Mechanism of Interconversion for Diastereomers of α,β-Bidentate $Cr^{III}(H_2O)_4(ADP)$: A Possible Model for Metal–Nucleotide Diastereomer Interconversions†

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ABSTRACT: The separation of the two diastereomers of α,β-bidentate CrADP by reverse-phase high-pressure liquid chromatography techniques is described. This technique provides complete resolution of the diastereomers within 10 min and relies on the use of ethanesulfonic acid (in the ionized form) as both the ion-pairing agent and isocratic buffer. Circular dichroism spectra of the two isomers were obtained, and the screw sense of each isomer was identified according to the assignments made by Dunaway-Mariano & Cleland [Dunaway-Mariano, D., & Cleland, W. W. (1980) *Biochemistry 19*, 1506–1515]. By use of the described separation technique, a scheme was devised for monitoring the interconversion of the isomers under varying conditions of pH, temperature, buffer type, and buffer concentration. Rate constants were calculated by using iterative descent methods, and a mechanism is proposed from the results. The data suggest that specific base catalysis is the primary process involved and that an S_N1CB- (substitution, nucleophilic, unimolecular, conjugate base) type mechanism is most probable.

Nucleotides play a vital role in the energetics of the cell because they are involved in a host of biological processes. Nucleotides exist in the cell most commonly as Mg^{2+} chelate and are utilized in this form by most enzymatic reactions. For the nucleotide adenosine 5′-diphosphate (ADP),[1] the Mg^{2+} (or any other metal ion) can, in theory, bind to either of the two phosphate groups (α or β) or both of them to yield mono- and bidentate coordination geometries. The binding of a metal ion to both phosphate groups of ADP leads to chirality in the α phosphate. This allows the possibility of stereospecificity of the two diastereomers for enzymes utilizing this coordination isomer. The elucidation of the stereoselectivity of enzymes is a useful tool in learning more precisely the exact mechanism of enzyme–nucleotide interactions. [Eckstein (1980) presents a more thorough discussion of metal–nucleotide interactions for the ATP molecule, and the reader is directed there.]

Metal–nucleotide complexes in vivo are, in most cases, in rapid dynamic equilibrium, and thus in vitro experiments using Mg^{2+}, Ca^{2+}, etc. with nucleotides yield only time-averaged signals with NMR. Therefore, the exact coordination isomer (let alone the proper stereoisomer) utilized by a particular enzyme cannot be easily deduced. Recently, work done by Huang & Tsai (1982) with ^{17}O NMR was established the macroscopic structure of MgADP and MgATP, but much more quantitative information is still required before the inclusion of enzymatic interactions can be done. To overcome these problems, the exchange-inert complexes of nucleotides with Cr(III) have proven to be useful in both stereospecific and mechanistic studies (Cleland, 1982).

In addition to the information that can be gained by the use of these substrate analogues with enzymes, it would also be informative to use these analogues as models from which to gain information about metal–nucleotide interactions in aqueous solutions. The importance of this kind of information can be appreciated when one realizes that an enzyme, which has a preference for either a specific geometric or stereoisomer or both, must deal with these constant, rapid interconversions. Bossard et al. (1982) addressed this point to an extent by measuring rates of conversion of monodentate CrADP to bidentate CrADP. In their paper, they also propose a possible mechanism that will be addressed in more detail under Discussion.

Besides mechanistic information, practical information as to the rates of interconversion from one diastereomer to the other is necessary. This is because it has been demonstrated by Gruys & Schuster (1982) and Dunaway-Mariano & Cleland (1980a) that, under enzymatic assay conditions, diastereomer interconversion occurs readily. This can, of course, be a potentially serious experimental handicap.

The purpose of this paper is therefore 2-fold. Primarily, data will be presented that will demonstrate the mechanism by which one diastereomer of α,β-bidentate CrADP interconverts to the other. This will serve as a possible foundation by which other metal–nucleotide interactions can be approached. Second, rate constants for the interconversion of the diastereomers under a variety of experimental conditions will be presented. These data will be useful for investigators to use as a reference for future experiments.

Materials and Methods

General. ADP (grade IX), Mes, and ion-exchange resins were purchased from Sigma Chemical Co.; sodium acetate and sodium chloride were from Fisher Scientific Co.; ethanesulfonic acid was from Alfa Products; $CrCl_3 \cdot 6H_2O$ was from Mallinckrodt Inc.

