Thermodynamic Quantities for the Dissociation Equilibria of Biologically Important Compounds

6. THE DISSOCIATION OF CALCIUM GLUCOSE 1-PHOSPHATE*

By H. B. CLARKE† and S. P. DATTA
Department of Biochemistry, University College, London

(*Received* 11 *June* 1956)

There is considerable evidence that the calcium salts of many multivalent acids are incompletely dissociated in solution (Martell & Calvin, 1952); among others the dissociation constant of calcium hydrogen phosphate has been measured (Greenwald, Redish & Kibrick, 1940; Davies & Hoyle, 1953). Since glucose 1-phosphate (G1-P) is known to occur in calcifying cartilage (see, for example, McLean & Urist, 1955) it is of interest to obtain detailed information about any interactions between its anions and Ca^{2+} ions.

The titration curves of K_2G1-P with hydrochloric acid in the presence of sodium chloride or calcium chloride of equal ionic strength (Fig. 1) show that Ca^{2+} ions interact with the species $G1\text{-}P^{2-}$ but not with $HG1\text{-}P^{-}$. These findings are similar to those with Mg^{2+} ions and G1-P (Clarke, Cusworth & Datta, 1954). It has therefore been assumed that the only complex formed in significant amounts is CaG1-P and the possibility that complexes such as $CaHG1\text{-}P^{+}$ or $Ca(HG1\text{-}P)_2$ were formed has been ignored.

Accordingly, 11 G1-P buffers containing calcium chloride were examined potentiometrically in cells without liquid junction of the type

$$\mathrm{Pt.H_2\ (1\ atm.)\,|\,KHG1\text{-}P\ (}m_a\mathrm{).K_2G1\text{-}P\ (}m_b\mathrm{).CaCl_2\ (}m_s\mathrm{)\,|\,AgCl.Ag}$$

at 10°, 20°, 30°, 40° and 50°. From the e.m.f. of these cells, together with the values of m_a, m_b and m_s, the dissociation constant of CaG1-P

$$K_s = \frac{a_{Ca^{2+}} \,.\, a_{G1\text{-}P^{2-}}}{a_{CaG1\text{-}P}} \qquad (1)$$

was calculated by the method used for the magnesium salt (Clarke *et al.* 1954).

The values of $-\log K_s$ at different temperatures were found to vary according to the Harned & Robinson (1940) equation

$$-\log K_s = \mathrm{A}/T - \mathrm{D} + \mathrm{C}T, \qquad (2)$$

where T is the absolute temperature and A, C and D are constants which were evaluated by the method of least squares. From these constants were calculated smoothed values of $-\log K_s$ and the thermodynamic quantities associated with the dissociation of CaG1-P.

METHOD

The apparatus, the general technique and the materials, except the $CaCl_2$, were the same as for MgG1-P (Clarke *et al.* 1954). A solution of $CaCl_2$ was made by dissolving a weighed amount of $CaCO_3$ (Specpure, Johnson Matthey and Co., London, E.C. 1) in a slight excess of HCl. The concentration of chloride in this solution was estimated gravimetrically as AgCl. The e.m.f.'s were measured in absolute volts and the constants used were as in Clarke, Datta & Rabin (1955).

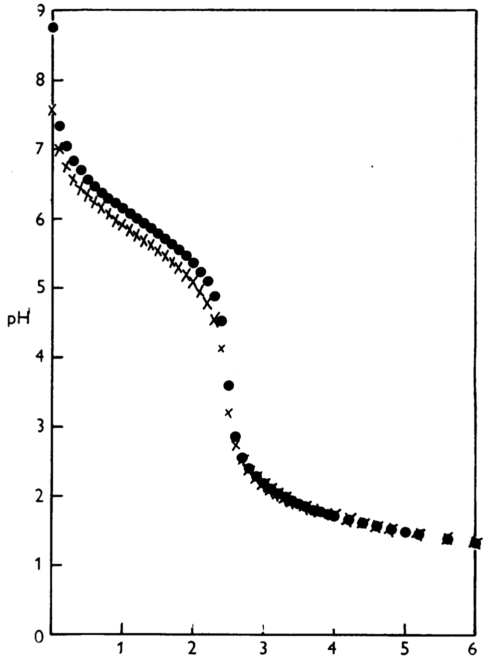

Fig. 1. Titration of dipotassium glucose 1-phosphate with HCl in the presence of NaCl and $CaCl_2$ of equal ionic strength. ●, 25 ml. of 0·1M dipotassium glucose 1-phosphate and 25 ml. of 0·3M-NaCl; ×, 25 ml. of 0·1M dipotassium glucose 1-phosphate and 25 ml. of 0·1M-$CaCl_2$.

