the apparent K_i, labeling was selective for the two largest virion polypeptides, λ_1 and λ_2. At saturation, there were only 10 high-affinity PLP binding sites per core in each of the λ polypeptide species. These findings implicate either or both λ polypeptide species in viral transcription and they indicate that a special population, representing no more than 10% of the total λ molecules in each core, participates in RNA synthesis.

Several types of RNA viruses carry a complete mRNA-synthesizing apparatus from cell to cell as required by the structure of their genomes (Raghow & Kingsbury, 1976). Although these viruses possess few genes, they contain a transcriptive apparatus that has many of the capabilities of the cell nucleus, including, besides a transcriptase, four or five additional enzymatic activities that cap, methylate, and polyadenylate the transcripts (Shatkin, 1976; Raghow & Kingsbury, 1976). The question arises of how these activities are apportioned among so few viral gene products. Little progress has been made toward an answer for any of these viruses, because it is difficult to obtain adequate amounts of viral proteins for study or to separate the proteins from the virus particles in an enzymatically active form.

Reovirus, which contains 10 genes in the form of separate segments of double-stranded RNA, is a case in point. Only four protein species have been identified in virus cores that display a full spectrum of RNA synthesizing and modifying activities (Joklik, 1974; Shatkin & Both, 1976), and only denaturing and inactivating treatments have, as yet, been able to dislodge any of these proteins from the core (White & Zweerink, 1976). This is unfortunate, because in other respects reovirus is an attractive subject for studies of virus transcriptase, being easy to grow and possessing a prodigious RNA synthetic capacity (Joklik, 1974).

For these reasons, we chose reovirus as the subject of this study to evaluate affinity labeling as a means of identifying the enzymatically relevant polypeptides in an intact and functioning viral transcriptive complex. In affinity labeling of enzymes, the aim is to form a covalent bond between the enzyme and a labeled analogue of a substrate or allosteric effector, identifying the site on the enzyme where the labeled compound binds (Singer, 1967). At another level of analysis, as in the present work, the method can identify the enzyme in a mixture of proteins, such as a multienzyme complex. We chose to investigate pyridoxal 5-phosphate (PLP) as a probe of reovirus transcriptase, because it is a compound with a record of application to affinity labeling of several nucleic acid polymerases (Bull et al., 1975; Martial et al., 1975; Modak, 1976; Papas et al., 1977; Venegas et al., 1973), among other types of enzymes (Colombo & Marcus, 1974; Marie, 1976; Piszikiewicz et al., 1977; Rippa et al., 1967; Schnackerz & Noltmann, 1971; Shapiro et al., 1968). PLP has a structural

† From the Division of Virology, St. Jude Children's Research Hospital, Memphis, Tennessee 38101. *Received July 3, 1979.* This work was supported by Research Grant AI 05343 and Cancer Center Support Grant CA 21765 from the National Institutes of Health and by ALSAC.

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resemblance to nucleotides, and its aldehyde group endows it with potential for covalent binding to amino groups in proteins (Marie, 1976; Martial et al., 1975).

Materials and Methods

Chemicals. Pyridoxal 5-phosphate, pyridoxal, pyridoxamine, pyridoxamine 5-phosphate, KBH_4, Hepes [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid], and Caps (cyclohexylaminopropanesulfonic acid) were products of Sigma Chemical Co. Nucleoside triphosphates were purchased from Boehringer Mannheim. [^{3}H]UTP (41.6 Ci/mmol) and [^{3}H]CTP (21.4 Ci/mmol) were obtained from New England Nuclear. Potassium [^{3}H]borohydride (7–15 Ci/mmol) was supplied by Amersham/Searle and New England Nuclear.

Virus. Initial stocks of the Dearing strain of reovirus type 3 and mouse L cells adapted to suspension culture were generously provided by Drs. H. J. Zweerink and B. N. Fields, respectively. Growth conditions and details of procedures for purification of the virus have been published (Smith et al., 1969). The main steps in virus purification were Freon (trichlorotrifluoroethane) extraction of infected cells and successive density gradient centrifugations in sucrose and cesium chloride. The cesium chloride was removed by dialysis against 0.15 M NaCl and 0.015 M sodium citrate (pH 6.8), and virions were stored in this solution at 4 °C. Concentrations of purified virions were determined by UV absorption, using the molar extinction coefficient of virus particles implicit in the data of Smith et al. (1969), namely, $\epsilon_{260} = 2.9 \times 10^8$ L mol^{-1} cm^{-1}. Good agreement was obtained by use of the Bio-Rad protein assay. Virions labeled with ^{14}C-labeled amino acids (Morgan & Zweerink, 1974) were purified as described above.

