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Mechanism of Lactic Acid Formation Catalyzed by a Macrocyclic Chromium(III) Complex. A Comparison with the Glyoxalase I Enzyme

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The transformation of methylglyoxal and of 1,3-dihydroxyacetone and glyceraldehyde into lactic acid can be catalyzed by metal complexes, and chromium(III) complexes of the macrocyclic 5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane tetraamine ligand, with two coordination sites in the *cis* position available for substrate coordination, are reasonably effective for the production of coordinated lactate.

The detailed stoichiometry of this process, including stereoselectivity studies using optically active complexes, has been investigated by a combination of ^{1}H and ^{13}C NMR, ion-exchange chromatography, deuterium labelling studies, and a single-crystal structure determination of one of the diastereomers formed from 1,3-dihydroxyacetone: *cis*-[Cr(cyc*b*)($C_3H_4O_3$)]ClO_4, which crystallizes in the orthorhombic space group *Fdd*2 with $a = 31.663(15)$, $b = 9.650(5)$, $c = 15.848(7)$ Å and $Z = 8$.

The suggested mechanism of the methylglyoxal transformation process involves bidentate substrate coordination followed by protonation, dehydration and carbocation formation, intramolecular 1,2-hydride shift, and deprotonation. This mechanism is discussed in relation to the zinc(II)-containing glyoxalase I enzyme, which performs an analogous substrate transformation in natural systems.

The transformations of 1,3-dihydroxyacetone and glyceraldehyde are stoichiometrically more complicated, and result for both substrates in coordinated lactate in which *one* hydrogen atom in the methyl group originates from solvent water.

Lactic acid is an important carbohydrate degradation product, and is widely distributed in biological systems as both the racemic and the optically active acid. One pathway for the production of lactic acid in natural systems is the glyoxalase enzyme system, which catalyzes an intramolecular methylglyoxal rearrangement process.[1] This reaction can formally be considered an OH/H interchange, and it was recently demonstrated that this type of transformation could also be catalyzed by simpler metal complexes than the zinc(II)-containing glyoxalase I enzyme.[2]

The present work describes a stoichiometric study of lactic-acid-formation processes catalyzed by chromium(III) complexes of the optically active 5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane, cyc*b*, tetraamine ligand (Fig. 1). Abbreviations for ligand and complex names are given in Table 1.

Table 1. Ligand and complex name abbreviations (see Fig. 1).

cyc*b*	5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane
ss-cyc*b*	(7*S*,14*S*)-5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane
rr-cyc*b*	(7*R*,14*R*)-5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane
lact	lactate: $CH_3CHOHCO_2^-$
r-lact	(*R*)-lactate
s-lact	(*S*)-lactate
rr,r complex	*cis*-[Cr(*rr*-cyc*b*)(*r*-lact)]$^{2+}$
rr,s complex	*cis*-[Cr(*rr*-cyc*b*)(*s*-lact)]$^{2+}$
ss,r complex	*cis*-[Cr(*ss*-cyc*b*)(*r*-lact)]$^{2+}$
ss,s complex	*cis*-[Cr(*ss*-cyc*b*)(*s*-lact)]$^{2+}$

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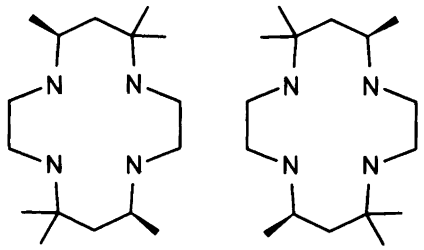

Fig. 1. (7*S*,14*S*)- and (7*R*,14*R*)-5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane.

Results and discussion

Reactivity. Red–violet dilute solutions of *cis*-[Cr(cyc*b*)$(OH_2)_2]^{3+}$ change colour to blue–violet after dissolution of methylglyoxal. At room temperature this transformation takes hours for completion, but at 100°C it is finished within a few minutes. Ion-exchange chromatography of such solutions shows the presence of components with charges of 1+ and 2+, in addition to the 3+ charged initial reactant, as judged from the fractionation behaviour in dilute acidic solution. Addition of perchlorate ions to the eluates causes precipitation of a brownish, dark green crystalline product in the 1+ charged eluate. Solid compounds were not obtained by this approach from the 2+ charged eluate.

Identification of chromium(III) complexes. Semiquantitative kinetic measurements in dilute acidic solutions indicate the deprotonated diaqua complex *cis*-[Cr(cyc*b*)$(OH_2)(OH)]^{2+}$ to be the dominant chromium(III) reactant. In neutral and acidic solutions this complex reacts with methylglyoxal to give products with spectral and acid/base properties characteristic of complexes of chelate lactate. These products with chelate lactate appear to be formed initially, but at longer reaction times complexes with properties characteristic of complexes coordinated with monodentate carboxylate and water are also present.