The equipment and instruments used in this study consisted of the following: the liquid-chromatograph equipment was a Beckman Model 341 isocratic liquid-chromatograph system (containing Model 112 pump, Model 160 fixed-wavelength detector, and Model 210 sample-injection valve), a Beckman ultrasphere-ODS reverse-phase column (4.6 mm × 25 cm), and either a Curken strip chart recorder (Model 250-2) or

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[1] Abbreviations: ATP, adenosine 5′-triphosphate; ADP, adenosine 5′-diphosphate; Mes, 2-(*N*-morpholino)ethanesulfonic acid; CD, circular dichroism; HPLC, high-performance liquid chromatography; ΔS^*, ΔH^*, and ΔG^*, thermodynamic parameters for activated complex; NMR, nuclear magnetic resonance.

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Spectra-Physics SP 4100 computing integrator. For CD studies, a Jasco J-41C spectropolarimeter interfaced to a Digital RX02 computer was used; for polarimetry, we used a Perkin-Elmer Model 141 polarimeter; and for concentration determinations, a Perkin-Elmer Model 552 UV–vis spectrophotometer was used. The fitting of interconversion data was done by an IBM main-frame computer (University of Nebraska Computer Network).

Preparation of α,β-bidentate CrADP was similar to the method described by Dunaway-Mariano & Cleland (1980a). A 100-mL solution 10 mM each in $CrCl_3$ and NaADP was heated to 80 °C in a boiling H_2O bath for 10 min, cooled rapidly by pouring it into a 250-mL Erlenmeyer flask (precooled in an ice–water bath), adsorbed onto a 15-mL column of Dowex 50 H^+ (a 20-cm^3 syringe is convenient for column use), and washed with water. Perchloric acid, 0.5 M, was then applied to the column until a visible separation between the unreacted $Cr(H_2O)_6$ top layer and the α,β-bidentate CrADP product was apparent. After this, the top band of the column was removed and discarded and the CrADP band transferred to a smaller 10-cm^3 syringe that contained 1 cm of fresh resin. The product, α,β-bidentate CrADP, was then eluted with 1 M $HClO_4$, brought to a pH of 4.0 with saturated $KHCO_3$, filtered, and stored at 4 °C.

Separation of the diastereomers of α,β-bidentate CrADP was performed similar to the method of Gruys & Schuster (1982), for the β,γ-bidentate CrATP diastereomers. Ethanesulfonic acid, 10 mM, pH 2.5 (adjusted with 5 N NaOH), was used as the isocratic buffer and ion-pairing agent. The column was equilibrated for a minimum of 0.5 h at a 1 mL/min flow rate prior to injection of the sample. For analytical work, a 20-μL loop was attached to the injector and then filled with either pure isomer or a mixture of the two. Detection of the nucleotide analogues was done by absorbance at 254 nm. The flow rate was 1 mL/min. For preparative work, a 350-μL loop was attached to the injector and filled with the Dowex-purified mixture of both diastereomers. Detection of the diastereomers was done by monitoring absorbance at 436 nm (at 254 nm, this amount of sample will saturate the absorbance range). This is close to one of the visible absorption maximas of bidentate CrADP (Bossard et al., 1982). The flow rate was 1 mL/min, and the diastereomers were collected as they eluted from the column. The purity of the isolated isomers was from 95 to 100%. For both the analytical and preparative scales, the peaks were assigned in the order in which they eluted from the column. Concentration of the isolated isomers was determined by the adenine absorbance at 259 nm ($\epsilon = 15.4 \times 10^3$).

CD spectra of the two diastereomers of α,β-bidentate CrADP were obtained at ambient temperature, at pH 2.5, and with 10 mM ethanesulfonic acid as the background buffer. A preparative-scale separation was performed, and the molar ellipticities were calculated by the adenine content of the isolated diastereomers.

Polarimetric measurements of the α,β-bidentate CrADP diastereomers were done at 546 nm with the Hg lamp, at ambient temperature, and with 10 mM ethanesulfonic acid as the background buffer. A preparative separation was performed and the concentration determined by the adenine absorbance. Since specific rotation values are expressed in degrees milliliter per decimeter gram $[[\alpha]_\lambda^T = \alpha/(lC)]$, a molecular mass of 549 g/mol was used along with the adenine content to calculate the concentration in grams per milliliter.

