Complexation between 3-Methylorotic (3-Methyluracil-6-carboxylic) Acid Dianion and Divalent Metal Cations. The Slow Acid Dissociation of Transition Metal Complexes and its Possible Relevance to Orotic Acid Phosphoribosylation

By **Denis Lalart, Jean Guillerez, Guy Dodin,** and **Jacques-Emile Dubois,*** Institut de Topologie et de Dynamique des Systèmes de l'Université Paris VII, associé au C.N.R.S., 1 rue Guy de la Brosse, 75005 Paris, France

3-Methylorotic (3-methyluracil-6-carboxylic) acid dianion gives 1 : 1 complexes with divalent metal ions. Their stability constants were determined by u.v. spectroscopy for Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}. The kinetics of formation of these complexes was investigated by T-jump relaxation in the alkaline pH range. The rate constants thus determined for complexation by Ca^{2+}, Mg^{2+}, Co^{2+}, and Cu^{2+} show that it fits the Eigen mechanism. Complex dissociation was studied by the stopped-flow technique in the acidic pH range. Surprisingly, the observed first-order rate constants for transition metal ions (Co^{2+}, Ni^{2+}, and Cu^{2+}) are found to be dependent on the final acidity. These results indicate a mechanism involving the slow protonation of these complexes ($k_{H^+} < 10^4$ l mol^{-1} s^{-1}). A possible explanation of the inhibiting properties of transition-metal ions in the enzyme-catalysed syntheses of 6-carboxyuridine 5′-monophosphate is inferred.

IN basic aqueous solutions, orotic (uracil-6-carboxylic) acid dianion is a mixture of two isomeric forms, N(3)H and N(1)H. Specific complexation occurs between the N(3)H isomer and divalent metal ions. A previous T-jump study [1] showed that complexation occurs much more quickly with Ca^{2+} than with Mg^{2+} ($k_{\mathrm{Mg}^{2}}$ 5.5×10^5 l mol^{-1} s^{-1}). These prior results provide some insight into the role played by metal ions in the phosphoribosylation of orotic acid (the fifth step in the *de novo* biosynthesis of pyrimidines). Indeed, phosphoribosylation involves the specific attachment of a phosphoribosyl group to the N(1) atom of orotic acid, in the presence of Mg^{2+}.[2] It is known [3] that Mg^{2+} acts as an activator, while Ca^{2+} is an ineffective bystander and the transition-metal ions Co^{2+}, Cu^{2+}, and Ni^{2+} act as inhibitors.

One of the roles thought to be played by the metal ions is making orotic acid available in the form of its reactive, N(3)H tautomeric dianion, where the N(1) atom is unsubstituted. Moreover, the difference in behaviour between Mg^{2+} and Ca^{2+} might be explained by the difference between the lifetimes of their complexes. This hypothesis is supported by kinetic studies carried out in this laboratory on OPRTase, the enzyme involved in phosphoribosylation,[4] which have established that the N(3)HMg^{2+} complex is the true substrate of the enzyme. However, this reasoning does not account for the inhibiting properties of the transition-metal ions. Indeed, these ions form complexes with the N(3)H tautomer of the dianion which are stabler than those formed with Mg^{2+}.[5,6]

Before seeking a broader explanation, it is necessary to delve into the mechanism(s) involved in the formation and dissociation between a divalent metal ion and the N(3)H tautomeric dianion, and to determine the kinetic parameters. In order to avoid kinetic coupling with the tautomerism [1] in orotic acid, the study reported here has been carried out on 3-methylorotic acid, whose dianion is a model for the N(3)H tautomer of orotic acid.

EXPERIMENTAL

Products.—3-Methylorotic acid was obtained as previously described,[7] and recrystallized from ethanol. Its purity was checked by u.v., mass spectroscopy (M^+ 170) and t.l.c. Metal salts were analytical grade (Merck; Prolabo).

Potentiometry.—pH Values were monitored by a Radiometer PHM 64 pH-meter equipped with a Radiometer G 202 C glass electrode.