Spectral characteristics of the 1+ charged complex vary as a function of the mode of purification, and a chromatographic analysis shows that the fraction of bidentate lactate complex contains two components. This is demonstrated in Fig. 2 for the product mixture formed from *cis*-$[Cr(cyc\mathit{b})(OH_2)(OH)]^{2+}$ and methylglyoxal.

Stoichiometry of diastereomer formation. Product mixtures similar to those formed from methylglyoxal and the macrocyclic chromium(III) complex are formed from other three-carbon atom substrates with the carbon atoms in the same average formal oxidation state. These transformations obviously require more extensive rearrangements, but coordinated deprotonated lactic acid is produced from both 1,3-dihydroxyacetone and glyceraldehyde and the chromium(III) complex. These latter substrate transformations are slower than that of methylglyoxal, but much faster than required if an uncatalyzed formation of methylglyoxal was the rate-determining step.

The similar chromium(III)-catalyzed rearrangement of glyoxal into coordinated deprotonated glycolic acid occurs via an intermediate supposed to contain bidentate deprotonated hydrated glyoxal. This intermediate is reasonably stable and has been isolated in solution and characterized kinetically and spectroscopically.[3] Attempts to isolate intermediate reaction products from the present three substrates have not been successful, and only the free substrate reactant and the chelate coordinated lactate reaction products are seen at the present limits of detection. Monodentate carboxylate-bound lactate is eventually present, but appears to be formed by a secondary process involving partial aquation of the bidentate complex.

The formation ratios of the diastereomeric lactate complexes were investigated as a function of temperature and hydrogen-ion concentration. A summary of the data involving methylglyoxal, 1,3-dihydroxyacetone and racemic and optically active glyceraldehyde is given in

Table 2. Summary of diastereomer formation ratios for chelate lactate complexes at 20 and 100°C.[a]

Substrate (concentration)	Complex (concentration)	H^+ concentration	Ratio
Methylglyoxal (3.5–30 mM)	*rac-*, *ss-* (1.5–2.5 mM)	10^{-6}–10^{-2} M	0.422(18)
1,3-Dihydroxyacetone (2.5–25 mM)	*rac-*, *ss-* (1.5–2.0 mM)	0.01–0.05 M	0.393(11)
Glyceraldehyde (2.4–20 mM)	*rac-* (1.5–2.0 mM)	0.01 M	0.388(16)
(*R*)-Glyceraldehyde (2.0 mM)	*ss-* (2.0 mM)	0.01 M	0.374(10)
(*R*)-Glyceraldehyde (2.0 mM)	*rr-* (2.0 mM)	0.01 M	0.432(10)

[a]The ratios given are the amount of substance in the first eluted fraction (*rr,s* and *ss,r* complexes) as compared to the total amount of substance of chelate lactate complexes. Standard deviations are shown in parenthesis.

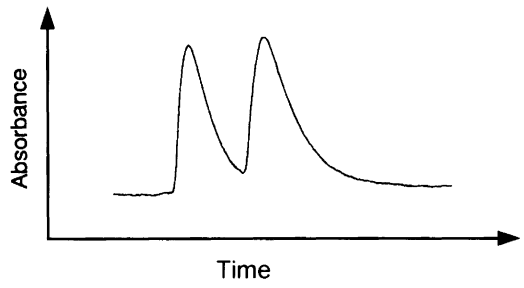

Fig. 2. FPLC separation of diastereomeric lactate complexes, using gradient elution from 0.01 M HBr to 0.01 M HBr + 0.20 M NaBr. Absorption monitored at 254 nm. The fraction first eluted contains *rr,s* and *ss,r* complexes. The second fraction contains *rr,r* and *ss,s* complexes.

Table 2. All substrates gave a mixture of diastereomers in roughly a 2:3 ratio. The diastereomer ratio is independent of temperature, of hydrogen-ion concentration and of the degree of conversion, but minor differences as a function of substrate and substrate chirality are observed.

Description of dominant reaction-product structure. Fractional crystallization of the perchlorate salt formed from the racemic macrocyclic chromium(III) complex and 1,3-dihydroxyacetone gives a product in which only minor amounts of the component first eluted at the FPLC chromatographic method (Fig. 2) are present, and crystals of *cis*-[Cr(cyc*b*)($C_3H_4O_3$)]ClO_4, containing doubly deprotonated lactic acid, could be obtained in a quality suitable for a single-crystal structure determination (cf. the Experimental section).

A view of the cation in this compound is shown in Fig. 3, and selected bond distances and angles are given in Table 3. The macrocyclic ligand is coordinated in the *cis* configuration with *gauche* and *chair* conformations to the five- and six-membered chelate rings. This conformation of the macrocyclic amine is very similar to that of four previously characterized chromium(III) complexes of this macrocyclic ligand, involving the carbonato,[4] the dihydroxo,[4] the dicyano[5] and the glycolato complexes,[6] and that which from various semiempirical rationalizations is estimated to be the most stable.[7]

The compound is a racemic mixture with (*R*) and (*S*) deprotonated lactate coordinated to chromium(III) complexes of the (7*R*,14*R*)- and (7*S*,14*S*)-tetraamine ligand (Fig. 1), respectively.