Transcriptase Assay. Purified virions were digested with 50 μg of chymotrypsin/mL for 60 min at 37 °C to produce virus cores and activate the transcriptase (Joklik, 1974). Reaction mixtures contained, in 0.1-mL volumes, 1.9×10^{-9} M cores (from 10 μg of virions), 100 mM Hepes (pH 8.0), 12 mM $MgCl_2$, 2 mM ATP, CTP, and GTP, 0.2 mM UTP, and 2.5 μCi of [^{3}H]UTP. Samples were incubated at 40 °C for the intervals indicated under Results. Reactions were stopped by the addition of ice-cold 5% trichloroacetic acid, and acid-insoluble radioactivity was measured (Portner & Kingsbury, 1972). More than 95% of the cores in our preparations contained active transcriptase, as shown by a shift to higher buoyant density in CsCl after incubation in the reaction mixture (Skehel & Joklik, 1969).

PLP and Borohydride Treatments. Just before use, 20 mM stock solutions of PLP were prepared in sterile water. Reovirus cores were incubated with various concentrations of PLP, as noted under Results, in 100 mM Hepes and 12 mM $MgCl_2$ (pH 8.0), for 30 min at 24 °C in the dark.

To determine the effect of borohydride reduction on transcriptase activity, we cooled PLP-treated cores to 4 °C and added KBH_4, freshly prepared as a 100 mM stock in 100 mM KOH, to a final concentration of 5 mM. There was no increase in pH of the core samples under these conditions. After 5 min, samples were assayed for transcriptase activity as described above.

To label the PLP–transcriptase complex, we used [^{3}H]-borohydride. Stock solutions were adjusted with unlabeled KBH_4 to a specific activity of 3 Ci/mmol in 100 mM KOH at a concentration of 300 mCi/mL. Stocks were stored in 25-μL portions in liquid nitrogen (Randerath & Randerath, 1973). Cores (9.5×10^{-9} M) were treated with PLP as described above. The samples were then cooled to 4 °C, and 5 μL of [^{3}H]borohydride (1.5 mCi) was added. After 5 min, samples were diluted to 3 mL with 100 mM Hepes (pH 8.0) and centrifuged through 2 mL of 1.2 g/cm^3 cesium chloride in an SW 50.1 rotor at 45 000 rpm for 90 min.

Polyacrylamide Gel Electrophoresis. Virus polypeptides were separated in 7.5% polyacrylamide gels containing 5 M urea (Martin & Zweerink, 1972). After electrophoresis, gels were fixed with acetic acid–methanol, cut into 1-mm slices, and counted by liquid scintillation.

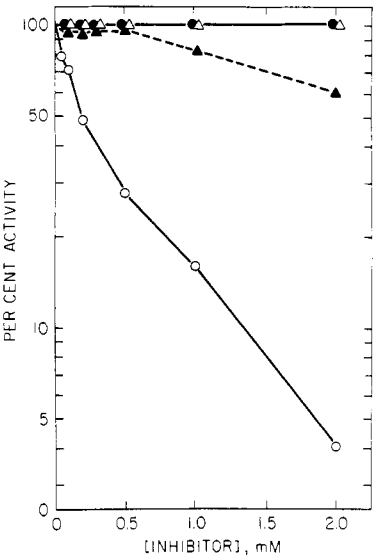

FIGURE 1: Inhibition of reovirus transcriptase by PLP. Reovirus cores (1.9×10^{-9} M) were incubated with the indicated concentrations of PLP or one of its congeners for 30 min. Transcription was then measured for 15 min without dilution of the inhibitor. (○) PLP; (▲) pyridoxal; (●) pyridoxamine; (△) pyridoxamine 5-phosphate.

