Complexes of 3,4-Dihydroxyphenyl Derivatives. 9. Al^{3+} Binding to Catecholamines and Tiron

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Abstract: In order to assess a possible role for Al^{3+} in dementias, its stability constants for binding to four catecholamines have been determined by potentiometric titration at 25 °C and 0.2 M ionic strength. Because Al^{3+} binds to the catecholate locus with the ammonium group still protonated, conventional treatments are not applicable and the concentration of the chelating microform requires estimation. Owing to the high catecholate basicity, uncertainty in the pK_a value for the last phenolic ionization, and the low concentration of the chelating microform, conditional stability constants applicable to pH 7.0 have also been calculated. For the addition of one through three ligands to Al^{3+}, the successive average pH 7.0 conditional stability constant logarithms are as follows: L-DOPA and dopamine, 8.05, 5.32, and 1.25; norepinephrine and epinephrine, 8.27, 5.69, and 1.94. The corresponding values for tiron (1,2-dihydroxybenzene-3,5-disulfonate) are significantly enhanced to 10.4, 7.9, and 4.6, making tiron the strongest catecholate Al^{3+} binder in neutral solutions. The conditional stability constants indicate that about a 1000-fold excess of catecholamine is required for its Al^{3+} binding to compete with that of citrate and a 100-fold excess for catecholamine–Al^{3+} binding to compete with that of ATP.

Following acknowledgment of Al^{3+} release from soils by acid rain[1] and association of Al^{3+} with dementias,[2] tracing modes of Al^{3+} binding in organisms has acquired new significance. In blood plasma, the predominant small molecule Al^{3+} carrier is citrate,[3-5] which occurs at 0.1 mM concentration, while the major protein carrier of Al^{3+} is transferrin, most of which is iron-free.[3,4,6] The action of Al^{3+} in dementias occurs in brain tissue, where the concentrations of citrate (2 μM) and transferrin are low to nonexistent. Other small molecules become possible Al^{3+} binders in brain and other tissues. With a chelating catechol moiety, the so-called catecholamines promise to bind Al^{3+} strongly. That they do so under physiological conditions is demonstrated by a histological fluorescent staining technique for catecholamines utilizing their complexes with Al^{3+} perfused into tissue.[7]

In this paper we evaluate the stability constants for Al^{3+} binding to L-DOPA (L-3,4-dihydroxyphenylalanine), dopamine, norepinephrine, and epinephrine and also include results for tiron (1,2-dihydroxybenzene-3,5-disulfonate). This work continues our study of metal ion complexes of catecholamines and related compounds.[8,9] In addition to reporting the normal stability constants for Al^{3+}, we also compare conditional stability constants applicable to physiological conditions.

Experimental Section

Potentiometric titrations were performed with a Radiometer PHM 64 pH meter and TTA 80 automatic titration unit equipped with G2040B glass and K4040 calomel electrodes. Ligand concentrations were 4, 6, and 9 mM and the Al^{3+} concentrations 1, 2, and 4 mM, with ligand to Al^{3+} molar ratios of 1, 2, 3, 4.5, 6, and 9. The temperature was 25 °C and the ionic strength 0.2 M controlled with KCl. All pH meter readings were made on a concentration scale. To convert pK_a values in this paper to the more common activity scale of NBS buffers,[10] add 0.13 log unit. Metal ion stability constants do not require a conversion.

At ligand to Al^{3+} ratios of 3 or greater, no precipitation occurred, equilibrium was reached promptly, and the fit between experimental (30–40 points) and calculated (PSEQUAD)[11] titration curves was excellent. With equimolar solutions, all four ligands in Table I formed precipitates by pH 7. At molar ratios of 3 or less, several minutes were required to reach equilibrium, and the fit was unsatisfactory. For the probable hydroxo complexes of Al^{3+} the stability constants (log β) assumed[12] were −5.52 for $Al(OH)^{2+}$, −13.57 for $Al_3(OH)_4^{5+}$, and −23.46 for $Al(OH)_4^-$. The dimerization constants in Table I improve the fits, but a wholly satisfactory fit at less than 3:1 ratios requires additional species including binary and ternary hydroxo complexes. The computer program rejected several other monomeric and polymeric species. At 3:1 or greater mole ratios dimer formation becomes less important, the fits are excellent, and the stability constant values in Table I bear standard deviations less than 0.03 log unit.