Deuterium labelling experiments. Further details of the substrate transformations were characterized by isolation of reaction products formed in D_2O. Decomposition of the isolated complexes of chelate lactate in an acidic solution containing chloride and perchlorate ions gave solid *cis*-[Cr(cyc*b*)Cl_2]ClO_4 and solutions of lactic acid which were sufficiently free of paramagnetic chromium(III) to be suitable for ^1H and ^{13}C NMR measurements.

The NMR spectra given in Fig. 4 show unambiguously incorporation of *one* deuterium atom from the solvent water into the methyl group of the lactic acid formed from both 1,3-dihydroxyacetone and glyceraldehyde. This incorporation is independent of whether the reaction has taken place in acidic or neutral solution. Minor amounts of undeuterated lactic acid also appear to be present. This is most likely the result of the decrease in isotopic purity of the solvent water caused by the dissolution of undeuterated reactants.

Similar deuteration experiments using methylglyoxal do not show any deuterium atom incorporation in the isolated lactic acid (Fig. 4).

Mechanism of lactic acid formation. The mechanism of formation of coordinated lactate arrived at, on the basis of the above experiments, is given in Fig. 5.

For methylglyoxal the mechanism is similar to that proposed for formation of coordinated glycolate from glyoxal, involving chelate substrate binding as the first

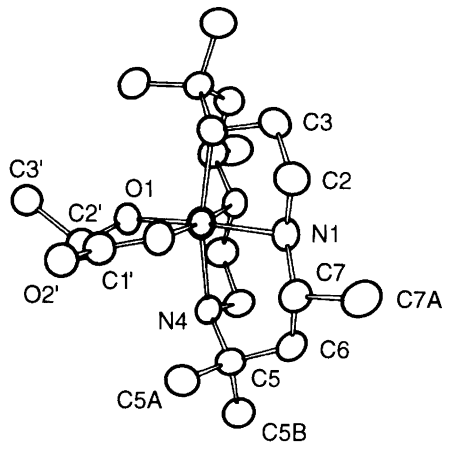

Fig. 3. cis-[Cr(cyc*b*)($C_3H_4O_3$)]$^+$ structure and numbering scheme, showing the *rr,r* complex enantiomer. The crystal structure is that of the racemic mixture.

Table 3. Summary of structural data for *cis*-[Cr(cyc*b*)($C_3H_4O_3$)]ClO_4.

	Range (std. dev.)
Distances/Å	
Cr–N	2.147–2.148(5)
Cr–O1	1.892(4)
C–C (cyc*b* ligand)	1.515–1.537(10)
C–N (cyc*b* ligand)	1.492–1.520(8)
C1′=O2′	1.223(15)
C1′–O1	1.288(15)
C2′–O1	1.486(16)
C2′–C3′	1.573(15)
C1′–C2′	1.516(15)
Angles/°	
O–Cr–O	85.54(19)
N–Cr–N (NCCN-unit)	83.44(18)
N–Cr–N (NCCCN-unit)	88.62(18)