Table I: Effects of PLP and Potassium Borohydride on Transcriptase Activity

addn	act. [pmol/(μg h)][a]
none	94.6
5 mM KBH_4	87.4
2 mM PLP	3.9
2 mM PLP + 5 mM KBH_4	1.4

[a] Reaction conditions are described in the legend of Figure 1. Activity is expressed as picomoles of UMP incorporated per microgram of reovirus protein per hour.

Results

PLP Is a Reversible Inhibitor of Reovirus Transcriptase. Reovirus cores were incubated with various concentrations of PLP and assayed for transcriptase activity in the presence of PLP. As shown in Figure 1 and Table I, PLP was an effective inhibitor of RNA synthesis, whereas its congeners pyridoxal, pyridoxamine, and pyridoxamine 5-phosphate had much less effect. Evidently, both the phosphate group and the aldehyde moiety are essential for optimal interference with reovirus transcriptase activity.

Inactivation of reovirus transcription by PLP was reversible (Figure 2). A 20-fold dilution of a mixture of cores and 1 mM PLP restored activity, whereas samples maintained in 1 mM PLP displayed their usual degree of inhibition. However, inhibition of transcription could be made irreversible by reducing the complex with KBH_4 (Figure 2). Since KBH_4 itself had no effect on transcriptase activity (Table I), we conclude that PLP inhibits by forming a Schiff base with a primary amino group on a protein that plays a role in transcription (Martial et al., 1975). Borohydride reduction enhanced the degree of inhibition produced by a given PLP concentration,

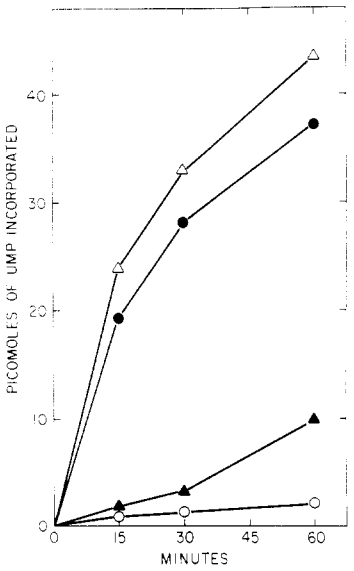

FIGURE 2: Reversal of PLP inhibition. Cores were incubated with 1.0 mM PLP for 30 min at 24 °C. The samples were then diluted 20-fold into complete transcriptase reaction mixture (●) or transcriptase reaction mixture containing 1.0 mM PLP (▲). A third sample was reduced with KBH_4 (5 mM final concentration) before dilution by the transcriptase reaction mixture (○). After dilution, samples were incubated at 40 °C for the indicated times and [^{3}H]UMP incorporation was measured in comparison to an untreated control (△).

Table II: Kinetic Constants of Reovirus Transcriptase and Its Inhibition by PLP

nucleotide	K_m (M)[a]	K_i (M)[b]
CTP	$7.5 (\pm 2.0) \times 10^{-5}$	$1.3 (\pm 0.6) \times 10^{-4}$
GTP	$6.3 (\pm 2.0) \times 10^{-5}$	$1.4 (\pm 0.5) \times 10^{-4}$
UTP	$4.0 (\pm 1.4) \times 10^{-4}$	$1.8 (\pm 1.1) \times 10^{-4}$
ATP	5.0×10^{-3}	[c]

[a] Molarity of each nucleotide. Values for CTP, GTP, and UTP are the means of eight determinations (plus or minus standard) errors), including six determinations in the presence of three different concentrations of PLP. The value for ATP is the mean of two determinations in the absence of PLP. In the presence of PLP, the K_m for ATP was $7.1 (\pm 3.5) \times 10^{-4}$ M (three concentrations of PLP, six determinations). [b] Molarity of PLP. Values for CTP, GTP, and UTP are the means (plus or minus standard errors) of 30 determinations. [c] This value varied with ATP concentration (see Table III).

reflecting the formation of an irreversible complex (Figure 2).