Table I. Catecholamine Acidity Constants and Their Al^{3+} Complex Stability Constants[a]

row	const	L-DOPA	dopamine	norepi[e]	epi[e]
1	pK_{1a}	8.80[b]	8.89[c]	8.58[d]	8.64[d]
2	pK_{2a}	9.83	10.41	9.53	9.84
3	pK_{3a}	13.4	13.1	12.9	13.1
4	log β_1	26.12	26.42	25.33	25.81
5	lob β_2	49.42	50.19	48.05	49.08
6	lob β_3	68.63	69.93	67.05	68.57
7	pk_1	8.98[b]	8.90	8.69[d]	8.67[d]
8	pk_2	9.19	9.98	9.11	9.38
9	pk_{12}	9.63	10.36	9.42	9.81
10	pk_{21}	9.42	9.29	9.00	9.10
11	k_1/k_2	1.6	12	2.6	5.1
12	pk_{13}	12.96	12.71	12.59	12.67
13	log K_1	16.03	15.63	15.60	15.57
14	log K_2	13.21	12.98	12.99	13.03
15	log K_3	9.12	8.95	9.27	9.25
16	$pK_{3(1a)}$	9.75	10.03	9.35	9.77
17	$pK_{3(2a)}$	10.15	10.50	9.76	10.22
18	$pK_{3(3a)}$	10.65	11.03	10.36	10.71
19	log K_d	−7.54	−7.82	−7.59	−7.69
20	pK_{da}	ppt	ppt	6.39	6.88

[a] At 25 °C and 0.2 M ionic strength. See text for definitions of constants. [b] Acidity constants from ref 15. [c] Macroconstants from ref 14. [d] Acidity constants from ref 16. [e] Norepi, norepinephrine; epi, epinephrine.

Results

Table I tabulates the results for catecholamines. The first three rows contain the stepwise macroscopic pK_a values. Rows 4–6 show

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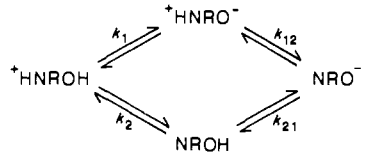
the overall stability constants in the standard log β_n format for ligand AH with pK_{1a} and pK_{3a} included in the determination. In order to properly interpret the overall stability constants, we must consider the form of the ligand chelating to the metal ion.

Of the three macroscopic pK_a values listed in the first three rows of Table I for the catecholamines, the third, pK_{3a}, corresponds virtually exclusively to ionization of the second phenolic group. As verified by several techniques, the macroconstants K_{1a} and K_{2a} cannot be assigned exclusively to the first phenolic and ammonium group deprotonation constants but are mixtures of them.[13-17] We define the microscopic acidity constants for the first two deprotonations in the following scheme



where the last subscript designates the group undergoing deprotonation. In the upper pathway a phenolic group deprotonates first, while in the lower pathway the ammonium group does so. The first two macroscopic acidity constants determined by titration are related to the four microconstants of the scheme by

$$K_{1a} = k_1 + k_2 \qquad K_{2a}^{-1} = k_{12}^{-1} + k_{21}^{-1}$$

and

$$K_{1a}K_{2a} = k_1k_{12} = k_2k_{21}$$

Since there are only three independent equations, additional information is needed to resolve the four microconstants. The course of the first phenolic deprotonation may be followed independently by ultraviolet or ^1H NMR spectroscopy leading to resolution of the four microconstants with the results presented in rows 7–10 in Table I. The concentration ratio of the phenolic to ammonium monodeprotonated species $[^+HNRO^-]/[NROH] = k_1/k_2$ appears in the 11th row of Table I and, for a single compound, is a constant independent of pH. Since for the four so-called catecholamines of Table I the ratio varies from 1.6 to 12 and is in all cases greater than unity, the upper or phenolic deprotonation pathway predominates, and the compounds are better described as ammonium catecholates.