U.v. Spectroscopy.—U.v. spectra were recorded on a Cary 118 u.v. spectrophotometer fitted with thermostatted cells. For the determination of complexation constants, suitable buffers were used, as previously described.[1] The following aqueous solutions were chosen: for pH 4.40, CH_3COOH–CH_3COONa (0.1M); for pH 5.00, KH_2PO_4–K_2HPO_4 (0.1M); for pH 7.32, primary NBS buffer–KH_2PO_4 (0.025M)–Na_2HPO_4 (0.025M); and for pH 9.18, primary NBS buffer–$Na_2B_4O_7$ (0.01M).

T-Jump Experiments.—For the experiments with magnesium, cobalt, or copper salts, the classical procedure described elsewhere [8] was used with T_{initial} 4 and T_{final} 13 °C; I 0.2 ($NaClO_4$). Since, as noted previously,[1] relaxations involving calcium binding with orotic acid and its derivatives generally last only a few μs, they are too fast to be investigated by classical procedures with a Joule T-jump spectrometer and require new operational conditions. Therefore, a 4 °C T-jump in less than 1 μs was obtained by using a 0.01 μF capacitor in a 0.5M-$NaClO_4$ solution.

U.v. spectroscopy was used to check the absence of ionic strength effects. A 0.2M-Tris buffer solution containing 7×10^{-5}M Cresol Red at pH 8 was used to determine the minimum time of rising temperature (0.8 μs) of the T-jump apparatus for which the sensitivity was 5×10^{-4} OD units. Relaxation signals were recorded in the digital mode over 512 points by a fast transient digitizer (Tektronix R 7912 equipped with a 7A13 plug-in unit). Three to six relaxation curves were then summed up in the memory of a PDP-11 computer (Digital) thereby increasing the signal : noise ratio.

Since, in many experiments, relaxation times are in the 10^{-6}—10^{-5} s range, the distortion of the signal by the instrumental response should be taken into account.[9,10] For accurate kinetic measurements, a deconvolution procedure by a curve simulation method was used. This numerical deconvolution method takes into account that the observed signal $F(t)$ is the convolution product of the true relaxation signal $f(t)$ and the apparatus response function $h(t)$ [equation (1)].

$$F(t) = \int_0^t f(t - x)h(x)\mathrm{d}x \qquad (1)$$

Stopped-flow Experiments.—Fast pH-jumps were performed by rapid mixing of solutions of complexes of 3-methylorotic acid with HCl solutions in a stopped-flow apparatus (Nortech SF-3A Mark IV) at 20 ± 1 °C. The solutions of complexes were initially at a pH high enough (*i.e.* pH 7.0 for transition-metal salts, or 9.0 for alkaline-earth metal salts) to ensure complete complexation which was checked by u.v. spectra. The acidic solutions were made from commercial Titrisol doses (Merck).

RESULTS

Definitions.—H_2L refers to the neutral form of 3-methylorotic acid, HL^- to the monoanion, L^{2-} the dianion, M^{2+} divalent metal cations, LM the complexes between M^{2+} and L^{2-}, and HLM^+ the complexes between M^{2+} and HL^-. C_L and C_M designate the analytical concentration of 3-methylorotic acid and of metal salt, respectively.

U.v. Spectroscopy.—The first and second dissociation constants, pK_{a_1} and pK_{a_2}, of 3-methylorotic acid at I 0.1 ($NaClO_4$) and 20 °C are 1.71 ± 0.13 and 10.11 ± 0.07, respectively. Adding divalent metal salts to solutions of 3-methylorotic acid dianion induces a significant bathochromic shift in the maximum absorbance wavelength, $\lambda_{max.}$ 298—*ca.* 310 nm. A similar effect is observed when alkaline-earth metals are added to solutions of 3-methylorotic acid monoanion in the neutral pH range, and when transition-metal salts are added to them even in acidic media.