PLP Acts Noncompetitively. An important property of an active-site inhibitor is a competitive relationship with substrate (Singer, 1967). This criterion was not met by the interaction between PLP and reovirus transcriptase, as shown by kinetic analyses (Figure 3). In these measurements, the concentration of a single, rate-limiting nucleoside triphosphate was varied, while the concentrations of the remaining three nucleoside triphosphates were held constant (Anthony et al., 1969). The apparent K_m values derived from the data in Figure 3 are summarized in Table II. There were marked differences in the affinities of individual substrate species for the enzyme: highest affinities were shown by CTP and GTP, whereas the affinity of UTP was an order of magnitude lower and that of ATP was another order of magnitude below UTP. When PLP was added to the reactions, there was no discernible effect on the K_m of CTP, GTP, or UTP, but V_{max} was reduced in each case. Thus, PLP was not competitive with any of these nucleoside triphosphates. In the case of ATP, both K_m and V_{max} were shifted proportionately in the presence of PLP, giving parallel lines in the double-reciprocal plots (Figure 3A). This

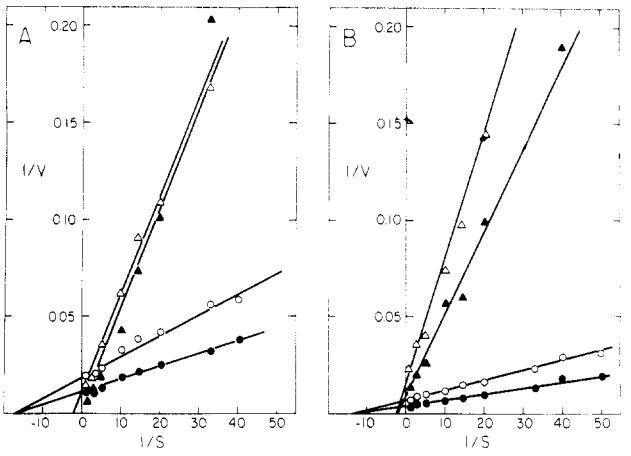

FIGURE 3: Kinetics of reovirus transcriptase and the effect of PLP. Transcriptase reaction mixtures were prepared as described under Materials and Methods, except the concentrations of three nucleoside triphosphates were kept at 2 mM while the concentration of the fourth was varied from 0.02 to 2 mM. The reactions also contained 2.5 μCi of either [^{3}H]UTP or [^{3}H]CTP. Ordinate: V (velocity) is expressed in picomoles of nucleoside monophosphate incorporated into RNA during a 15-min incubation at 40 °C. Abscissa: S (substrate concentration) is given in millimolarity. (A) Transcriptase activity with various concentrations of ATP (▲), ATP + 0.1 mM PLP (△), GTP (●), and GTP + 0.1 mM PLP (○). (B) Transcriptase activity with various concentrations of UTP (▲), UTP + 0.1 mM PLP (△), CTP (●), and CTP + 0.1 mM PLP (○).

Table III: Dependence of the K_i for PLP on ATP Concentration

	K_i at ATP (mM)[a]			
PLP (mM)	0.02	0.07	0.40	2.0
0.05	0.93	0.28	0.16	0.077
0.50	6.0	1.3	0.34	0.25

[a] The data were obtained from experiments like those in Figure 3. K_i values were calculated as described under Results.

appears to be an example of "uncompetitive" inhibition (Westley, 1969), suggesting that ATP, alone among the substrates, actually regulates the ability of the transcriptase to be inhibited by PLP. The situation may be similar to that described for poxvirus and rhabdovirus transcriptases, for which ATP performs several functions besides serving as a substrate (Gershowitz et al., 1978; Testa & Banerjee, 1979). When the data for the three noncompetitive nucleoside triphosphates were analyzed by the method of Hunter & Downs (1945), using

$$K_i = i[v_i/(v - v_i)] \quad (1)$$

where i is the inhibitor concentration and v and v_i are the velocities in the presence and absence of the inhibitor, apparent K_i values of the order of 0.2 mM PLP were obtained (Table II). In contrast, the apparent K_i for PLP varied inversely with ATP concentration (Table III), in agreement with our interpretation of the data in Figure 3A.