The interconversion of the diastereomers was monitored with the liquid chromatograph system set up for analytical

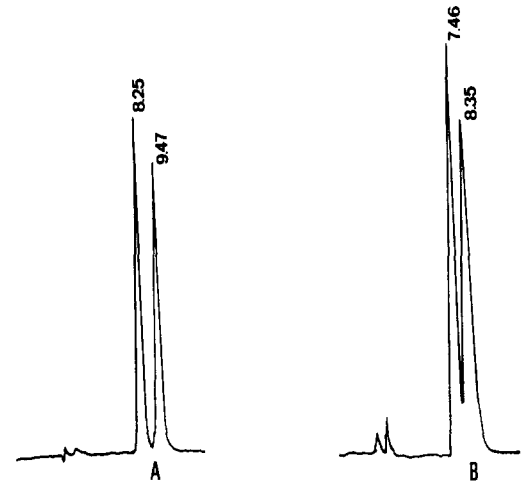

FIGURE 1: (A) Elution profile from chromatography of α,β-bidentate CrADP reaction mixture on an Altex ODS, C_{18} reverse-phase column with 10 mM ethanesulfonic acid, pH 2.5, as eluting buffer. A 20-μL loop was used for analytical separations. The flow rate was 1 mL/min, and the detector wavelength was 254 nm. (B) Elution profile for a preparative separation with a 350-μL loop and a detection wavelength of 436 nm. The conditions were the same as in (A). For both (A) and (B), the numbers indicate retention times.

analysis. The Spectra-Physics 4100 computing integrator was then used to monitor the exact percent of each isomer during the course of the interconversion. The reactions were initiated by the addition of 50 μL of pure isomer I or II to 950 μL of 20 mM buffer at a particular pH and temperature. The extent of interconversion was then monitored by periodic injections of the reaction mixture into the HPLC system (which, at a pH of 2.5, terminated the reaction) or by a series of samples initiated and then stopped by the addition of 50 μL of 1 N H_2SO_4 at specific time intervals. The former procedure was followed when the experimental conditions produced slow interconversions, and the latter procedure was followed when rapid changes occurred. Specific conditions for the interconversions are noted in the figure legends.

The calculation of rate constants of the data was accomplished by iterative descent methods as outlined by Fletcher & Powell (1963) and Powell (1964, 1965). These methods will find the local minimum for a large number of variables of a particular function, even if only poor initial approximations to the solution are known. When these methods are applied to chemical kinetics, the integrated solutions to the differential equations are required. Statistical subroutines were added to the general program so that a general "goodness of fit" could be evaluated for different mechanisms.

Two different mechanisms were used to fit the data: a reversible binary mechanism of two components and a reversible ternary mechanism of three components, i.e.

$$A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \text{ and } A \underset{k_2}{\overset{k_1}{\rightleftharpoons}} B \underset{k_4}{\overset{k_3}{\rightleftharpoons}} C$$

respectively.

Results

Separation of Diastereomers of α,β-Bidentate CrADP. Figure 1 demonstrates the ease and power of the described separation techniques. Previously, asymmetric adsorption chromatography with cycloheptaamylose as described by Cleland (1982) was required for the separation. This technique provides good separation but requires a synthetic procedure for cross-linking the cycloheptaamylose and a minimum of 24 h for complete separation. As can be seen in Figure 1, ex-

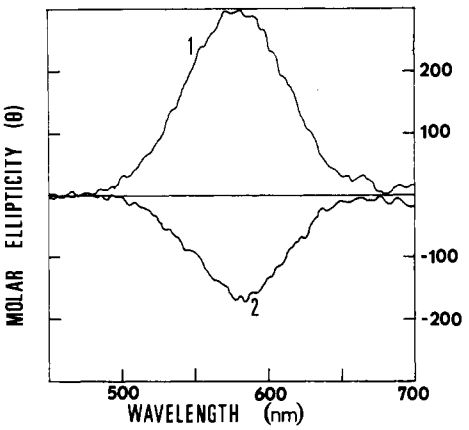

FIGURE 2: CD spectra of the two diastereomers of α,β-bidentate CrADP in 10 mM ethanesulfonic acid, pH 2.58 at ambient temperature. The spectra are numbered in the order in which the isomers elute from the C_{18} reverse-phase column.