A tripositive ion such as Al^{3+} prefers oxygen donors and is expected to chelate to the catecholate locus of catecholamines rather than bind at the amino group. Even for DOPA with its strongly chelating glycinate locus, the crossover pH from glycinate-like to catecholate binding of Al^{3+} occurs at pH < 5.[18] The Al^{3+}–norepinephrine complex inhibits enzymatic O-methylation but not N-methylation by catechol-*O*-methyl transferase.[19] Thus, even in acidic solutions, Al^{3+} complexation to catecholamines occurs primarily at the catecholate moiety, with the ammonium group remaining protonated.

To calculate a proper stability constant it is necessary to estimate the concentration of the microspecies with both phenolic groups ionized and the ammonium group still protonated.[20,21] This monoanionic microspecies occurs in too low a concentration to be measured directly. We may approximate its concentration by estimating the microconstant k_{13} for loss of the second phenolic proton from the microspecies with a protonated ammonium group.

Table II. Conditional Stability Constants at pH 7.00

ligand	log K_{1c7}	log K_{2c7}	log K_{3c7}	μ^a
catechol	7.66	4.90	0.81	0.6[b]
	8.20	4.97	0.29	0.1[c]
L-DOPA	8.08	5.26	1.17	0.2[d]
dopamine	8.01	5.36	1.33	0.2[d]
norepinephrine	8.31	5.70	1.98	0.2[d]
epinephrine	8.22	5.68	1.90	0.2[d]
gallic acid	8.70	5.59	2.42	0.6[e]
1,2-dihydroxynaphthalene-4-sulfonate	8.59	6.16	2.58	0.6[f]
catecholmonosulfonate	9.3	6.0	2.0	0.1[g]
tiron	10.38	7.92	4.57	0.2[d]

[a] Ionic strength. All values refer to 25 °C unless otherwise stated. [b] Reference 28. [c] Reference 27. [d] This research. [e] Reference 25. [f] Reference 30. [g] At 20 °C. Reference 29.

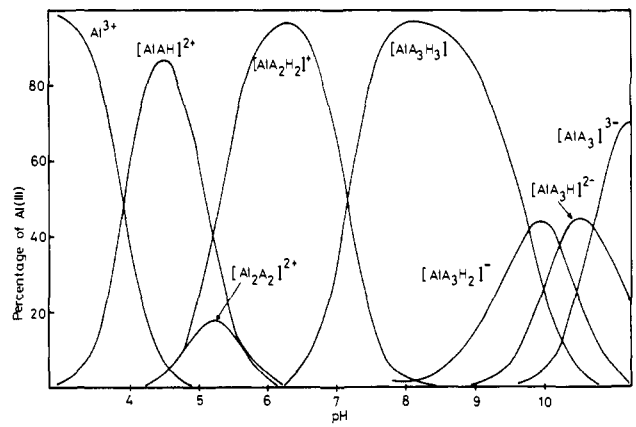


Figure 1. Mole fraction, Al(III) basis, versus pH for a solution containing 1 mM total Al(III) and 9 mM epinephrine.

The effect of a protonated ammonium group on a phenolic ionization may be expressed as the difference $pk_{21} - pk_1 \simeq 0.4$ for the ligands in Table I and tyrosine.[22] We correct pK_3 assigned exclusively to the second phenolic ionization in a molecule with a deprotonated amino group by the difference for each ligand according to $pk_{13} = pK_3 - (pk_{21} - pk_1)$. The resulting values appear in the 12th row of Table I. The sum $pk_1 + pk_{13}$ is now used to calculate the concentration of the microspecies with two anionic phenolates and a protonated ammonium group.

Successive stepwise stability constants for Al^{3+} chelated at the diionized catecholate locus with the ammonium group protonated appear as log K_1, log K_2, and log K_3 in the 13–15th rows in Table I. These constants are calculated from the overall stability constants in rows 4–6 by taking into account the differences between the microconstant sum $pk_1 + pk_{13}$ and the macroconstant sum $pK_{1a} + pK_{3a}$. The differences between the successive stepwise constants span narrow ranges for the four ligands across Table I: log $(K_1/K_2) = 2.54$–2.81 and log $(K_2/K_3) = 3.72$–4.09. The surprising point is that the last ratio is not much greater. The K_3 constant implies a hexacoordinate complex having three bidentate catecholate ligands with a 3^- charge at the coordination site; this complex contrasts with that for hydroxide which forms only up to tetrahedral[3,23] $Al(OH)_4^-$ and not $Al(OH)_6^{3-}$ or even $Al(OH)_5^{2-}$. Similar hexacoordinate complexes appear with other catecholate complexes (Table II). Therefore, catecholate binding allows six coordination of Al^{3+} much more easily than does hydroxide ion. A distribution diagram of complexes present in 9:1 solution containing epinephrine and Al^{3+} as a function of pH appears in Figure 1.