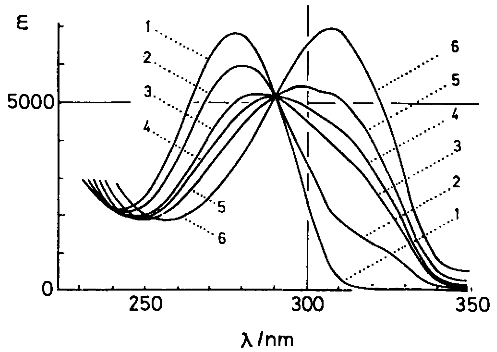


FIGURE 1 Effect of the addition of $NiCl_2$ on the u.v. spectra of 3-methylorotic acid: C_L 2.0 × 10^{-4}M; t 20 ± 1 °C; I = 0.1M(KH_2PO_2–K_2HPO_4); pH 5.0 (1—5) and 7.0 (6); C_{Ni} 0 (1), 1 × 10^{-3}M (2); 5 × 10^{-3}M (3); 1 × 10^{-2}M (4); 5 × 10^{-2}M (5); 1 × 10^{-1}M (6)

The isosbestic point obtained indicates the existence of an equilibrium (*cf.* Ni^{2+} in Figure 1). Benesi–Hildebrandt plots of the inverse of the absorbance $(A - A_0)^{-1}$ *versus* C_M^{-1} can be used when it is possible to apply the hypothesis of buffered concentration of metal cations and to neglect the formation of metal hydroxides. These conditions are met when experiments are performed in the presence of metal salt in excess, at a sufficiently low pH. The linear relationships thereby obtained (Figure 2) make it possible to determine the stability constants (Table 1) *via* equation (2).

$$\frac{1}{A - A_0} = \frac{1 + \dfrac{[H^+]}{K_{a_2}}}{\varepsilon_{LM}(1 + \dfrac{[H^+]}{K_{a_2}}) - (\varepsilon_L + \varepsilon_{HL}\dfrac{[H^+]}{K_{a_2}})} \left[1 + (1 + \frac{[H^+]}{K_{a_2}} \times \frac{1}{C_M K_M{}^L})\right] \qquad (2)$$

In methanol, complexation is greatly enhanced and the stoicheiometry between the ligand and the metal salt in the complex is found to be 1 : 1.

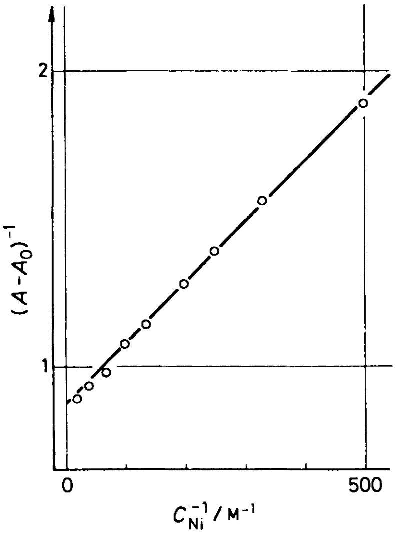


FIGURE 2 $(A - A_0)^{-1}$ *versus* C_{Ni}^{-1}: C_L 2.0 × 10^{-4}M; t 20 ± 1 °C; I = 0.1M(KH_2PO_4–K_2HPO_4); λ 310 nm

Kinetic Experiments by T-Jump.—In the pH range 7—11, two relaxations are observed.

Fast relaxation. The amplitude of the fast relaxation does not vary significantly with the nature of the metal ion, but is considerably pH-dependent and is a maximum around pH = pK_{a_2}. In experiments with the calcium salt, use of deconvolution method gives a relaxation time τ_1 of *ca.* 0.4 μs at pH 10.3 and C_L 3 × 10^{-4}M.

Slow relaxation. Since the slow relaxation depends on the nature of the metal ion, two cases must be distinguished. First, for alkaline-earth dications (Ca^{2+} and Mg^{2+}), the signal corresponds to an increase of optical density at 312 nm which has a pH-dependent amplitude. When metal is added, the amplitude is enhanced and the pH of the maximum is shifted to lower values. The relaxation time τ_2 is in the microsecond range (Ca^{2+}) or in the millisecond range

TABLE 1

Metal salt	log $K_M{}^L$
$CaCl_2$	3.40 ± 0.18 [a]
$Mg(ClO_4)_2$	4.11 ± 0.15 [a]
$CoCl_2$	6.70 ± 0.12 [b]
$NiCl_2$	7.77 ± 0.12 [b]
$CuCl_2$	9.14 ± 0.15 [c]

Measured at 20 ± 1 °C. [a] pH 9.18, I 0.01. [b] pH 5.00, I 0.1. [c] pH 4.40, I 0.1.

