Interactions of some metal ions with Nitrogenous bases present in Nucleotides

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Abstract: -

Acidity constants at 30c^{o} and 0.125 ionic strength have been determined for the Nitrogous bases of nucleic acid; cytocine, uarcil and thymine, and found to be 3.55 x10^{-19}, 1.44 x10 $^{-19}$ and 7.24 x10 $^{-20}$ respectively.

Stability constants of these bases with Thorium and uranyl ions have been determined. Results showed that metal ions Thorium and uranyl ions behave as hard acids and the nitrogenum bases behave as Hard bases according to Pearson's definition .Hardness – softness parameters for these ligands were calculated ,stability constants of complexes with metal ions could be arranged as follows :- Cytosine > Uracil > Thymine .

Introduction

There are several biological molecules form complexes with metal ions, the study of metal complex formation and their stabilities are an important subjects, since more than 3% of the human body consists of metals, life depends on these metals. Transition elements in particular, even though some are present in only trace amounts, generally appear in the active centers of enzymes that catalyze substrates to form aggregate molecules, and so an understanding of the properties of the metal complexes that marshal their conjoining is required (1).

It is a well –known fact that metal ions are essential in a large variety of biological processes, including those with nucleic acids and their derivatives (2) . For the DNA polymerase which contains tightly bound Zn2+ .There is evidence that this metal ion binds the enzyme to DNA, to fulfill its function the enzyme must also be activated by a divalent cation such as Mg 2+ or Mn2+ , and these metal ions bind the nucleoside triphosphate substrate to the enzyme (3). Thus the interplay between metal ions and nucleotiedes or their derivatives, is receiving much attention at present (4) .

There are two broad types of bonding interaction of inorganic species with nucleic acids (5),first ,polynuceotides present to a metal center three major types of ligating regions ,namely ,the ribose moiety ,the phosphate oxygens of the phosophodiester linkages and the hetrocyclic ring nitrogen atoms and the exocyclic functional groups of the purine and pyrimidine bases .Second,in many instances metal ions or complexes will coordinate preferentially to one of the four common bases in DNA or RNA .

Metal compounds are often selective as to which of these ligating regions (or combination of regions) will form the strongest bond. Intervention of metal ions between the nitrogenous bases of nucleic acid of the DNA or the RNA may strengthen ring structure or it may not ,depending on the Hard–Soft acid-base interaction of Pearson(6), thus in order to evaluate these interactions, stability constant measurements of these

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complexes are needed .In this research we have chosen two heavy metal ions ,namely ,Thoriom and Uranyl ions and two nitrogenous bases of perimidine type that found in the DNA ,the cytosine and the thyamine while a third nitrogenous base of the same type found in the RNA ,the uracil moity.

Experimental

Materials:-

Standard solution of thorium Nitrate ,uaranyl Nitrate and Sodium perchlorate were prepared from analyar grade qualities ,Freshly prepared solutions of cytosine ,uarcil and Thymine were made from AR grades. All solution were made with deionized water and the carbonate free alkali solutions were standardized against potassium hydrogen phthalate (6 , 7) .

Apparatus and procedure

pH measurements were made using practironic ph –meter, equipped with glass electrode and standard silver –silver chloride electrode. The pH –meter was standardized before each run against buffer solution of known pH values and was checked at end of each run.

Titrations were carried out in a closed vessel provided with magnetic stirrer. The titrant was delivered from a semi micro burette and the pH value was recorded as soon as it becomes steady .Temperature control of solution was made with Townson and Mercer Thermostated bath of 0.05 c^o sensitivity.

Steps in evaluating Hard and Soft parameters of Ligands

1. Determination of pKa of the ligands

Since we are dealing with bases of nucleic acid, therefore equivalent quantities of perchloric acid should be added before titration with alkali is taking place.

$$B.2HClO_4 + H_2O \longrightarrow BH_2^{++} + 2ClO_4^{-} + H_2O$$

$$BH_2^{++} + H_2O \overset{Ka_1}{\longleftrightarrow} BH^{+} + H_3O^{+}$$

$$BH^{+} + H_2O \overset{Ka_2}{\longleftrightarrow} B + H_3O^{+}$$

Typically results and titration curves are shown in table (1) and figures (1, 2, 3).From these measurements an overall “Ka” values of the ligands were obtained and are summarized in table (2).

2. Determination of stability consonant of complexes

Titration with alkali to mixtures of metal ions and ligands at constant ionic strength and temperature were made in Nitrogen atmosphere following the same procedure a dopted for complexes of other ligands (8,). Typical results are summarized in tables (3,4). Calculation of metal chelate formation constants were made according to J.Bjerum,s summation equation (9)and explained fully in previous papers(10,11).