---

* Part 5: Clarke, Datta & Rabin (1955).

† Present address, Services Electronic Research Laboratory, Baldock, Herts.

Table 1. *Calcium glucose* 1-*phosphate. Molalities* (*m*) *and ionic strengths* (*I*) *of solutions, e.m.f.'s of cells* (*E*), *absolute volts* (*corrected to* 1 *atm. pressure of hydrogen and rounded temperatures*) *and extrapolation functions* (*y*)

m_a = molality of KH glucose 1-phosphate, m_b = molality of K_2 glucose 1-phosphate, m_s = molality of $CaCl_2$,

$$m_{H^+} = \text{antilog}\left[\frac{(E-E_0)_F}{2{\cdot}3026RT} - \log m_{Cl^-} + \frac{2AI^{\frac{1}{2}}}{1+I^{\frac{1}{2}}}\right],\quad m_{A^{2-}} = \text{antilog}\left[\log K_a + \log m_{HA^-} - \log m_{H^+} + \frac{4AI^{\frac{1}{2}}}{1+I^{\frac{1}{2}}}\right],$$

$$I = m_a - m_b + 3m_s - 2m_{H^+} + 4m_{A^{2-}},\quad y = -\log m_{Ca^{2+}} - \log m_{A^{2-}} + \log m_{CaA} + \frac{8AI^{\frac{1}{2}}}{1+I^{\frac{1}{2}}}.$$

In these solutions $m_{Cl^-} = 2m_s + m_a$. For definition of symbols see Ashby, Crook & Datta (1954).

	$10^3 m_a$	1·0367	1·2912	1·5736	1·6723	2·0235	2·5115	2·6175	3·1744	3·7396	4·0966	4·6388
	$10^3 m_b$	2·9414	3·6634	4·4646	4·7445	5·7409	7·1255	7·4264	9·0062	10·6097	11·6226	13·1610
	$10^2 m_s$	0·47001	0·58539	0·71340	0·75812	0·91734	1·1386	1·1867	1·4391	1·6953	1·8572	2·1030
	$10^7 m_{H^+}$	3	3	3	3	4	4	4	5	5	5	5
10°	E	0·71910	0·71258	0·70654	0·70461	0·69889	0·69223	0·69095	0·68479	0·67973	0·67686	0·67299
	$10^3 m_{A^{2-}}$	2·2089	2·6983	3·2150	3·3834	4·0336	4·8961	5·0819	6·0000	6·9728	7·5683	8·4843
	$10^2 I$	2·2067	2·7273	3·2944	3·4877	4·1960	5·1639	5·3736	6·4515	7·5620	8·2559	9·3143
	y	2·4357	2·4271	2·4298	2·4355	2·4285	2·4316	2·4324	2·4477	2·4491	2·4531	2·4560
20°	E	0·72988	0·72309	0·71674	0·71473	0·70879	0·70185	0·70055	0·69418	0·68885	0·68588	0·68187
	$10^3 m_{A^{2-}}$	2·1615	2·6372	3·1307	3·2933	3·9251	4·7579	4·9438	5·8429	6·7675	7·3473	8·2388
	$10^2 I$	2·1878	2·7029	3·2607	3·4516	4·1526	5·1086	5·3183	6·3887	7·4799	8·1675	9·2161
	y	2·4845	2·4766	2·4838	2·4894	2·4821	2·4862	2·4852	2·4980	2·5038	2·5071	2·5097
30°	E	0·74043	0·73338	0·72675	0·72465	0·71852	0·71131	0·70998	0·70330	0·69772	0·69466	0·69052
	$10^3 m_{A^{2-}}$	2·0973	2·5574	3·0293	3·1844	3·7989	4·6014	4·7847	5·6360	6·5130	7·0765	7·9404
	$10^2 I$	2·1621	2·6709	3·2201	3·4081	4·1021	5·0460	5·2547	6·3059	7·3781	8·0592	9·0968
	y	2·5460	2·5374	2·5457	2·5515	2·5422	2·5461	2·5441	2·5601	2·5682	2·5704	2·5720
40°	E	0·75076	0·74342	0·73646	0·73432	0·72789	0·72050	0·71913	0·71216	0·70621	0·70289	0·69876
	$10^3 m_{A^{2-}}$	2·0268	2·4669	2·9108	3·0640	3·6430	4·4253	4·6026	5·4100	6·2091	6·7061	7·5722
	$10^2 I$	2·1339	2·6348	3·1727	3·3599	4·0398	4·9756	5·1818	6·2155	7·2565	7·9110	8·9495
	y	2·6110	2·6034	2·6149	2·6180	2·6125	2·6116	2·6092	2·6264	2·6418	2·6506	2·6452
50°	E	0·76089	0·75323	0·74576	0·74354	0·73690	0·72905	0·72779	0·72071	0·71449	0·71108	0·70684
	$10^3 m_{A^{2-}}$	1·9372	2·3512	2·7454	2·8890	3·4360	4·1407	4·3323	5·1180	5·8590	6·3341	7·1615
	$10^2 I$	2·0980	2·5885	3·1066	3·2899	3·9570	4·8617	5·0737	6·0987	7·1165	7·7622	8·7852
	y	2·6889	2·6831	2·7048	2·7074	2·7003	2·7072	2·6979	2·7075	2·7243	2·7314	2·7252