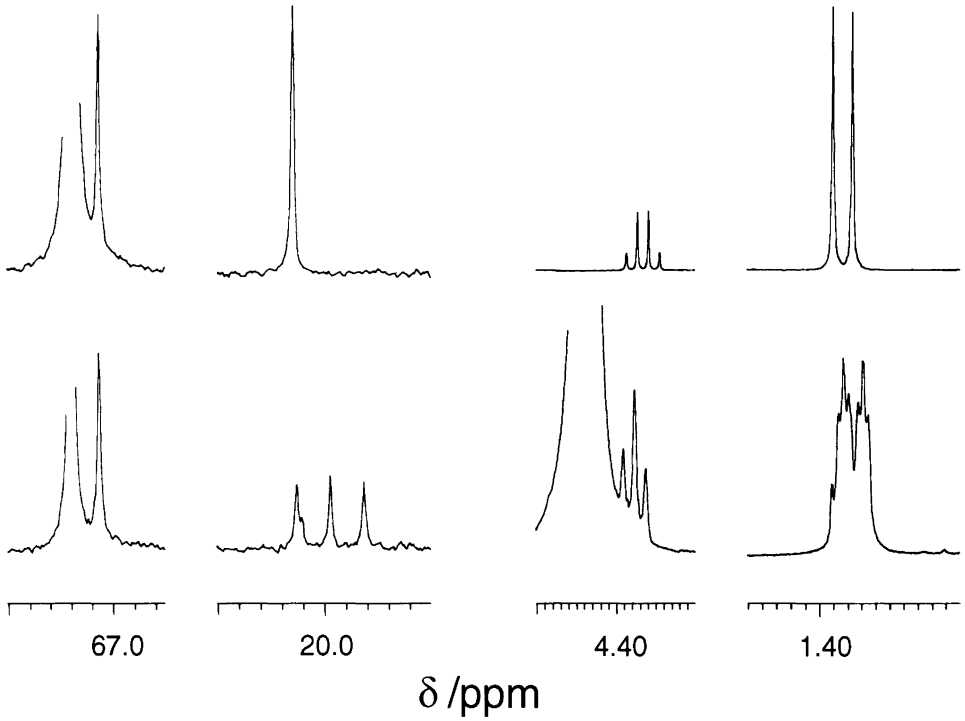

Fig. 4. NMR spectra of lactic acid in acidic D_2O. Left part: ^{13}C spectra. Right part: ^{1}H spectra. Upper part: lactic acid formed from methylglyoxal in D_2O. Lower part: lactic acid formed from 1,3-dihydroxyacetone in D_2O. Different concentrations of chromium(III) in the two solutions cause minor shifts of the resonance positions. This is particularly prominent in the ^{1}H spectrum for the solvent water resonance to the left of $\delta = 4.4$ ppm. Differences in the lactic acid resonance positions were always significantly smaller. Units on the abcissa correspond to δ-value differences of 0.2 ppm (^{13}C) and 0.02 ppm (^{1}H).

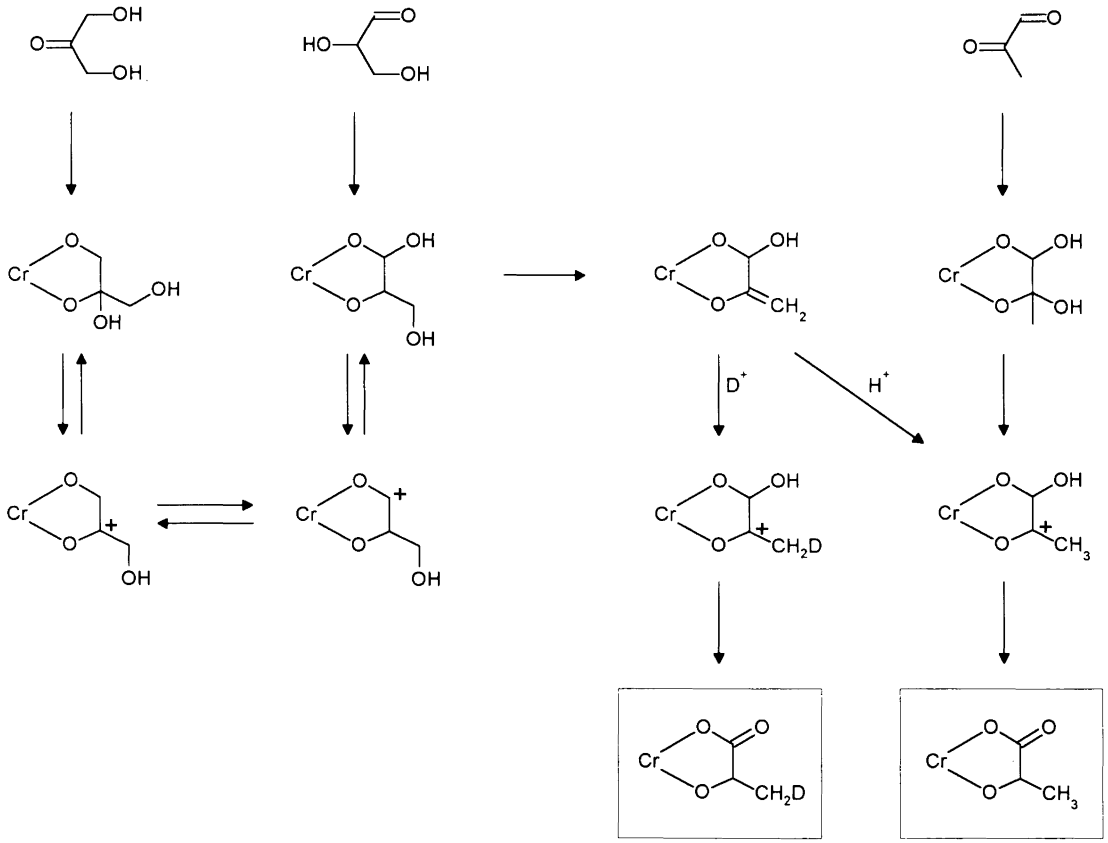

Fig. 5. Suggested mechanism of lactate formation catalyzed by *cis*-$[Cr(cyc\mathit{b})(OH_2)(OH)]^{2+}$.

essential step.[3] Protonation, dehydration, carbocation formation, intramolecular 1,2-hydride shift and deprotonation complete the transformation.

Unlike glyoxal, it has not been possible to isolate the intermediate chelate complex of hydrated methylglyoxal. This may be caused by a more efficient carbocation formation, governed by an increased carbocation stability induced by the increased electron donation of the methyl group as compared to a hydrogen atom.