(Mg^{2+}). It is slightly dependent on pH and C_M (*cf.* Mg^{2+} in Figure 3). These experimental variations in τ_2 (Figure 4) can be expressed by equation (3). The rate constants k_2

$$\tau_2 = \frac{k_2 C_M}{1 + \frac{[H^+]}{K_{a_2}}} + k_{-2} \tag{3}$$

and k_{-2} are given in Table 2.

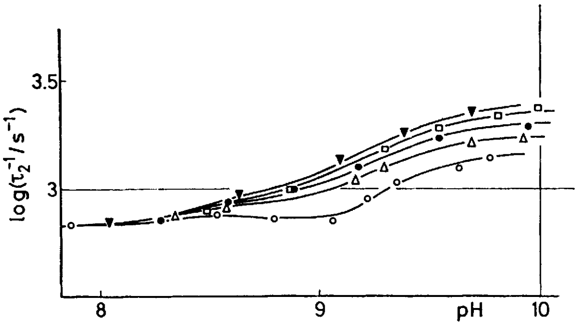


FIGURE 3 Relaxation time of 3-methylorotic acid dianion complexation by Mg^{2+}: — $\log(\tau_2)$ *versus* pH in H_2O: I 0.2 ($NaClO_4$); $t_{initial}$ 4 °C; t_{final} 13 °C; C_L 3.6 × 10^{-4}M; C_{Mg} 1.8 × 10^{-3}M (○), 3.6 × 10^{-3}M (△), 5.4 × 10^{-3}M (●), 7.2 × 10^{-3}M (■), 9.0 × 10^{-3}M (▼)

For transition metal salts, a decrease in optical density at 312 nm is observed with cobalt (in the millisecond range), and with copper (in the microsecond range), but no signals are detected with nickel. Although the amplitude is also pH-dependent, it decreases when metal ions are added, thereby preventing a study of relaxation time with metal salt in excess. The relaxation is slightly catalysed by OH^- or by metal salt addition. Since these variations in τ_2 are qualitatively the same as with alkaline-earth metals, the results are adjusted to fit equation (3), thereby giving the complexation rate constants (Table 2).

TABLE 2

Metal salt	k_2/l mol^{-1} s^{-1}	k_{-2}/s^{-1}	log (k_2/k_{-2})
$CaCl_2$ [a]	(8.7 ± 2.1) × 10^7	(8.5 ± 1.5) × 10^4	3.0
$Mg(ClO_4)_2$ [b]	(4.3 ± 0.4) × 10^5	(7.1 ± 0.5) × 10^2	2.8
$CoCl_2$ [c]	(1.1 ± 0.3) × 10^6	(2.9 ± 0.2) × 10^2	3.6
$CuCl_2$ [d]	(1.5 ± 0.7) × 10^7	(3.0 ± 0.3)	6.7

[a] Measured for I 0.5, T_f° 9°, C_M/C_L 10—100. [b] Measured for I 0.2, T_f° 13°, C_M/C_L 10—50. [c] Measured for I 0.2, T_f° 13°, C_M/C_L 3—10. [d] Measured for I 0.2, T_f° 13°, C_M/C_L 1—3.