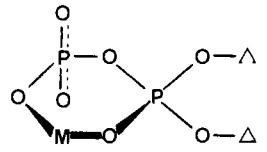

FIGURE 3: Model of stereochemical configuration of α,β-bidentate metal–ADP complex. Λ and Δ represent where the rest of the molecule is attached. Λ signifies left-handed screw sense, whereas Δ signifies right-handed screw sense [adapted from Dunaway-Mariano & Cleland (1980a)].

cellent resolution is obtained for both preparative and analytical work. More significantly, the time involved for complete separation has been reduced to less than 10 min. Since no synthetic procedure is required for the column-packing material, we would also assume the reverse-phase HPLC technique described under Materials and Methods to be considerably easier than the cycloheptaamylose method.

Polarimetric Measurements and CD Spectra of Isolated Diastereomers. Figure 2 shows the visible CD spectra of the isolated diastereomers. In comparison to the CD spectra presented by Dunaway-Mariano & Cleland (1980a), it is evident that the two eluting isomers in this paper are in reverse order to the two eluting isomers in the Dunaway-Mariano & Cleland paper.

Dunaway-Mariano & Cleland (1980a,b) have assigned the diastereomer with negative ellipticity as the Δ isomer on the basis of the inhibitory powers of the isolated diastereomers on creatine kinase. The Δ screw-sense isomer is the preferential substrate of creatine kinase as evidenced by the specificity for MgATPαS, isomer B, and CdATPαS, isomer A (Burgess & Eckstein, 1980). Therefore, eluting isomer 2 in this paper is the Δ isomer and eluting isomer 1 is the Λ isomer.

Cleland (1982) has assigned the notation of Λ and Δ to the left- and right-handed screw sense, respectively, as shown in Figure 3. Our assignments of configuration are based upon this same criterion.

The specific rotation values ($[\alpha]^{25°C}_{546nm}$) for both of the isolated diastereomers were found to be −59.5 and −13.5 for the Λ and Δ isomers, respectively (units are deg·mL/dm·g). Other asymmetric centers in the α,β-bidentate CrADP molecule besides the chromium–phosphate bonds contribute to the overall specific rotation value. This is evident since both values of the isolated diastereomers are negative.

Calculation of Rate Constants. Of the two mechanisms used to fit the data, the reversible binary mechanism with only two components proved to be far superior statistically than

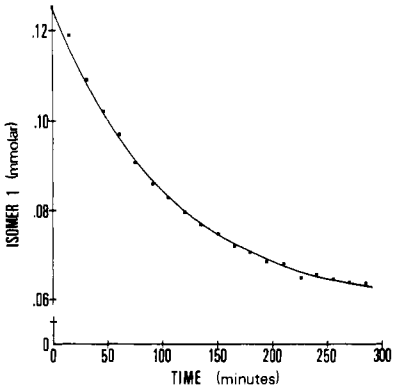

FIGURE 4: Data for conversion of isomer 1 (the Λ isomer) toward the equilibrium concentration of both isomers at pH 6.0 and 26.5 °C. The solid line is the computer fit for the data by using the binary, two-component mechanism. 50 μL of pure isomer 1 was added to 950 μL of 20 mM Mes, pH 6.0, and incubated in a temperature-controlled water bath at 26.5 °C. The extent of interconversion was monitored by 15 min intervaled injections of the reaction mixture into the HPLC system. The HPLC system was set up for analytical analysis (20-μL loop, 254-nm filter, and the SP 4100 computing integrator).

the more complicated mechanism of three components. The fit for the more complicated mechanism produced rate constants that were erroneous according to their σ values. The simple mechanism, however, produced rate constants with very reasonable σ values. For example, the calculated rate constants for the simple mechanism at pH 6.0 and 26.5 °C are k_1 = 0.00503 ± 0.00008 min^{-1} and k_2 = 0.00439 + 0.00017 min^{-1}. The same statistical results were found for all other experimental data.

Figure 4 demonstrates how well the two-component mechanism fits the experimental data. The calculated rate constants produce the solid line from which the observed data deviate very slightly. The final sum of squares from the residuals is 1.379×10^{-3}, and the number of residual runs is 7. The data from Figure 4 in actuality are the poorest statistically of all the experimental data that were fitted to the two-component mechanism. Thus, it is clearly evident that the reported rate constants are statistically accurate.