The mechanism proposed for the glyceraldehyde and 1,3-dihydroxyacetone transformations follows the pattern established for methylglyoxal, but is more complicated. Essential steps here seem to be coordinated substrate interconversion through a carbocation rearrangement process, followed by enol formation and proton incorporation from the solvent to give a carbocation identical to that formed from methylglyoxal.

In Fig. 6 is shown the detailed stereochemistry of the methylglyoxal rearrangement process for the minimum movement of the transferred hydrogen atom, i.e. a hydrogen atom movement from one side of the chelate ring to the other is not considered to be a likely event. It is seen that the chirality of the coordinated lactate eventually formed is a function of the spatial position of the transferred hydrogen atom only, i.e. the hydrogen atom of the hydrated aldehyde group, and that the chirality around carbon atom 2 is unimportant. This mechanism explains a preferred formation of the *ss,s* and *rr,r* complexes, even though these products intuitively would be expected to be less stable than the *ss,r* or *rr,s* complexes, because of an unfavourable hydrogen–hydrogen interaction between a methyl group of the macrocyclic ligand, shown as ellipses in Fig. 6, and the methyl group of the lactate ligand (cf. Fig. 3).

The stereochemistry of the latter steps of the glyceraldehyde and 1,3-dihydroxyacetone rearrangements is shown in Fig. 7. The pattern of these steps is seen to follow closely that of the methylglyoxal rearrangement process, in agreement with a very similar diastereomer formation ratio, cf. the data in Table 2.

Comparison with the glyoxalase I enzyme. The conversion of methylglyoxal into lactic acid in natural systems is catalyzed by the glyoxalase I and II enzymes. Glutathione acts as a cofactor in forming a hemithioacetal which, in an intramolecular redox reaction catalyzed by the glyoxalase I enzyme, is transformed into a thioester. The catalytic cycle is completed by hydrolysis of the ester to give lactic acid and regenerate glutathione. This latter process is catalyzed by the glyoxalase II enzyme.

The glyoxalase I enzyme contains hexacoordinated zinc(II) at the active site, coordinated to the polypeptide

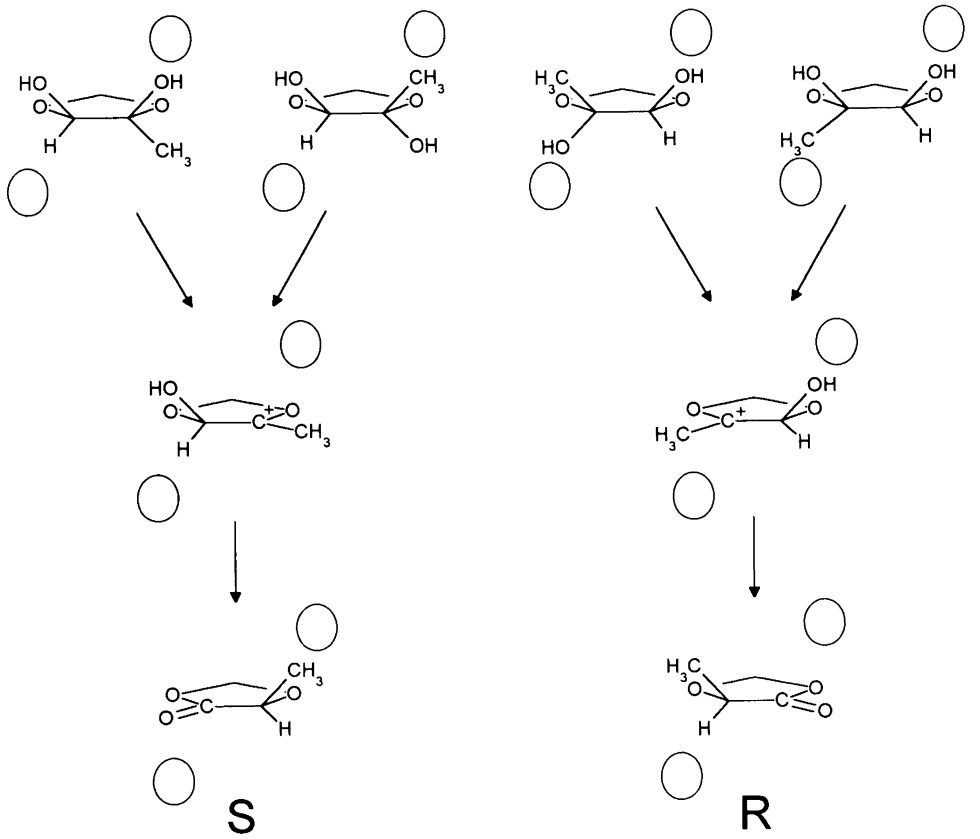

Fig. 6. Suggested detailed stereochemistry of diastereomeric lactate formation from the four possible coordination possibilities for chelate hydrated methylglyoxal, cf. Fig. 5. Ellipses above and below the coordinated substrate indicate protruding methyl groups of the *ss*-cyc*b* ligand, cf. Fig. 3.