For the 3:1 complex $Al(LH)_3^0$ the acidity constants for successive loss of noncoordinated ammonium group protons are listed

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as $pK_{3(na)}$ in the 16–18th rows of Table I. To allow for the statistical effect of three equivalent protons in $Al(LH_3)^0$ and three equivalent proton binding sites in AlL_3^{3-}, we consider the intrinsic constants $pk_{3(1a)} = pK_{3(1a)} + \log 3$, $pk_{3(2a)} = pK_{3(2a)}$, and $pk_{3(3a)} = pK_{3(3a)} - \log 3$. For each of the four ligands the three intrinsic constants are nearly equal, indicating deprotonations from equivalent and independent ligands with little electrostatic and other interactions of the other ligands on ammonium group deprotonations in the 3:1 complex. For the four ligands across Table I, the average intrinsic $pk_{3(na)}$ values are 10.18, 10.52, 9.82, and 10.23. These average values are notably less acidic than the corresponding pk_2 and pk_{12} constants in Table I representing ammonium group deprotonations from unbound ligands with no and one ionized phenolic group. Indeed, the average intrinsic $pk_{3(na)}$ value for ammonium group deprotonations in the 3:1 complex corresponds most closely to the microconstant pk_{132} for ammonium group deprotonation from the free ligand microspecies with two anionic phenolate groups. Similar to the method described above for pk_{13}, we estimate $pk_{132} = pk_{12} + pk_{21} - pk_1$, and since $pk_{21} - pk_1 = pk_{12} - pk_2$, we also obtain $pk_{132} = 2pk_{12} - pk_2$, to give the following values for the four ligands across Table I: 10.07, 10.74, 9.73, and 10.24. The close agreement of these values with the average intrinsic $pk_{3(na)}$ values listed above indicates that the uncoordinated ammonium group in the 3:1 complex deprotonates as if from a ligand with two anionic phenolate groups; the presence of Al^{3+} has little effect. This comparison demonstrates once again the much greater polarizing power of the proton over any metal ion;[24] compare $pK_a = -1.7$ for $H(H_2O)^+$ with $pK_a = 5.5$[3,4] for $Al(H_2O)_6^{3+}$.

Computer fits at less than 3:1 ligand to Al^{3+} molar ratios are markedly improved (but not perfected) by introduction of a dimer and its deprotonation according to

$$2AlLH^+ \rightleftharpoons Al_2L_2 + 2H^+ \quad (1)$$

$$Al_2L_2 \rightleftharpoons Al_2(L)(LH_{-1})^- + H^+$$

For these reactions we designate the equilibrium constants as K_d and K_{da}, and their values appear in the last two rows of Table I. For L-DOPA and dopamine, precipitate formation at pH 5 in equimolar solutions precludes obtaining the acidity constant values K_{da} for proton loss from a coordinated water molecule in the dimer. The dimer may be a dihydroxo bridged complex formed by proton loss from a coordinated water in $AlLH^+$ (protonated ammonium group) and subsequent dimerization. For the analogous dimerization with aqueous Al^{3+}, $\log K_d = -7.7$,[25] similar to the values listed in Table I with coordinated ammonium catecholate.

Stability constant results were also obtained for tiron (1,2-dihydroxybenzene-3,5-disulfonate). For the free ligand we find $pK_3 = 7.56$ and $pK_4 = 12.26$. The successive stability constants of the −4 ligand species are $\log K_1 = 16.31$, $\log K_2 = 13.85$, and $\log K_3 = 10.50$. Hydroxo bridged dimer formation occurs by eq 1 with $\log K_d = -7.66$ and $pK_{da} = 5.94$.