In almost all experiments, unless designated otherwise, the calculated rate constants were derived from monitoring the conversion of isomer 1 to isomer 2 (Λ to Δ). However, in order to be sure that the reverse reaction produced the same rate constants in each different type of experiment (varying pH, temperature, buffer concentration, etc.), at least one duplicate was done with the reverse reaction. In all cases, the rate constants were approximately the same (data not reported).

Interconversion vs. pH. One of the characteristics of chromium(III) is to form polymeric species at neutral or basic pH values (Rollension, 1973). In addition, rates of hydrolysis and decomposition of chromium(III) complexes of ATP have been reported at various alkaline pHs (McClaugherty & Grisham, 1982). These behaviors were apparently only at pH values above 6.5, where an observed cloudy green precipitate formed at rates possibly competitive with the interconversions. However, under the conditions chosen for this investigation, there was little if any CrADP lost to side reactions. This is based on the observation that the total area under the integrated peaks of the chromatograms remained relatively constant.

Table I illustrates the results found when the interconversion was monitored and the rate constants were calculated at pH values 5.0–6.5. When the data are plotted as log k_{obsd} vs. log $[OH^-]$ (not shown), a linear relationship is found (correlation

Table I: Effect of pH on Calculated Rate Constants for Interconversion of Diastereomers of α,β-Bidentate CrADP[a]

$$\Lambda \text{ isomer} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \Delta \text{ isomer}$$

pH	$k_{1,obsd}$ (min^{-1})	$k_{2,obsd}$ (min^{-1})	K_{eq}
5.0	3.61×10^{-4}	3.18×10^{-4}	1.14
5.5	1.24×10^{-3}	1.10×10^{-3}	1.13
6.0	5.03×10^{-3}	4.39×10^{-3}	1.15
6.5	1.48×10^{-2}	1.24×10^{-2}	1.19

[a] All rate constants, except k_2 for pH 5.0 and 5.5, were calculated from the conversion of the Λ isomer to the Δ isomer. The two exceptions were found by the reverse reaction. All reactions were done at 26.5 °C. 50 μL of pure isomer 1 or 2 was added to 950 μL of 20 mM Mes or 20 mM acetate, depending on the desired pH. For the reaction at pH 6.5, the reaction was stopped by the addition of 50 μL of 1 N H_2SO_4 at specific time intervals. For the reactions at pH 5.0, 5.5, and 6.0, the extent of interconversion was monitored by periodic injections into the HPLC system. The HPLC system was set up for analytical analysis (20-μL loop, 254-nm filter, and the SP-4100 computing integrator).

Table II: Effect of Buffer Concentration on Calculated Rate Constant for Conversion of Λ Isomer to Δ Isomer[a]

[Mes] (mM)	[Na$^+$Mes] (mM)	[NaCl] (mM)	μ (mM)	k_{obsd} (min^{-1})
3.09	6.91	193	200	2.89×10^{-2}
6.20	13.8	186	200	2.82×10^{-2}
12.4	27.6	172	200	2.69×10^{-2}
24.7	55.3	145	200	3.02×10^{-2}

[a] All reactions were done at pH 6.5 and 30 °C. 50 μL of pure isomer 1 (the Λ isomer) was added to the above solutions and in incubated in a temperature-controlled water bath at 30 °C. The reactions were stopped by the addition of 50 μL of 1 N H_2SO_4 at specific time intervals. The extent of interconversion was monitored by the analytical HPLC technique described under Materials and Methods. The symbol μ is ionic strength.

coefficient 0.9989). The slope of this plot is 1.09, which indicates that the rate of interconversion goes up by a factor of 10 per pH unit. This is similar to the findings of Bossard et al. (1982), who found that the rate of conversion of monodentate CrADP to bidentate CrADP had the same pH dependence.

It is interesting to note that the rate constants reported in Table I result in K_{eq} constants greater than 1 for all pH values tested (the average K_{eq} being 1.15). Thus, the kinetic data are in agreement with the CD spectra of the isomers in that the isomers cannot be exact mirror images of each other.

Interconversion vs. Concentration of Buffer. To help determine whether or not the buffer in the reaction mixture was in actuality one of the catalyzing species, an experiment was devised that is outlined in Table II. If indeed the buffer is part of the catalysis scheme, a rate dependence upon the buffer's concentration should be seen (Moore & Pearson, 1981). Table II clearly shows that if the ionic strength is maintained, no apparent rate dependence on the concentration of buffer is evident (the average rate constant of all four buffer concentrations being $2.9 \times 10^{-2} \pm 0.001$ min^{-1}).