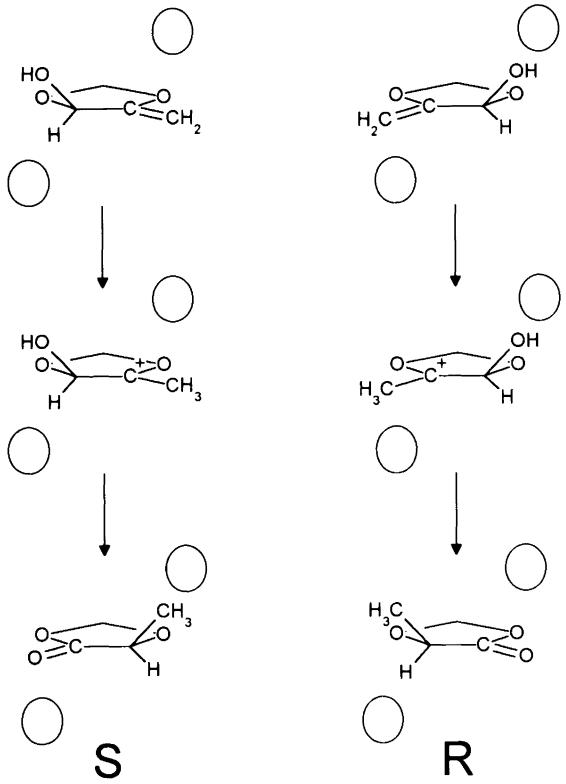

Fig. 7. Suggested detailed stereochemistry of latter stages of diastereomeric lactate formation from glyceraldehyde and 1,3-dihydroxyacetone, cf. Fig. 5. Ellipses above and below the coordinated substrate indicate protruding methyl groups of the *ss*-cyc*b* ligand, cf. Fig. 3.

chain through carboxylate and imidazole units and with two coordination positions occupied by water ligands. The catalytic mechanism for the function of the enzyme has been proposed to involve substrate polarization by hydrogen bonding to the metal-coordinated water ligands, followed by a base-assisted proton transfer from carbon atom 1 to carbon atom 2 in an enediol intermediate protected from the solvent.[1]

The present work demonstrates an entirely different catalytic mechanism for the macrocyclic chromium(III) complex. Firstly, there is a direct metal-ion coordination of the substrate, and secondly, the internal redox reaction takes place via a 1,2-hydride shift. Intuitively such a mechanism could also be expected to provide an attractive pathway for the enzymatic process, in that two positions are apparently available for substrate coordination in the enzyme, and a direct metal ion coordination could be expected to be far more effective for substrate activation than interactions via hydrogen bonding.

Evidence presently available does not, however, support direct metal-ion coordination of the substrate. Firstly, an NMR study of an equilibrium mixture of the product thioester and the manganese(II) or cobalt(II) substituted enzymes shows no entry of the reaction product into the first coordination sphere of the metal ion.[8] Secondly, it has been estimated that the upper limit for the rate of product release in a hypothetical inner-sphere complex would be too low to be compatible with the catalytic efficiency of the enzyme.[8] These results, however, are only valid for the enzyme equilibrium conformation adopted in the presence of the thioester reaction product, and could relate to a later stage in the progress of the enzymatic process. Conformational enzyme changes as the result of interactions at the active site have been characterized,[9] and the dissociation of the converted substrate from an inner-sphere complex could in principle be coupled with, and assisted by, a conformational change of the enzyme.

The 1,2-hydride shift mechanism demonstrated for the present macrocyclic chromium(III) complexes is not compatible with the suggested proton-transfer mechanism of the enzyme. The experimental basis for this mechanism seems mainly concentrated on enzyme catalysis of hydrogen-atom incorporation from solvent water,[10] a reaction which is not observed for the simple chromium(III) complexes investigated in this work. Also, the enzymatic catalysis of hydrogen-atom exchange at the carbon atom 2 of the thioester reaction product,[8] and the characterization of a primary hydrogen vs. deuterium isotope effect on the partition ratio for transformation of fluoromethylglyoxal into fluorolactate and pyruvate,[11] have been taken as evidence for the proton-transfer mechanism. These first two observations could both, however, be caused by an improved tendency for deprotonation and carbanion formation of a metal-ion coordinated substrate, or converted substrate, at the strongly hydrophobic environment at the active site of the enzyme, and the partition ratio isotope effect may also be explained by a metal-ion coordinated substrate and processes analogous to those shown in Fig. 5.

In conclusion, arguments may be advanced for the operation of a mechanism for the glyoxalase I catalyzed transformation of methylglyoxal into lactic acid analogous to that operating for a macrocyclic chromium(III) complex. This involves substrate coordination at the metal centre followed by a 1,2-hydride shift.