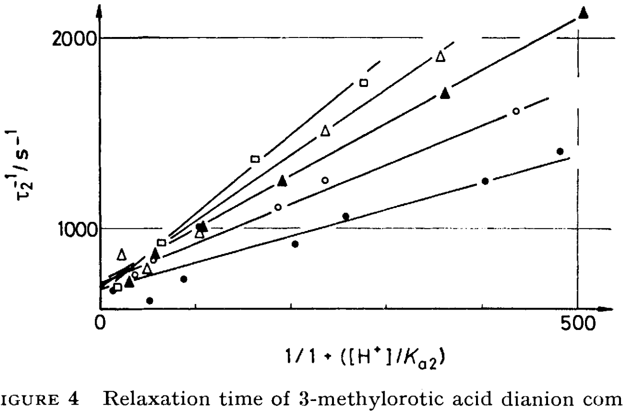


FIGURE 4 Relaxation time of 3-methylorotic acid dianion complexation by Mg^{2+}: τ_2^{-1} *versus* $1/(1 + ([H^+])/K_{a2})$ in H_2O: I 0.2 ($NaClO_4$); $t_{initial}$ 4 °C; t_{final} 13 °C; C_L 3.6 × 10^{-4}M; C_{Mg} 1.8 × 10^{-3}M (●), 3.6 × 10^{-3}M (○), 5.4 × 10^{-3}M (▲), 7.2 × 10^{-3}M (△), 9.0 × 10^{-3}M (□)

Kinetic Experiments by Stopped-flow.—Fast pH-jump studies of the dissociation of the LM complexes from the neutral to the acid pH range indicate that for transition metals (Ni^{2+}, Co^{2+}, and Cu^{2+}) the kinetics are of the first order. The observed rate constants vary linearly with the final acidity (*cf.* Ni^{2+} in Figure 5). The slope k_H and intercept k_0 of these correlations are given in Table 3. For a pH jump from 7.0 to 1.1 in a solution of LNi, the variation of the amplitude relative to the wavelength lies between the differential spectra of LNi–H_2L and LNi–HL^-.

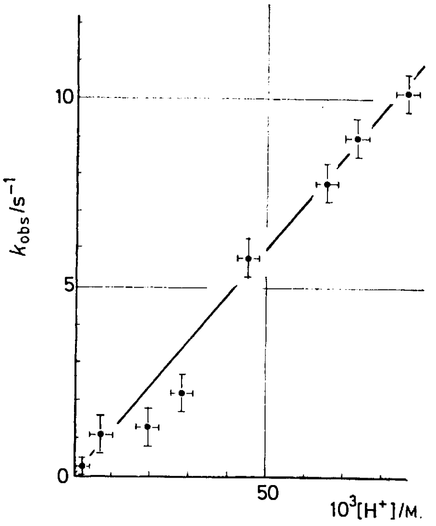


FIGURE 5 Fast mixing of a (3-methylorotic acid + $NiCl_2$) solution with 1N-HCl. First-order rate constants *versus* $[H^+]_{final}$. Experimental conditions after mixing: t 20 ± 1 °C; C_L 5 × 10^{-5}—5 × 10^{-4}M; C_M 5 × 10^{-4}—5 × 10^{-3}M

TABLE 3

Metal salt	k_H/l mol^{-1} s^{-1}	k_0/s^{-1}
$CoCl_2$	(3.4 ± 0.8) × 10^3	36 ± 10
$CuCl_2$	(1.7 ± 0.5) × 10^2	15 ± 5
$NiCl_2$	(1.2 ± 0.3) × 10^2	$\simeq 0$

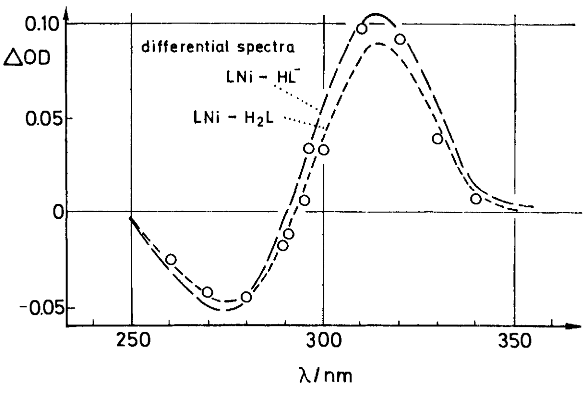
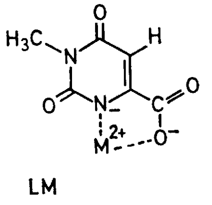


FIGURE 6 Fast mixing of a (3-methylorotic acid + $NiCl_2$) solution with 1N-HCl. Amplitude of the signal (○) *versus* wavelength. Experimental conditions after mixing: t 20 ± 1 °C; C_L 1.2 × 10^{-4}M; C_{Ni} 5.0 × 10^{-4}M; pH 1.1. The dashed lines are the differential spectra LNi–HL^- and LNi–H_2L for the same concentration in 3-methylorotic acid

Dissociation also occurs with alkaline-earth metals, but the observed kinetics are too fast to be time-resolved.

