Determination of Dissociation Constants of Pyrimidine Bases by Ionophoretic Technique

A.P. Mishra, S.K. Mishra and K.L. Yadava

Electrochmeical Laboratories, Department of Chemistry, University of Allahabad, Allahabad - 211 002 (India)

Ionophoretic technique developed for the assessment of dissociation constants of aminoacids is extended to the pyrimidine bases viz. uracil, thymine and cytosine. The values agree with those detrmined by the other standard methods earlier by other workers.

Ionophoretic technique has been applied earlier for the determination of dissociation constants of α- amino acids [1]. In this paper, the method is extended for determining the dissociation constants of a few biologically important compounds[2] namely cytosine, thymine and uracil. The usual draw backs of this technique like variation in the temperature during the electrophoresis, capillay flow on the paper, electroosmosis, adsorption and molecular sieving affecting the mobility of charged moieties, are quite well known [3]. The technique described here is almost free from these vitiating factors.

EXPERIMENTAL

Instrument as described in reference 1

Chemicals

Uracil, thymine, cytosine (B.D.H.); perchloric acid (BDH AnalaR): Caustic soda (Merck); Electro osmotic indicator - Glucose(BDH). Filter paper Whatman No.1 (for chromatography);

Pyrimidine base spots were detected under UV light λmax 260-290nm. Glucose spot was detected as black spot by the use of aqueous silver nitrate in acetone and 2% ethanolic caustic soda.

Procedure

The level of the hollow base plate in the instrument was made horizontal with a spirit level. 150 ml of 0.1 M perchloric acid was taken in each tank of the electrophoretic apparatus. The levels of the two tank solutions were made same by siphoning them through a bent tube. These precautions were taken to check any gravitational and hydrodynamic flow. Paper strips (Whatman No.1) of 30 x 1 cm^2 size were soaked in the background electrolyte and then excess of electrolyte solution was blotted by pressing them gently within the folds of dry filter paper sheets. The strips in duplicate were then spotted with an aqueous solution (0.01M) of pyrimidine bases and glucose solution (0.01M) in the centre with a micropipette and were subsequently placed on the base plate and sandwiched under the upper hollow metallic plate with end of strips dipping into the background electrolyte as described in ref.[1]. The temperature was maintained at 35°C. The strips were allowed to remain as such for 15 minutes. Then electrophoresis experiment was carried out for 60 mts. The mobility was calculated as described earlier[1].

RESULTS AND DISCUSSION

A plot of overall mobility against pH (Fig. 1) gives curves with three plateaus in cytosine and two in uracil and thymine. The first plateau of the cytosine lies in the positive range of the mobility whereas those of the other two bases have the zero-mobility. Obviously, cytosine becomes protonated whereas the remaining two bases remain neutral in acidic background electrolyte solution. Cytosine attains zero mobility around pH 5.2 indicating that the positively charged protonated cytosine loses its protons and becomes neutral species. With further increase of pH, all the three bases get deprotonated to give the corresponding anions and mobility becomes negative.

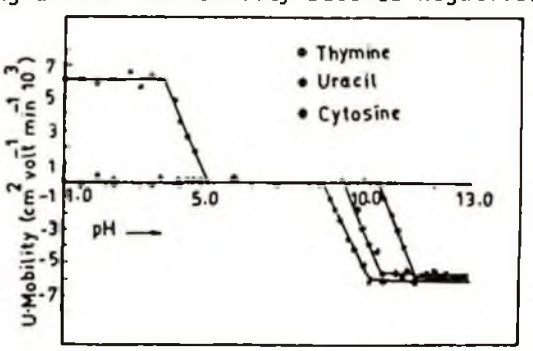
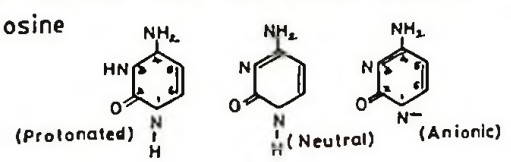