PLP-Directed Labeling of Core Polypeptides. Although the noncompetitive kinetics of transcriptase inhibition suggested that PLP was not an active-site inhibitor, this did not impute lack of specificity to its interaction with the proteins in reovirus cores (Shapiro et al., 1968; Marie, 1976). Therefore, it was of interest to identify the polypeptides which interacted with PLP under these conditions. As shown in Figure 4, there was a clear selectivity for core polypeptide species λ_1 and λ_2 in the PLP-directed incorporation of tritium from [^{3}H]borohydride (panel C) and λ_2 had a slightly greater affinity for PLP than λ_1. As evidence of chemical specificity, 1 mM pyridoxal, which

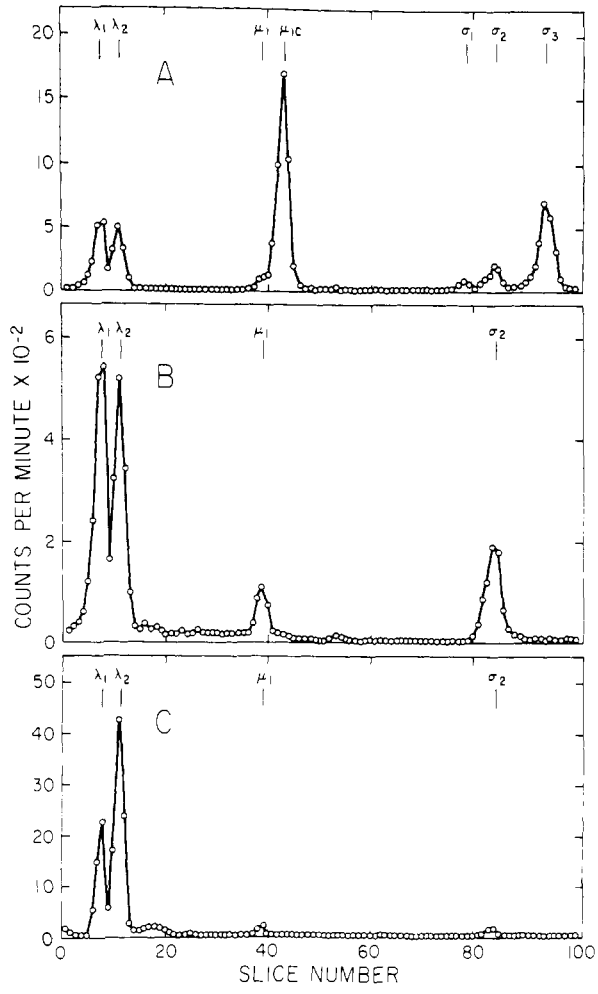

FIGURE 4: PLP-directed labeling of core polypeptides. Cores from 50 μg of reovirions were incubated with 1 mM PLP for 30 min, treated with [^{3}H]borohydride, purified, and subjected to polyacrylamide gel electrophoresis as described under Materials and Methods. ^{3}H radioactivity in core polypeptides (C) is compared with polypeptides from virions (A) and cores (B) labeled biosynthetically with ^{14}C-labeled amino acids. Migration is from left to right.

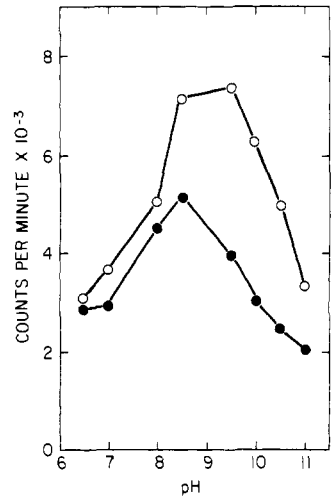

FIGURE 5: Effect of pH on PLP-directed labeling of λ_1 and λ_2. Buffers were 0.1 M Hepes below pH 9.0 and 0.1 M Caps above pH 9.0. The PLP concentration was 0.1 mM and incubation was for 30 min at 24 °C before addition of [^{3}H]borohydride. Cores were then centrifuged through CsCl and analyzed by polyacrylamide gel electrophoresis. (●) Radioactivity in λ_1; (○) radioactivity in λ_2.

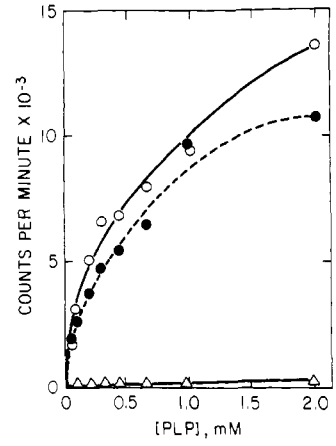

FIGURE 6: Labeling of core polypeptide species as a function of PLP concentration. After incubation with the indicated concentrations of PLP, cores were reduced with [^{3}H]borohydride and analyzed as described in Figures 4 and 5. (●) λ_1; (○) λ_2; (△) σ_2.

inhibited transcriptase slightly (Figure 1), directed only 10% as much label into the λ polypeptides, and [^{3}H]borohydride alone did not label any of the polypeptides in the core (data not shown).