DISCUSSION

The two dissociation constants of 3-methylorotic acid correspond to ionization of the carboxy-group (pK_{a_1} 1.71) and to cleavage of the N–H bond of N(1) (pK_{a_2} 10.11), respectively. Since the values of the two constants obtained here lie between those determined by Fox *et al.*[11] ($pK_{a_1} < 1$, pK_{a_2} 10.52) and those reported by Davidenko *et al.*[12] (pK_{a_1} 2.22, pK_{a_2} 9.81), they seem to be realistic.



LM

The dianion of 3-methylorotic acid forms 1 : 1 complexes, LM, with metal dications. Their stability constants determined by u.v. spectroscopy are close to those obtained recently by potentiometry.[12] These complexes are slightly more stable than those with orotic acid dianion,[1,5,6] *i.e.* $\Delta = \log\ (K_M{}^L)^{3\text{-Me-orot}} - \log\ (K_M{}^L)^{\text{orot}} = 0.3 \pm 0.2$. This difference could be explained by the tautomerism of orotic acid dianion. If the stabilities of 3-methylorotic acid complexes are assumed to be equal to those of the N(3)H tautomer, then $1 + K_T{}^{-1} = 10^{\Delta}$ and $K_T \simeq 0.9$, which, given the approximations made, is consistent with the value $K_T = 0.4$ obtained from u.v. spectra.[1]

In the pH range 7—10, the LM complexes form *via* reaction (4). This reaction corresponds to the two relaxations observed by T-jump experiments.

$$M^{2+} + HL^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} M^{2+} + L^{2-} + H^+ \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} LM + H^+ \quad (4)$$

The fast process is proton exchange, for which the relaxation time is given by equation (5). The value of τ_1 obtained by deconvolution in the presence of calcium salt gives k_{-1} *ca.* 2×10^{10} l mol^{-1} s^{-1}, which is a diffusion-controlled rate. It can be assumed that, in the presence of the other metal salts, the proton exchange fits the same kinetic law with a similar rate constant.

$$(\tau_1)^{-1} = k_1 + k_{-1}([L^{2-}] + [H^+]) \quad (5)$$

The slower process is metal-ion exchange, for which the relaxation amplitude A can be expressed by equation (6), assuming C_M and H$^+$ to be buffered. This expression satisfactorily explains the observed variations relative to those of [H$^+$] or C_M. Thus, the difference between alkaline-earth and transition metals stems from the difference between the stability constants of the complex. Moreover, it is possible to use equation (6) to estimate the relative order of enthalpy variations for complexation: $\Delta H_{Mg} < \Delta H_{Ca} < 0 \simeq \Delta H_{Ni} < \Delta H_{Cu} < \Delta H_{Co}$. The relaxation time for the formation of the metal complex LM from the dianion when there is an excess of metal salts is expressed by a relationship similar to equation (3). The values of the rate constants for metal-ion exchange are those assigned to k_2 and k_{-2} in Table 2. The rate constants for complexation, k_2, are close to those determined by Hague and Eigen [13,14] for other substrates. This suggests that the rate-limiting step in the complexation is also the replacement of water molecule(s) on the metal ion by the ligand.

$$A = \frac{C_o{}^L\, \Delta H\, \Delta T\, K_M{}^L\, C_M \left[(\varepsilon_L - \varepsilon_{LM}) + \frac{[H^+]}{K_{a_2}} (\varepsilon_{HL} - \varepsilon_{LM}) \right]}{RT^2 \left[1 + \frac{[H^+]}{K_{a_2}} + K_M{}^L\, C_M \right]^2} \quad (6)$$

The ratio of k_2 with the reciprocal constants, k_{-2}, agrees well with $K_M{}^L$ equilibria constants for alkaline-earth metals. However, for transition metals, the observed discrepancies between this ratio and $K_M{}^L$ constants might be related to the fact that the hypothesis of buffered metal ion concentration was not obeyed.