Discussion

The high stability constants recorded in Table I fail to furnish a realistic impression of binding strengths because of the high pK_a values of the catecholate ligands. In neutral solutions the ligands are protonated, and Al^{3+} must compete with protons for catecholate binding sites on a ligand. In acidic and neutral solutions the first reaction occurring with ammonium catechols and the free Al^{3+} species is

$$Al^{3+} + HLH_2^+ \rightleftharpoons HLAl^{2+} + 2H^+$$

with the ammonium group remaining protonated. For formation of the equimolar complex we define the conditional stability constant[3,4] by $K_{1c} = \alpha K_1$, where α is the fraction of free ligand that contains two ionized catecholate oxygens available for chelation of Al^{3+}. Since K_1 is known (see Table I) and the fraction $\alpha = k_1k_{13}/([H^+]^2 + K_{1a}[H^+] + K_{1a}K_{2a})$, K_{1c} may be calculated for any pH. Values of K_{1c} calculated for pH 7.00 and labeled K_{1c7} are tabulated in Table II. Since at pH 7, $(H^+) \gg K_{1a}$ for the catecholamines (but not for tiron) the expression for K_c simplifies to

$$\log K_{1c} = \log K_1 - pk_1 - pk_{13} + 2pH$$

By extension, the conditional second and third stability constants are defined by $K_{2c} = \alpha K_2$ and $K_{3c} = \alpha K_3$, and the calculated values at pH 7.00 also appear in Table II. Comparison of the conditional constants $\log K_{nc7}$ for the four catecholamines in Table II reveals only slightly stronger Al^{3+} binding to the epinephrines than to DOPA and dopamine at pH 7.0.

Conditional stability constants provide several important advantages over normal stability constants for ligands such as catecholamines. (1) For ligands with protonated donor groups, conditional constants indicate directly the stability at a specific pH (such as 7.00 in Table II). (2) In cases where an acidity constant is uncertain as is the high pK_{3a} value for catecholamines, compared to the normal stability constants, conditional stability constants provide a more reliable comparison of metal ion binding capability among a group of ligands (Table II) and between teams of investigators. Use of different acidity constants results in correspondingly different normal stability constants, the difference canceling out upon conversion to conditional stability constants. (3) When the ligand microform actually complexing to the metal ion fails to occur appreciably in the absence of metal ion, the free ligand microconstant basis affects the numerical value of the normal stability constant. Again, conversion to a conditional constant basis cancels out the pK_a and normal stability constant differences. Thus no matter what the acidity constant basis for calculation of normal stability constants, conditional constants remain independent of the basis and directly comparable among ligands and between investigators.

For catechol itself at 25 °C and 0.1 M KCl, reportedly $pK_{1a} = 9.26$ and $pK_{2a} = 13.43$,[26] and from the successive stability constants $\log K_n$ of 16.89, 13.66, and 8.98,[27] we calculate the conditional stability constants at pH 7.00 that appear in the second row of Table II. At a high ionic strength of 0.6 M NaCl where $pK_{1a} = 9.20$,[28] we deduce the conditional stability constants in the top row of Table II. The values in the first two rows of Table II are consistent with each other and reveal the impact of the ionic strength difference. The catechol 0.1 ionic strength value of $\log K_{1c7} = 8.20$ falls within the range of the catecholamine values for the same constant in Table II. Correspondingly, a plot (Figure 2) of the stability constant $\log K_1$ for catechol and catecholamines versus the sum of the two phenolate pk values yields a slope of essentially unity, 0.95 ± 0.12. Addition of the second and third ligands as indicated by K_{2c7} and K_{3c7} becomes significantly weaker for catechol compared to the catecholamine values in Table II. The slope of a $\log K_2$ versus $\sum pk$ plot shows 0.48 ± 0.08, while that of $\log K_3$ versus $\sum pk$ actually becomes negative at −0.17 ± 0.11 (Figure 2).

The values for the conditional stability constants at pH 7.00 show a general strengthening from top to bottom of Table II. In neutral solutions substituted catechols bind Al^{3+} more strongly than catechol itself. The strengthening actually is greater the more negatively charged the ligand, and greater in $\log K_{3c7}$ than $\log K_{1c7}$ despite the buildup of high negative charge in the 3:1 complexes.