The results in Figure 4 were obtained when borohydride labeling was done at pH 8, the optimum for transcriptase activity (Joklik, 1974). Figure 5 shows that the specificity of the reaction was independent of pH. From pH 6 to 11, only the λ polypeptide species were labeled. Optimum labeling of λ_1 occurred at pH 8.5, whereas λ_2 had a broader optimum at about pH 9. These optima are 1 to 2 pH units below the average pK values of lysine ε-amino groups in proteins, approaching the pK values of lysine residues in the active sites of several enzymes (Piszikiewicz et al., 1977; Tanford, 1962).

Stoichiometry of PLP Binding. The data in Figure 4 were obtained at a PLP concentration of 1 mM, at which transcriptase activity was inhibited 80–90% (Figure 1). A wider range of PLP concentrations, spanning 0.01–2 mM, was then examined as a measure of labeling specificity (Figure 6). Consistently, negligible radioactivity went into μ_1 or σ_2, and there was a slight preference for labeling of λ_2 compared to λ_1.

Since the previous findings indicated that PLP was capable of saturating sites on λ_1 and λ_2 at concentrations relevant to transcription inhibition, a more extensive series of PLP-directed borohydride-labeling experiments was subjected to Scatchard analysis. Although there was some scatter, straight lines could be fitted with reasonable confidence to the data for the individual λ proteins (Figure 7). As shown by the slopes of these regression lines, PLP bound to λ_2 with slightly greater affinity than to λ_1, and there appeared to be an equal number of high-affinity PLP binding sites in each protein species, ~10/virion. This represents ~10% of the λ protein molecules in each virion and supports the idea that the binding is related to transcriptase inhibition in a specific manner.

Discussion

This study revealed high-affinity PLP binding sites on the two largest protein species in the reovirus core, λ_1 and λ_2. This binding accompanied a noncompetitive inhibition of core transcriptase activity. PLP may not be an active-site label for reovirus transcriptase, but it was still possible to implicate the λ proteins in transcription by its use.

Careful evaluation of previous reports on the interaction of PLP with nucleic acid polymerases indicates that a noncompetitive interaction such as we found with reovirus may not be exceptional. It has been concluded that PLP was a competitive inhibitor of nucleoside triphosphate binding to the

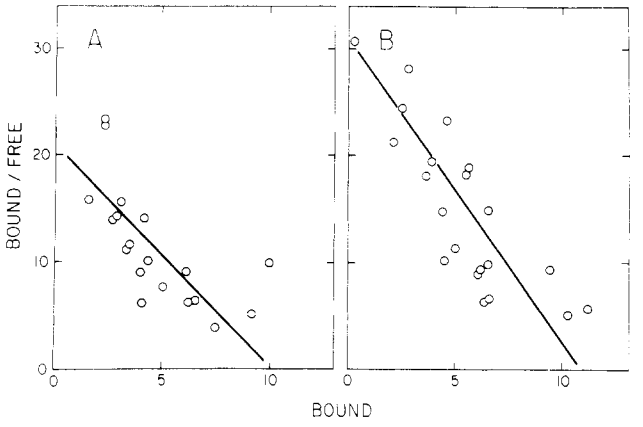

FIGURE 7: Scatchard analysis of PLP binding. Cores from 50 μg of reovirions were used to obtain each data point. They were incubated with various concentrations of PLP, labeled with [^{3}H]borohydride, and analyzed by polyacrylamide gel electrophoresis as described earlier. Abscissa: "BOUND" is the number of moles of PLP bound per mole of reovirus cores, as determined from the specific activity of the [^{3}H]borohydride used. Ordinate: "BOUND/FREE" is the ratio of each abscissa value to the concentration of PLP (millimolarity) used to obtain that value. (A) λ_1; (B) λ_2.