Dissociation of the LM complexes was studied in acidic media by stopped-flow experiments. As the final pH is in the 0—3 range, near pK_{a_1}, the medium at the end of the reaction is a mixture of the neutral species H_2L and of the monoanion HL$^-$. Therefore, the wavelength-dependent variation of the amplitude indeed corresponds to the differential spectra between the initial and the end product: thus, the intermediate species remain in small amounts during the reaction.

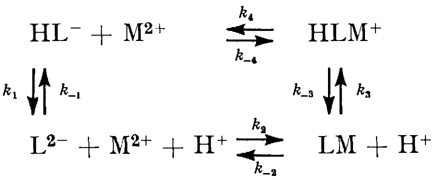
The acidity-dependence of the kinetics obtained by stopped-flow experiments is not explained by the previous simple mechanism. This suggests that there is an alternative pathway involving the protonation of the LM complex, thereby yielding the intermediate species HLM$^+$ which in turn gives the monoanion HL$^-$, and then the neutral species H_2L [reaction (7)]. At this stage it is not possible to identify *a priori* the rate-limiting step.

$$LM + 2H^+ \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} HLM^+ + H^+ \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} HL^- + H^+ \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} H_2L + M^{2+} \quad (7)$$

If, at first, it is assumed that both protonations are faster than the dissociation of protonated complex HLM$^+$, one obtains an expression of the first-order rate constant which fits the linear relationship observed between k_{obs} and [H$^+$]. However, the values of k_4 and k_{-4} resulting therefrom are either unrealistic or in total disagreement with those obtained by Eigen's theory [13] when using the $K_M{}^{HL}$ equilibrium constants determined by potentiometry.[12] It would be more realistic to assume that the protonation is slow. As the intermediate species, HLM$^+$, has not been detected by u.v. spectroscopy, and its stability constants [12] are always low ($\log K_M{}^{HL} < 3$), it reasonably follows that HLM$^+$ is in much smaller concentrations than the other species, and the steady-state approximation can be applied. This would also very simply explain the identity of the variation of the amplitude *versus* the wavelength, with the differential spectra HL^-–LM or H_2L–LM. Under these conditions, k_{H^+} can be assumed to be the ratio $(k_3k_4):(k_{-3} + k_4)$ which reduces to k_3 when using the values of stability constants.[12]

The values of k_3 thus obtained are surprisingly low for a protonation with a rate which is generally diffusion-limited. However, these results should be compared to those obtained for multidentate ligands, such as aminopolycarboxylic complexes.[15] In the latter cases, the mechanism involves a fast protonation (but not at the reactive site), followed by a fission of the metal–nitrogen bond, and then by a transfer of the proton to the reactive site.[16] Such a mechanism might also satisfactorily explain the slowness of the protonation of the 3-methylorotic acid complex.

Thus, two distinctive pathways for the complexation may occur depending on the pH: *via* dianion L^{2-} when in the alkaline range, and *via* protonated complex HLM^+ when in the acidic range [reaction (8)]. In neutral solution, which is also the physiological condition, the two pathways should be competitive. Such a mechanism is classical,[13] but the protonation pathway is generally neglected when studying the influence of metals in biochemical processes. However, if, as our results indicate, it indeed plays a role in this process, this could account for the optimum pH of 8 for phosphoribosylation and of 6 for the reverse reaction.[17]



$$\begin{tikzcd}
HL^- + M^{2+} \arrow[r, shift left, "k_4"] \arrow[d, shift right, "k_1"'] & HLM^+ \arrow[l, shift left, "k_{-4}"] \arrow[d, shift right, "k_{-3}"'] \\
L^{2-} + M^{2+} + H^+ \arrow[u, shift right, "k_{-1}"'] \arrow[r, shift left, "k_2"] & LM + H^+ \arrow[l, shift left, "k_{-2}"] \arrow[u, shift right, "k_3"']
\end{tikzcd} \quad (8)$$

Conclusions.—These kinetic studies on 3-methylorotic acid have led to two conclusions: that the kinetics of dianion complexation by divalent metal ions occurs *via* the classic Eigen mechanism,[13] and that the attachment of a proton to transition ion-bearing complexes is especially slow.