For tiron the successive conditional stability constants at pH 7.00 appear at the end of Table II. These values substantially exceed those of the catecholamines and of catechol. For catecholmonosulfonate[29] the successive pH 7.0 conditional constants (Table II) lie intermediate between those of tiron on one hand and catechol and the catecholamines on the other. In a plot of

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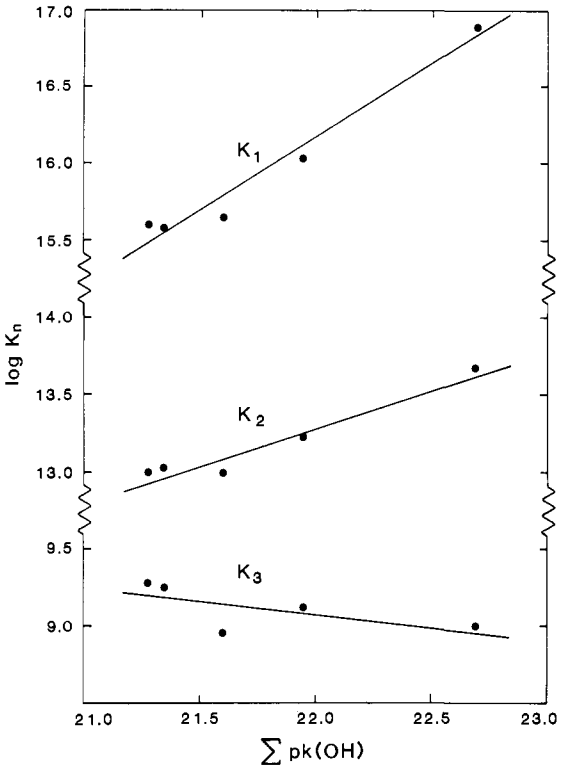

Figure 2. Logarithm of stepwise stability constants versus sum of two phenolic pK_a values ($pk_1 + pk_{13}$ for catecholamines). From left to right the ligands are norepinephrine, epinephrine, dopamine, DOPA, and catechol.

the normal stability constant log K_1 versus $\sum pk$ for the catecholate moiety, the point for tiron rides 2.2 log units too high and that for catechol monosulfate 1.1 log units too high. Thus each sulfonate group enhances log K_1 by 1.1 log units. This difference appears in the log K_{1c7} column of Table II. With allowance for the ionic strength difference, the results for 1,2-dihydroxynaphthalene-4-sulfonate[30] are consistent with those for catecholmonosulfonate.[29]

Lesser but still significant enhancements appear for tiron in similar plots of log K_2 and log K_3 versus $\sum pk$. On the basis of comparison among catechol, its monosulfonate, and tiron, it was suggested that the K_1 and K_2 values of catechols are practically independent of acidity.[29] The above comparison of normal stability constants of catechol and the four catecholamines suggests, however, that log K_1 and log K_2 do depend upon basicity as measured by the $\sum pk$ with slopes of 1.0 and 0.5, respectively. It is the sulfonate-containing ligands that deviate from the straight lines with abnormally high stability constants. This pronounced trend is illustrated by noting the high pH 7.0 conditional stability constants for tiron at the end of Table II. Enhanced binding of tiron also occurs with other metal ions; tiron is the strongest catecholate metal ion binder in neutral solutions.

The conditional stability constants of log $K_{1c7} \simeq 8.2$ in Table II furnish a direct measure of catecholamine to Al^{3+} binding strength at pH 7.0 and may be compared directly to conditional constants for other ligands of biological importance. For the Al^{3+}–citrate complex, the pH 7.0 conditional stability constant of log $K_{1c7} = 11.7$ allows for loss of a proton from the complex that strengthens the Al^{3+}–citrate interaction.[3-5] For the Al^{3+} complex of ATP and other nucleoside triphosphates, the pH 7.0 conditional stability constant has been estimated as log $K_{1c7} = 10.8$.[3,4] Consistent with the relative values of these constants, citrate extracts Al^{3+} from ATP.[31] For both citrate and nucleoside triphosphates the pH 7.0 conditional constants greatly exceed those for the catecholamines, and for the last to compete for Al^{3+} it would have to occur in 3000-fold excess over citrate and in 400-fold excess over ATP. These are realizable conditions in some tissues where mixed-ligand complexes are also likely to occur.

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