Experimental

Caution. The perchlorate salts described here are potentially explosive and should be handled accordingly.

Chemicals. Cis-[Cr(cyc*b*)$(OH_2)_2$]$Br_3 \cdot$ aq, *cis*-[Cr(cyc*b*)-Cl_2]Cl,[12] *cis*-[Cr(*ss*-cyc*b*)Cl_2]ClO_4 and *cis*-[Cr(*rr*-cyc*b*)-Cl_2]ClO_4[13] were prepared by available methods. Methylglyoxal (aqueous solutions; Fluka 25% or Sigma 40%), 1,3-dihydroxyacetone (Ega), glyceraldehyde (Ega) and D-glyceraldehyde (Aldrich or Sigma) were commercial products which were used without further purification (see below). Sodium bromide and aqueous hydrogen bromide were the best available commercial grades, which were purified as necessary: $NaBr \cdot 2H_2O$ was

recrystallized from water. HBr (47%, aq) was distilled from metallic tin.

Substrate purity and interconversion processes. The purity of the commercial substrates was controlled by ^{1}H and ^{13}C NMR using 0.1 M solutions in aqueous 0.01 M $DClO_4$. Immediately after dilution of the concentrated methylglyoxal solution or dissolution of the solid 1,3-dihydroxyacetone or glyceraldehyde, solutions contain appreciable amounts of unidentified polymeric substances. For the latter two substrates these species disappear almost completely after keeping the solutions for 3 min at 100°C. For methylglyoxal, however, the depolymerization processes are significantly slower, and some polymeric species are still present after such treatment. After 1 h at 100°C about 5% of monodeuterated methylglyoxal was formed in the 1,3-dihydroxyacetone and glyceraldehyde solutions. At room temperature less than 1% of methylglyoxal was formed in 2 weeks. The dilute methylglyoxal solutions in D_2O, both at 100°C and at room temperature, did not show any deuterium atom incorporation in the methyl group.

Commercial products of D-glyceraldehyde were found to contain appreciable amounts of 1,3-dihydroxyacetone and small amounts of methylglyoxal. The accurate substrate distributions of such products were determined and taken into account in the analysis of the diastereomer formation, since both methylglyoxal and 1,3-dihydroxyacetone react faster with the chromium(III) complex than glyceraldehyde.

Commercial products of methylglyoxal were found to contain appreciable amounts of formaldehyde. This was not found to interfere with the stoichiometric studies.

Structure determination. Cis-$[Cr(cyc\textit{b})(C_3H_4O_3)]ClO_4$ crystals occur in dark green, strongly pleochroitic, diamond-shaped plates where the large face is (100) and the *b*- and *c*-axes are along the diagonals. The space group could be assigned to *Fdd*2 through Weissenberg and precession photographs. Data were collected on a Picker FACS-1 diffractometer with Mo*K*α radiation at room temperature. The data were not corrected for absorption. The heavy atoms should be placed on two-fold axis and were located from the Patterson function. The atoms of the macrocyclic ligand were revealed through Fourier maps. The space group requires the complex to have a two-fold axis, which turned out not to be possible. Instead a disordered deprotonated chelate lactate ligand was assumed. The accurate geometry of this ligand was established by introducing population parameters of 0.5 for the uncoordinated ligand atoms. All atom parameters, except those of the disordered deprotonated lactate ligand and hydrogen, were refined anisotropically. Parameters for hydrogen atoms connected to the C7A carbon atom were not refined. Instead the positional parameters were taken as those calculated for an idealized geometry, and a common isotropic thermal parameter of 0.080 $Å^2$ was employed. Hydrogen atoms of the disordered deprotonated lactate ligand were not included in the calculation. The maximum peak in the final difference map was 0.5 e $Å^{-3}$, and the maximum parameter shift in the final refinement cycle was 0.95σ. Crystal data and data collection and reduction characteristics are given in Table 4 and fractional coordinates are given in Table 5. The X-RAY76 program system[14] was used for the refinements.

Table 4. Crystal data, and data collection and reduction characteristics, with standard deviations shown in parenthesis.

Formula	$CrClO_7N_4C_{19}H_{40}$
Space group	*Fdd*2
MW/g mol^{-1}	524.0
Cell parameters:	
a/Å	31.663(15)
b/Å	9.650(5)
c/Å	15.848(7)
Z	8
V/Å^3	4842(4)
μ(Mo*K*α)/cm^{-1}	4.28
Crystal size/mm^3	0.15 × 0.22 × 0.05
No of reflections:	
Independent	2743
Observed [$F^2 > 2\sigma(F^2)$]	1958
Used in calculations ($\sin\theta/\lambda < 0.6$)	1135
No. of parameters	203
$R = \Sigma(F_{obs} - F_{calc})^2$	0.044
w^{-1}	$\sigma^2(F) + 0.25\,\lvert F\rvert$
$R_w = \Sigma w(F_{obs} - F_{calc})^2$	0.059

Stoichiometric studies. Diastereomer formation ratios were investigated by reacting the chromium(III) complex with the substrate in a weakly acidic aqueous solution. The product formation was followed by FPLC chromatography. The amount of substance of the

Table 5. Fractional coordinates and equivalent isotropic thermal parameters. Standard deviations in parenthesis.