Interconversion vs. Temperature. Figure 5 is a plot of log K_{obsd} vs. the reciprocal of the absolute temperature for the conversion of the Λ isomer to the Δ isomer. By use of the well-known Arrhenius equation [$K_{obsd} = Ae^{-E/(RT)}$], the energy of activation is calculated to be 28.1 kcal/mol, and the preexponential factor A is 1.66×10^{18} min^{-1}.

Eyring (1935) has given a quantitative treatment of the activated complex such that application of transition-state theory to rates of reactions in solution can be done. For a

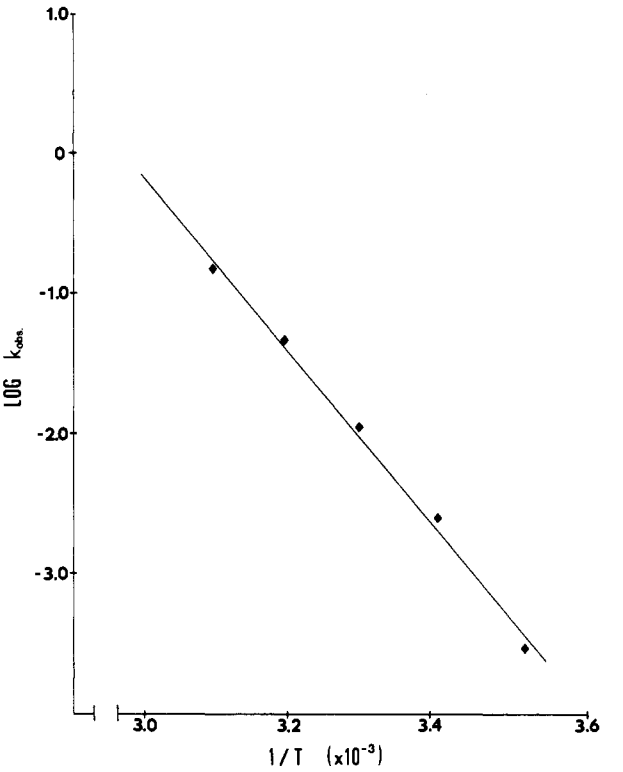

FIGURE 5: log of calculated first-order rate constants for conversion of isomer 1 to isomer 2 as a function of inverse of absolute temperature. 50 μL of pure isomer 1 was added to 950 μL of 20 mM Mes, pH 6.0, and incubated at the designated temperatures in a water bath. The reactions were stopped at specific time intervals with 50 μL of 1 N H_2SO_4 and then analyzed for isomer content with the HPLC system set up for analytical work (20-μL loop, 254-nm filter, and the SP 4100 computing integrator).

reaction at a given temperature, the free energy of activation can be interpreted in terms of thermodynamic parameters. Therefore, at pH 6.0 and 26.5 °C, the following thermodynamic parameters have been calculated for the conversion of isomer 1 (Λ isomer) to the activated complex:

$$\Delta S^{\ddagger} = 14.7 \text{ cal K}^{-1} \text{ mol}^{-1} \qquad \Delta H^{\ddagger} = 27.5 \text{ kcal mol}^{-1}$$
$$\Delta G^{\ddagger} = 23.1 \text{ kcal mol}^{-1}$$

Discussion

Gruys & Schuster (1982), Connolly et al. (1982), and M. Cohn et al. (personal communication) have all demonstrated the usefulness of reverse-phase HPLC techniques in the separation of Co(III) and Cr(III) nucleotide diastereomers. A study such as the one in this paper requires a method for precise differentiation between diastereomers within a reasonable time period. The HPLC separation techniques described here and by the above authors meet these requirements.

Recognizing the fact that the rates of interconversion were base catalyzed, it became pertinent to elucidate the particular type of base catalysis. Generally, two basic types of base catalysis can be observed experimentally. If catalysis is caused only by the concentration of hydroxide ion present, it is then dependent on the pH only and is independent of the concentration of buffer. This type of catalysis is called specific base catalysis, while another type of base catalysis evolves a dependence upon the nature and concentration of the buffer. This is designated general base catalysis (Jencks, 1969). Kinetically detailed descriptions of the above two cases are outlined by Wilkinson (1980) and Moore & Pearson (1981).