active site of a polymerase because a partial reversal of inhibition by nucleoside triphosphates was obtained (Martial et al., 1975; Modak, 1976; Papas et al., 1977; Venegas et al., 1973), but the kinetic analyses necessary to establish the point have not always been performed. Nucleic acid polymerases are subject to a wide variety of regulatory interactions with proteins, such as repressors and activators (Losick & Chamberlin, 1976). Furthermore, polymerases must interact not only with substrates but also with template and product nucleic acids, even in the absence of modulating proteins. Thus, there are several sites besides the catalytically active site that are at risk to perturbation by nucleotide analogues. In addition, PLP may inactivate transcriptase by binding to a site that has no direct relationship to the binding of nucleotides, RNA, or protein. It may bind to an exposed amino group that plays an entirely different role in the enzyme, such as stabilizing its tertiary structure.

The topography of the reovirus core is incompletely defined. There is evidence that λ_1 is the major outer capsid component of cores, that μ_1 and σ_2 are internal, and that polypeptide λ_2 composes the 12 spikes at the fivefold vertices of the core (Martin & Zweerink, 1972; White & Zweerink, 1976). A fifth species, λ_3, is thought to be present in small amounts in cores, but its location is unknown (Shatkin & Both, 1976). A relationship between λ_2 and RNA synthetic activity is suggested by evidence that newly synthesized RNA is extruded through the spikes of the core (Bartlett et al., 1974) and removal of λ_2 destroys transcriptase activity (White & Zweerink, 1976). However, strongly alkaline solutions were necessary to release λ_2, making it likely that the proteins remaining in the core were denatured. There is no previous evidence bearing on a role for λ_1 in transcriptase activity. However, RNA polymerases are commonly composed of several protein species (Losick & Chamberlin, 1976). Therefore, both λ_1 and λ_2 might participate in transcription, since different protein species might contain the sites for catalysis, template binding, and product attachment. We should also not overlook the possibility that one or both of the λ species are involved in one of the other enzymatic activities that modify viral transcripts (Shatkin, 1976). Indeed, evaluating this possibility is the next priority in our research.

PLP typically interacts with proteins by forming a Schiff base between its aldehyde group and primary amino groups, most commonly the ε-amino groups of lysine residues (Martial et al., 1975). The inactivity of pyridoxamine against reovirus transcriptase demonstrates the importance of the aldehyde moiety in the action of PLP. However, the possession of an aldehyde group with Schiff base forming capability was not the only requirement. Pyridoxal was relatively ineffective as an inhibitor and has a lower affinity than PLP for core proteins, judging by its inefficiency as a vector for [^{3}H]borohydride labeling. This underscores the importance of the phosphate group in the interaction of PLP with enzymes that utilize nucleotides (Venegas et al., 1973; Martial et al., 1975).

The widespread occurrence of lysine groups in proteins and the ability of PLP to direct hydrogen into them have sometimes been employed to achieve more generalized labeling than we aimed for in the present study. The limitation of labeling to the λ polypeptides indicates that we achieved the desired specificity; the other polypeptide species in the core, μ_1 and σ_2, contain lysine residues that can be labeled by amino-group reagents under different conditions. For example, dansyl chloride was found to label all four polypeptide species in intact cores in stoichiometric amounts (Martin et al., 1973). Moreover, PLP inactivated all of the transcriptase in the cores coincident with binding to a very limited number of sites, ~10 in each λ species (Figure 7). Amino acid analyses indicate that λ_1 contains ~50 lysine residues and that λ_2 contains ~70 (White & Zweerink, 1976). There are ~110 molecules of λ_1 and ~90 molecules of λ_2 in each core (Smith et al., 1969). Thus, PLP inactivated the transcriptase by binding to no more than 20 out of a maximum of 11 000 potential sites. Appropriate measurements showed that more than 95% of the cores were active in transcription (Materials and Methods), so the limitation of PLP binding does not reflect the existence of a large population of enzymatically inert protein molecules in our samples.