Atom[a]	*X*	*Y*	*Z*	100 *U*/Å^2
Cr	0.25	0.25	0.25	2.84(3)
O1	0.21361(13)	0.3090(5)	0.3377(3)	4.01(13)
N1	0.20293(15)	0.3141(5)	0.1606(3)	3.44(16)
N4	0.27205(14)	0.4592(5)	0.2358(3)	3.49(15)
C2	0.20202(20)	0.4682(7)	0.1686(5)	4.4(2)
C3	0.24707(22)	0.5209(7)	0.1652(5)	4.2(2)
C5	0.31885(19)	0.4928(6)	0.2295(4)	3.9(2)
C5A	0.33875(23)	0.4689(8)	0.3153(5)	4.9(2)
C5B	0.32522(23)	0.6465(7)	0.2073(5)	5.0(2)
C6	0.33932(19)	0.4033(7)	0.1604(4)	4.2(2)
C7	0.34065(19)	0.2468(7)	0.1730(5)	4.2(2)
C7A	0.37294(22)	0.1821(8)	0.1126(6)	6.2(3)
C1′	0.2218(5)	0.2744(15)	0.4142(9)	3.9(3)
C2′	0.2620(5)	0.1894(16)	0.4178(9)	5.8(4)
C3′	0.2914(6)	0.2565(19)	0.4867(12)	4.5(3)
O2′	0.2005(4)	0.3070(14)	0.4758(8)	6.2(3)
Cl	0.25	0.75	0.4294(2)	5.62(9)
O3	0.2362(4)	0.6612(15)	0.3664(9)	17.7(7)
O4	0.2835(4)	0.7000(18)	0.4732(7)	17.1(7)

[a] Atoms marked with a prime have population parameters of 0.5.

different diastereomers was determined by the FPLC chromatogram peak areas, by comparisons with calibration curves constructed analogously from the pure diastereomers. A summary of the data obtained is given in Table 2.

Deuterium labelling experiments. Deuterium labelling was carried out by reacting 0.2 mmol of chromium(III) complex with 1.0 mmol substrate in 3.5 ml D_2O. Experiments were conducted in 0.01 and $10^{-6.6}$ M acid obtained by adding $DClO_4$ or 2,6-dimethylpyridine, respectively, to the initial solution. The more acidic solutions were reacted at room temperature for about a week, and the 2,6-dimethylpyridine buffered solution at 100°C for about 3 min. Lactate complexes were isolated by ion-exchange chromatography using SP Sephadex C-25 by elution with 0.25 M $NaClO_4$ + 0.001 M $HClO_4$. The fraction containing lactate complexes was evaporated to dryness at 40°C, dissolved in D_2O and evaporated to dryness. The resulting product was reacted with 1.0 ml 1 M $DClO_4$ + 2 M NaCl at 100°C until an almost colourless lactic acid solution and a precipitate of *cis*-[Cr(cyc*b*)Cl_2]ClO_4 had formed. The precipitate was removed by filtration, and the resulting solution was used for the NMR experiments.

Instrumentation. Diastereomer formation ratios and product purities were controlled by FPLC chromatography using a Pharmacia FPLC apparatus equipped with a Mono S HR 5/5 column. Gradient elution from aqueous 0.01 M HBr to aqueous 0.01 M HBr + 0.20 M NaBr was used to separate the chelate lactate complexes. Unreacted chromium complex was removed with 0.01 M HBr + 1.0 M NaBr.

The absorption spectra were recorded on a Perkin-Elmer Lambda 17 spectrophotometer controlled by an RC-Partner computer.

Proton-decoupled ^{13}C NMR spectra were recorded at 62.896 MHz with a Bruker AC 250 MHz Fourier-transform spectrometer. Data, typically about 3×10^4 transients, were recorded at 300 K using a pulse width of 2 μs (45°), a sweep width of 14286 Hz and 32K data points, giving a digitizer resolution of 0.872 Hz/point in the final spectrum. ^1H NMR spectra were recorded at 250.134 MHz using a pulse width of 4 μs (45°), a sweep width of 3703 Hz and 32K data points, giving a resolution of 0.226 Hz/point. Under these conditions the acquisition times are 1.147 s (^{13}C) and 4.424 s (^1H) per free induction decay, and a relaxation delay between pulses was not found to be necessary. Chemical shifts are reported on the δ-scale with reference to internal 1,4-dioxane at δ = 67.4 ppm (^{13}C) and δ = 3.7 ppm (^1H).

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