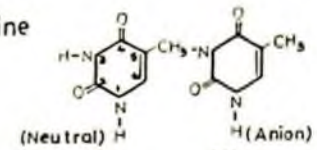
Fig.1 Plot pH Vs Mobility

The different species of cytosine have been suggested earlier [4] and given below:

Cytosine

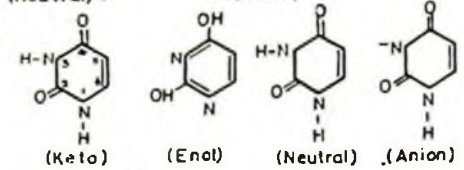


Thymine



(Neutral) (Anion)

Uracil



(Keto) (Enol) (Neutral) (Anion)

In case of cytosine the protonation in presence of acid would occur either on amino nitrogen or imino nitrogen N(3). However there is overwhelming evidence in favour of the latter site [6,4,10,11,12]. On increase of pH the attached proton is split off (second plateau). Imidonitrogen N(1) subsequently splits off proton on increase of pH and gives the anionic species.

As regards the site of deprotonation of uracil and thymine there has been a contraversy. However, a number of workers [5,9] have lately given evidence for the deprotonation preferably from N(3) to C(4).

The Table No.1 gives calculated dissociation constants. These values are fairly in agreement with literature values. Divergence in the values is partly due to variation in experimental temperature and ionic strength. Thymine possesses greater basicity [9,13,14] than uracil because of inductive effect of methyl group. Cytosine is most basic among the three, obviously because of the active imidonitrogen.

TABLE 1

pK VALUES FOR URACIL, THYMINE & CYTOSINE
Temp. 35°C, μ = 0.1 M

Base	Present Values	Ref9	Ref6	Ref15	Ref16
		[45° 0.1M KNO_3]	[25°μ=0]	[25° μ = 0.1M]	
Uracil	9.33 ±0.07	8.8	9.46	9.45	9.16
Thymine	9.80 ±0.05	9.1	9.9	9.9	9.65
Cytosine					
pK_1	4.51 ±0.08	4.2	4.58	-	4.4
pK_2	10.8 ± 0.1	10.0	12.15	-	11.71

REFERENCES

1. S. Singh, H.L. Yadava, P.C. Yadava and K.L. Yadava, *J. Electrochem. Soc. India.*, 34(2) (1985), 125; R.K.P. Singh and K.L. Yadava, ibid, 128; S. Singh and P.C. Yadava, ibid 181.

2. E. Tate, *Biochem. J.*, 195(2)(1981) 419.

3. D.J. Shaw, *Electrophoresis*, Acadamic Press (1969) 99 - 103.

4. L. Jardetsky, P. Pappas and N.G. Wade, *J. Am. Chem. Soc.*, 85(1963) 1657.

5. J.J. Fox and D. Shugar, *Biochem. Biophys. Acta*, 9 (1952) 369.

6. J.J. Christensen, H.J. Rytting and R.M. Izatt *J. Phys. Chem.*, 71(1967) 2700.

7. H.T. Miles, *Biochem. Biophys. Acta*, 22(1956) 247.

8. S. Lewin and D.A. Humphreys, *J. Chem. Soc. B*, 210 (1966).

9. M.M. Taquikhan and C.R. Krishnamoorthy, *J. Inorg. Nucl. Chem.*, 36(1974) 711.

10. T. Ueda and J.J. Fox, *J. Am. Chem., Soc.*, 85(1963) 4024.

11. H.T. Miles, *Proc. Natl. Acad, Sci., U.S.*, 47(1961) 791.

12. H.T. Miles, R.B. Bradley and E.D. Becker, *Science.*, 142(1963) 1569.

13. N.F. Hall and M.R.Sprinkle, *J. Am. Chem. Soc.*, 54(932) 3469.

14. A. Albert and E.P. Serjeant, *Ionization Constt.*, Wiley, New York (1962).

15. P.A. Levene, L.W. Bass and H.S. Simms *J. Biol. Chem.*, 70(1929) 229.

16. M. Robert Smith and E. Arthur Martell, *Critical Stability Constants Vol.2; Amines* 264, 271, 342; Plenum Press New York & London (1975).