By analogy, these observations can be extended to orotic acid and can serve to shed light on the role played by metals in the biochemistry of this compound. Since the complexation specifically stabilizes the N(3)H tautomer, it therefore seems likely that it contributes to the orientation of phosphoribosylation on the N(1) site. Moreover, recent work carried out in this laboratory on the kinetics of OPRTase suggests that the dianion–metal complex might be the substrate recognized by the enzyme.[4] The variety of behaviours observed for different metals (no effect for Ca^{2+}, catalysis for Mg^{2+}, inhibition for Cu^{2+}, Co^{2+}, and Ni^{2+}) might therefore be related to the kinetic properties of their complexes.

The fact that calcium is not a cofactor in phosphoribosylation could indicate that a rearrangement of the enzyme–metal–substrate complex might occur after the recognition of the substrate.[13] As the lifetime of complexes with calcium is shorter than that of all the other complexes, it would seem that dissociation might occur before the enzyme has the structure required for phosphoribosylation.

Moreover, the results reported herein indicate that proton attachment to the transition-metal ion complexes is particularly slow. If, as is indeed the case in the attachment of the proton to the complex in water, the attachment of the phosphoribosyl group to the metal ion–orotic acid dianion complex in the enzyme is assumed to be an electrophilic addition, such an analogy would indicate that phosphoribosylation on transition metal complexes is also very hindered. This would account for the inhibiting properties of the transition metal ions.

Kinetic studies involving the overall enzymatic system are currently under way in order to test the validity of these conclusions.

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REFERENCES

[1] G. Dodin and J. E. Dubois, *J. Am. Chem. Soc.*, 1980, **102**, 3049.
[2] J. Lieberman, A. Kornberg, and E. S. Simms, *J. Biol. Chem.*, 1955, **215**, 403.
[3] J. Victor, L. B. Greenberg, and D. L. Sloan, *J. Biol. Chem.*, 1979, **254**, 2647.
[4] G. Dodin, D. Lalart, and J. E. Dubois, *Bioinorg. Chem.*, submitted for publication.
[5] E. R. Tucci, C. H. Ke, and N. C. Li, *J. Inorg. Nucl. Chem.*, 1967, **29**, 1657.
[6] N. K. Davidenko and N. N. Zinich, *Koord Khim.*, 1979, **5**, 3.
[7] M. Bachstez, *Chem. Ber.*, 1930, **63**, 100.
[8] M. Dreyfus, G. Dodin, O. Bensaude, and J. E. Dubois, *J. Am. Chem. Soc.*, 1975, **97**, 2369.
[9] J. Aubard and J. E. Dubois, *J. Phys. Chem.*, 1980, **84**, 1413.
[10] J. Aubard, Doctoral Thesis, Université Paris VII, 1981.
[11] J. J. Fox, N. Yung, and I. Wempen, *Biochim. Biophys. Acta*, 1957, **23**, 295.
[12] N. K. Davidenko and N. N. Zinich, *Russ. J. Inorg. Chem.*, 1979, **24**, 891.
[13] D. N. Hague and M. Eigen, *Trans. Faraday Soc.*, 1966, **62**, 1236.
[14] D. N. Hague, in ' Chemical Relaxation in Molecular Biology,' eds. I. Pecht and B. Rigler, Springer Verlag, New York, 1977.
[15] D. L. Rabenstein, *J. Am. Chem. Soc.*, 1971, **93**, 2869.
[16] P. Lekteman and J. B. Westmore, *J. Chem. Soc., Dalton Trans.*, 1975, 480.
[17] J. G. Flaks, *Methods Enzym.*, 1961, **6**, 136.