3. Evalution of Hard soft interactions

From the first association constant of the complexes obtained and the Hard –Soft parameters of the metal ions (Th^{4+} and UO_2^{2+})calculated using the proper atomic parameters (12).It is possible to evaluate Hard and Soft parameters of the nitrogenous bases of nucleic acid using Mesono,s equation (13) .Typical results are shown in table (5).

Table (1)Titration of Mixtures of (Pyrimidine (0.015M) + 9.5ml 0.5MNa ClO4 + 37ml water). Ionic strength 0.125 Temp30C^{o}

ml 0.1M KOH	pH		
	Cytosine	Uracil	Thymine
0.00	1.61	1.47	3.03
1.0	1.63	1.49	3.73
2.0	1.69	1.52	4.06
3.0	1.72	1.56	4.28
4.0	1.76	1.59	4.48
5.0	1.81	1.64	4.66
6.0	1.88	1.68	4.86
7.0	1.93	1.73	5.04
8.0	2.00	1.78	5.34
9.0	2.09	1.85	5.86
10.0	2.20	1.93	8.08
11.0	2.39	2.03	8.36
12.0	2.65	2.16	8.48
13.0	3.14	2.33	8.58
14.0	3.63	2.72	8.64
15.0	3.95	6.61	
16.0	4.18	7.68	
17.0	4.37	8.29	
18.0	4.58	8.54	
19.0	4.81	8.75	
20.0	5.05	8.92	

Table (2)Over all ionization constant of conjugate acid of the pyrimidines at ionic strength of 0.125 and Temperature 30C^{o}

	Pyrimidine		
	Cytosine	Uracil	Thymine
Ka	3.55×10^{-19}	1.44×10^{-19}	7.24×10^{-20}
pKa	18.45	18.84	19.14

Table (3)Titration of Mixtures of pyrimidine (0.015M)and 0.005 MThorium Nitrate .Ionic strength adjusted with 0.5MNaClO4 to 0.125 ,Total volume 47.5ml,Temperature 30C^{o}

ml 0.1M KOH	PH		
	Cytosine	Uracil	Thyamine
0.00	1.63	1.50	2.77
1.00	1.64	1.54	2.83
3.00	1.67	1.59	3.02
5.00	1.73	1.66	3.22
7.00	1.79	1.76	3.45
9.00	1.86	1.88	3.93
11.00	1.96	2.06	4.28
13.00	2.07	23.4	4.57
15.00	2.19	2.72	4.88
17.00	2.38	2.93	5.30
19.00	2.61	3.07	6.96
20.00	2.73	3.17	
21.00	2.86		
23.00	3.03		
25.00	3.14		
27.00	3.26		
30.00	3.56		

Table (4)Ttration of Mixtures of Pyrimidine (0.01M) and 0.005 M Uranyl Nitrates .Ionic strength adjusted with 0.5M NaClO4 to 0.125,Total volume 47.5 ml ,Temperature 30C^{o} .

ml 0.1M KOH	PH		
	Cytosine	Uracil	Thymine
0.00	1.64	1.45	2.96
1.00	1.65	1.47	3.38
5.00	1.73	1.62	4.06
7.00	1.81	1.71	4.30
10.00	1.88	1.84	4.70
13.00	2.03	2.34	5.22
16.00	2.29	3.68	ppt
19.00	2.84	4.44	
20.00	3.13	4.96	
22.00	3.55	ppt	
24.00	3.82		
26.00	4.00		
28.00	4.18		
30.00	4.35		

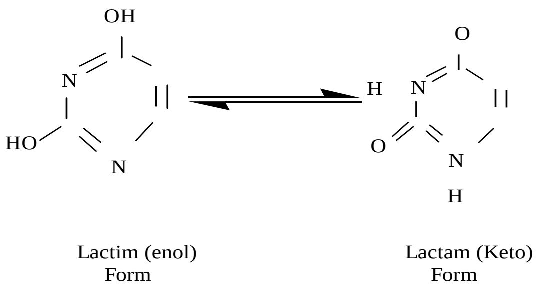
Table (5)Misiono,s Metal –ligand Parameter calculated according to the equation LogK = α x + β γ +Ɣ for the first association