The stoichiometry of 10 PLP binding sites per λ species that we deduced (Figure 7) provides a striking correlation with the fact that each virion contains 10 molecules of double-stranded RNA. Therefore, we hypothesize that transcription of each segment of RNA is mediated by a complex of one molecule of λ_1 and one molecule of λ_2, the remaining molecules of these proteins having structural roles or other functions. The alternative, which we cannot rule out at this point, is that only a minority of the PLP binding sites that we detected is relevant to transcriptase inhibition. Irrelevant sites might reside in only one of the λ polypeptide species or in both species. We can identify the number of PLP-occupied sites in each λ species by peptide mapping (Shapiro et al., 1968), but it is more difficult to devise a test for the involvement of both species in transcription. Further work with other affinity probes may help to decide that question.

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Enzymatic Synthesis and Carbon-13 Nuclear Magnetic Resonance Conformational Studies of Disaccharides Containing β-D-Galactopyranosyl and β-D-[1-^{13}C]Galactopyranosyl Residues†

H. A. Nunez and R. Barker*

ABSTRACT: Partially purified UDPgalactosyltransferase (EC 2.4.1.22) from bovine milk has been used to synthesize millimolar amounts of compounds such as Galβ(1→4)Glc, Galβ(1→4)GlcNAc-β-hexanolamine, and Galβ(1→4)-GlcNAcβ(1→4)GlcNAc. The same method has been used to prepare similar compounds containing ^{13}C-enriched galactopyranosyl moieties. Galβ(1→4)GlcNAc-β-hexanolamine was also synthesized in a solid-phase system in which the GlcNAc-β-hexanolamine glycoside was covalently linked to agarose beads. At pH 7.0 and at 1–5 mM Mn^{2+} the yields of the galactosyl saccharides are greater than 90% by using 10% excess of UDPGal donor. The use of a 90% enriched [1-^{13}C]galactosyl residue allowed the determination of the most abundant conformer about the galactopyranosyl–glycoside linkage by analysis of the carbon–carbon coupling constants from C1 of Gal to the C3′, C4′, and C5′ of GlcNAc or Glc.

The carbohydrate structures of the cell-surface glycoproteins and glycolipids are involved in cell–cell recognition, the immune response, hormone receptor functions, and internalization of various macromolecular materials. All are highly specific phenomena, and the specificity seems to be due, at least in part, to the structure of the oligosaccharide moieties involved. The nature of the individual sugar components and their sequence, linkage position, anomeric configuration, and overall configuration all appear to be important (Hughes, 1975). A well-documented case is the relationship of carbohydrate structure to the immune response elicited by the ABO and other antigenic groups of animal cell surfaces. However, even in this case very little is known about the conformation of the monosaccharide moieties about the glycosidic linkages of the oligosaccharide and the role that this may play in the interaction betwen antigen and antibody.

In general, the glycosubstances can be obtained in small amounts from biological sources, making studies of their physical, chemical, and biochemical properties difficult. Synthetic approaches are complex, exemplified by the elegant works in the laboratories of Lemieux (Lemieux & Driguez, 1975), Jeanloz (N-U-Din et al., 1974), Schuerch (1972) and their co-workers. In this paper we describe an approach using a partially purified UDPgalactosyltransferase for the preparation in high yield of millimolar amounts of a number of disaccharides containing D-Gal[1] in β(1→4) linkage. The ap-

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[1] Abbreviations used: ^{13}C NMR, ^{13}C nuclear magnetic resonance spectroscopy; Gal, α- or β-D-galactopyranose; Galβ(1→4)Glc (lactose), 4-*O*-(β-D-galactopyranosyl)-D-glucopyranose; Galβ(1→4)GlcNAc (*N*-acetyllactosamine), 4-*O*-(β-D-galactopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranose; Galβ(1→4)GlcNAcβ(1→4)GlcNAc, 4-*O*-(β-D-galactopyranosyl)-2-acetamido-2-deoxy-4-*O*-(β-D-glucopyranosyl)-2-acetamido-2-deoxy-D-glucopyranose; Galβ(1→4)GlcNAc-β-hexanolamine, 6-amino-1-hexyl 4-*O*-(β-D-galactopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranoside; GlcNAc, 2-acetamido-2-deoxy-D-glucopyranose; GlcNAc-β-hexanolamine, 6-amino-1-hexyl 2-acetamido-2-deoxy-β-D-glucopyranoside; GlcNAcβ(1→4)GlcNAc (chitobiose), 2-acetamido-2-deoxy-4-*O*-(β-D-glucopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranose.

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