Of course, reactions need not follow just one of the two schemes mentioned above but may be more intermittent in

nature. A generalized rate constant, which would account for this, would simply be

$$k_{obsd} = k_0 + k_{OH^-}[OH^-] + \sum_i k_i[B_i] \quad (1)$$

where k_0 is the noncatalyzed rate constant in solvent alone and B is a catalyzing base. Since OH^- and B are not being consumed and remain constant in reactions of this type, the observed rate constant is pseudo first order.

According to eq 1, if a reaction is catalyzed by hydroxide concentration only and if k_0 is small, then a plot of log k_{obsd} vs. log [OH^-] should be linear with a slope of 1. A plot of the data in Table I shows this type of relationship. In addition, Table II clearly demonstrates that there is no rate dependence on the concentration of the buffer Mes. Indeed, at low pH values where acetate was used as the buffer, no deviations in the data are apparent. Therefore, from these data and the above assumptions, we would conclude the interconversion of the diastereomers of α,β-bidentate CrADP to be specific base catalyzed.

A plot of k_{obsd} vs. hydroxide concentration is also linear and results in a slope equal to k_{OH^-}. This value is 4.7×10^5 min^{-1}. The y intercept of this plot should be equal to k_0, and the value for this is -6.8×10^{-5} min^{-1}, which for all practical purposes is zero. The interpretation of these two results is that hydroxide ion is a very potent catalyzing agent and that without its presence the reaction will not proceed.

Though experimental observations conclude that specific base hydrolysis is occurring, the large catalytic effectiveness of the hydroxide ion may have caused catalysis by other added bases to be difficult, if not impossible, to detect. It is certainly obvious that the hydroxide ion's catalytic capabilities compensate for its low concentration. It is possible that detectable interconversion might occur at low pH values with sufficiently added base (one with a proper pK_a value so that it would be completely ionized). However, since this study was concerned with effects on diastereomer interconversion under enzymatic assay conditions, extremely low pH studies were not done. It is pertinent to point out that under storage conditions (10 mM $CH_3CH_2SO_3^-$, pH 2.5, and 4 °C) no detectable interconversions occurs. This is true even up to a period of 3 months.

A positive $\Delta S^{\ddagger}$ term from the energy of activation data simply states that there is an increase in rotational and vibrational freedom in the activated complex. The large positive value that was found implies that the reaction follows a dissociative pathway. However, great care must be taken in interpretation of entropy changes. A positive entropy change could result from the solvent effects alone. A dissociative pathway is consistent though with the mechanisms proposed previously for the coordination of several organic ions to hexaaquo Cr(III) (Hamm et al., 1958) and the unifying mechanism proposed by Garrick (1937). It is also interesting to note that in the paper by Hamm (1958), the same pH dependence was seen as was observed in Table I, regardless of the anion.

Figure 6 is the mechanism proposed from the experimental data. This mechanism is very similar to Garrick's unifying mechanism (1937), which is conveniently called an S_N1CB mechanism (substitution, nucleophilic, unimolecular, conjugate base). In the case of the interconversion of the α,β-bidentate CrADP diastereomers, an isomerization is occurring that is only a special case of S_N1CB reaction.

The mechanism in Figure 6 starts with an acid–base equilibria that is assumed to be rapid according to the pH dependence. Aquo complexes of chromium are known to be slightly acidic due to the equilibrium reaction $[Cr(H_2O)_6]^{3+} \rightleftarrows [Cr(H_2O)_5(OH^-)]^{2+} + H^+$, with a first hydrolysis constant of 10^{-4} (Rollension, 1973). The hydroxyl group has a labilizing effect on a replaceable ligand, and so the chromium–oxygen bond from the phosphate is able to break, and a pentacoordinate intermediate is formed. This is substantiated by the positive entropy term and the known mechanisms of model compounds. Breakage of this bond (k_3 and k_6) is probably the rate-limiting step. Rotation followed by re-formation of the octahedral complex with the other available phosphate oxygen results in the opposite screw-sense isomer. The last step is a return to the acid–base equilibria. This is, of course, all reversible.