Ligand / M^{n+}		Uracil	Thymine	Cytosine	Metal Parameter	
	Ka	1.44×10^{-19}	7.24×10^{-20}	3.55×10^{-19}	X	γ
Th^{4+}	β_1	2.85×10^{19}	2.81×10^{18}	1.02×10^{20}	8.93	2.75
	β_2	1.15×10^{38}	2.62×10^{35}	1.56×10^{36}		
UO_2^{2+}	β_1	2.58×10^{20}	6.94×10^{17}	1.26×10^{22}	20.32	1.86
	β_2	6.45×10^{37}	5.26×10^{33}	2.06×10^{38}		
Ligand Parameter	α	0.485	0.35	0.58		
	β	5.13	5.19	5.033		
	γ	1	1	1		

Discussion

Nucleic acid constitvents can be considered as naturally occuring ligands possessing a variety of metal ion binding sites . Not only do the ribos and ribophosphate groups of the nucleosides and nucleotides contain potential coordination sites but also the purine and primidine bases possess several potential N-and O- donor atoms (14)

In solution, enol perimidino derivaties exist in the keto form (Lactam)or enol form (Lactim) depending on the pH of the Medium .The Lactam shape is the one predominating in the living cell.



In terms of the hard and soft acid and base (HSAB) principle (15) ,natural distinction exist between the type of coordination encountered with the hard donor atoms such as Oxygen and that experienced with the softer nitrogen atoms. Soft heavy metal ions such as Hg (II), Pt (II), and Ag (I) are therefore expected to preferentially bind to the N atoms of the base moiety .As Glassman and co-workers point out (16) ,further diversity in binding characteristics arise because of the varying degree of " hardness" or "Softness" of individual base donor atoms .

Regarding perimidine bases ,cytocine moity contains three nitrogen atoms (3 soft centers)and one oxygen atom(hard center) which makes it softer ligand where as uracil and Thymine moieties have two nitrogen atoms and two oxygen atoms respectively which make them harder ligands, as it is seen in fiure(4)

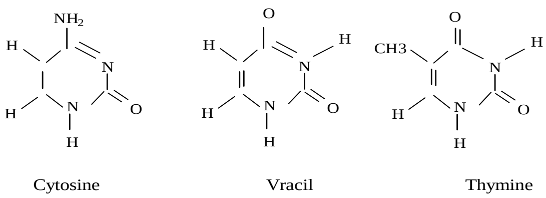


Fig (4): Chemical structure of permidines

Considering Hard –Soft interaction of metal ions exhibited by thorium and Uranyl ions, and as it can be seen from table (5), Thorium ion has smaller hardness parameter (X) than Uranyl ion, Also Thorium ion has larger softness parameter (Y) than Uranyl ion, which makes, Thorium harder ion than uaranyl .[17]

Now; stable complexes occur when (hard –hard)interactions and (soft –soft) interactions take place between metal ions and ligands [15] .With regard to uranyl ions (the soft ion),stable complexes occure with cytosine (the soft ligand) ,while thorium ion (The hard ion)shows stable complexes with uarcil and thymine (the hard ligands).

Again, results from table (5) show that metal ion complexes of (Th^{4+} and UO_2^{2+})with uarcil ligand are more stable than complexes of the same ions with Thymine ligand ,this difference in behavior is due to steric effect of the methyl group of the thymine ligand.

Finally, taking into consideration the first association constant of the complexes studies, it could be possible to arrange the stabilities of Metal – pyrimidine complexes according to the following sequences:

Stability of Metal – Ligand complexes

Metal ion	*Pyrmidine Ligands*
Th^{4+}	**Cytosine > Uracil > Thymine**
UO_2^{2+}	**Cytosine > Uracil > Thymine**

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تداخلات بعض الايونات الفلزية مع القواعد النتروجينية الموجودة في النيوكليتيدات

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الخلاصة :-

تم في هذا البحث تعيين الثوابت الحامضية (Ka) في درجة حرارة 30 م^{o} وقوى ايونية 0.125 لكل من السيتوسين واليوراسيل والثايمين وكانت على التعاقب 3.55×10^{-19}, 1.44×10^{-19} , 7.24×10^{-20} كذلك تم تعيين ثوابت الاستقرارية للمعقدات التي تكونها القواعد النتروجينية هذه مع كل من ايونات الثوريوم واليورانيل. لقد اثبتت نتائج البحث بان الايونات الموجبة تتصرف بمثابة حوامض صلدة (Hard acids) ،وان القواعد النتروجينية للحوامض النووية تتصرف وكأنها قواعد صلدة (Hard base) حسب تعريف بيرسون (Pearson) .لقد امكن حساب معاملات النعومة والصلادة لها وامكن ترتيب ثوابت الاستقرارية لها مع ايونات الثوريوم واليورانيل حسب النمط التالي: السيتوسين > اليوراسيل > الثايمين .