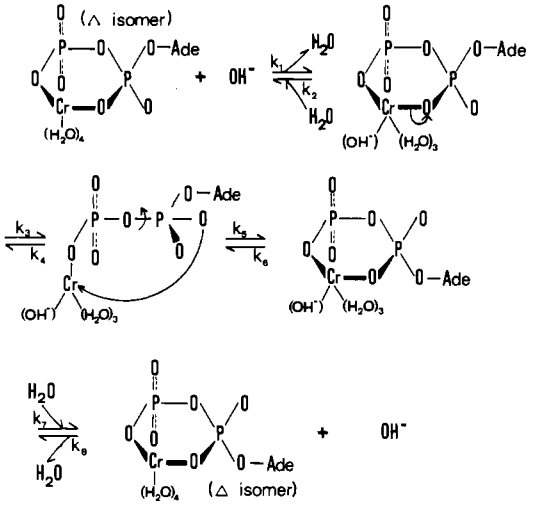

FIGURE 6: A possible mechanism illustrating interconversion of diastereomers of α,β-bidentate CrADP.

The proposed mechanism by Bossard et al. (1982) for the conversion of monodentate CrADP to bidentate CrADP follows the same general scheme as is outlined above. That is, deprotonation of the complex followed by a dissociation to a pentacoordinate intermediate occurs enroute to product. The pH dependence of the reaction also leads to the hypothesis that the rate-limiting step is the formation of the pentacoordinate intermediate.

An analysis of the interconversion in Figure 6 in the forward direction can lead to a simplified rate law if steady-state and equilibrium assumptions are used. If one assumes that the reaction starts with 100% of the Λ isomer and that the pentacoordinate intermediate is at a steady-state concentration (this is valid if the intermediate is very reactive and therefore low and constant in concentration), the initial rate of conversion is

$$\text{rate} = \frac{k_3 k_5 [\Lambda_{OH^-}\ \text{isomer}]}{k_4 + k_5} \quad (2)$$

Furthermore, if k_1 and k_2 are fast enough so that a facile equilibrium can occur, then the rate equation can be further simplified to

$$\text{rate} = \frac{k_3 k_5 K_{eq} [\Lambda\ \text{isomer}][B]}{(k_4 + k_5)[BH^+]} \quad (3)$$

and

$$\text{rate} = \frac{k_3 k_5 K_{eq} [\Lambda\ \text{isomer}][OH^-]}{(k_4 + k_5) K_b} \quad (4)$$

In this case, B replaces OH^- and represents any base that catalyses the reaction, and BH^+ is its conjugate acid and replaces the water molecule. As can be seen, eq 3 and 4 are

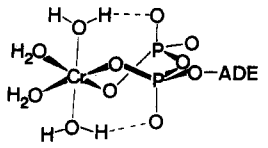

FIGURE 7: Pseudoequatorial position for Λ isomer of α,β-bidentate CrADP [adapted from Dunaway-Mariano & Cleland (1980a)].

of the forms such that a linear dependence on the hydroxide concentration would be expected.

The mechanism in Figure 6 does not precisely represent the exact structures of the bidentate CrADP molecule. It is felt that the true structures are ring conformers stabilized by strong hydrogen bonds between coordinated waters and phosphate oxygens (Dunaway-Mariano & Cleland, 1980a). A possible structure is shown in Figure 7. Unfortunately, to date, this structure is still speculative and unconfirmed. It is evident that the exact structure, when confirmed, must be taken into account for the overall mechanism. One reviewer has suggested that if the structure in Figure 7 is correct, then the coordinated water molecules most likely to be deprotonated would be those in the trans position. This is because the cis waters are involved in hydrogen bonding.

The need for proper models for metal–nucleotide interactions has clearly been demonstrated. Structural models for metal–nucleotide binding have been developed by X-ray crystal structure determination of inert complexes (Merritt et al., 1978, 1981), but little mechanistic work for isomer interconversions had previously been done. It is hoped that this paper has brought some new insights into the complexities of metal–nucleotide interactions and that this work will serve as a basis for future investigation.

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Registry No. $Cr^{III}(H_2O)_4$(ADP) (Λ isomer), 73000-97-2; $Cr^{III}(H_2O)_4$(ADP) (Δ isomer), 73037-58-8; $Cr^{III}(H_2O)_4$(ADP), 58642-43-6; $CrCl_3$, 10025-73-7; NaADP, 1172-42-5.

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