Table 2. *Calcium glucose* 1-*phosphate. Observed and calculated values of* $-\log K_s$ *at rounded temperatures, together with values of* 'β' *the slopes of the extrapolations, the constants of the equation* $-\log K_s = A/T - D + CT$, *where* T *is the absolute temperature, the minimum value of* $-\log K_s$ *and the temperature at which it occurs*

Temp.	$-\log K_s$ (obs.)	β	$-\log K_s$ (calc.)
10°	2·418$_0$	0·39	2·420
20	2·468$_5$	0·44	2·467
25	—	—	2·495
30	2·528$_4$	0·48	2·526
37	—	—	2·573
40	2·590$_0$	0·64	2·596
50	2·677$_6$	0·59	2·675
			$\sigma = 0{\cdot}005$

$A = 1517$
$D = 9{\cdot}445$
$C = 0{\cdot}02298$
$-\log K_{s\,(\text{min.})} = 2{\cdot}36$
$T_{(\text{min.})} = 257°\ K$

Table 3. *Thermodynamic quantities associated with the dissociation of calcium glucose* 1-*phosphate*

	ΔG^0		$-\Delta H^0$		$-\Delta S^0$		$-\Delta C_P^0$	
Temp.	Cal.	J	Cal.	J	Cal. deg.$^{-1}$	J deg.$^{-1}$	Cal. deg.$^{-1}$	J deg.$^{-1}$
25°	3 403	14 240	2 403	10 050	19·5	81·5	63	262
	σ4·6	19	83	346	0·90	3·8	11	46
37	3 652	15 280	3 170	13 260	22·0	92·0	65	273
	σ4·4	19	106	442	1·07	4·5	11·4	48

RESULTS

The measured e.m.f.'s, together with the values of the molalities of the various species and the value of the extrapolation function

$$y = -\log K_s + \beta I = -\log m_{\mathrm{Ca}^{2+}} . m_{\mathrm{G1\text{-}P}^{2-}} + \log m_{\mathrm{CaG1\text{-}P}} + \frac{8\,\mathrm{A}I^{\frac{1}{2}}}{1+I^{\frac{1}{2}}} \quad (3)$$

are given in Table 1. The extrapolated values of $-\log K_s$ at infinite dilution, the slopes β of the extrapolations, the values of A, C and D (equation 2) and the smoothed values of $-\log K_s$ are given in Table 2. The thermodynamic quantities associated with the dissociation of CaG1-P at 25 and 37° are given in Table 3. The standard errors of the results given in Tables 2 and 3 were calculated according to Please (1954).

DISCUSSION

The dissociation constants of CaG1-P are very similar to those of MgG1-P over the temperature range studied, the two constants being the same at 37·6°. At 37° the values of ΔG^0 for the dissociation of the calcium and magnesium salts are virtually identical (15 280 and 15 290 J/mole respectively), but the values of ΔH^0 and ΔS^0 are both less negative for the calcium salt than for the magnesium salt (ΔH^0 −13 260 and −15 200 J/mole; ΔS^0 −92 and −98 J/mole/degree respectively). These differences possibly reflect the greater degree of hydration of the Mg^{2+} ion and the greater decrease in the entropy of the solvent caused by this ion consequent on its smaller crystallographic radius, Mg^{2+} 0·65 Å, Ca^{2+} 0·99 Å (Pauling, 1945). That the Mg^{2+} ion is in fact more hydrated than the Ca^{2+} ion in aqueous solution is indicated by conductivity data, which give hydration numbers of 12 and 10 respectively (Robinson & Stokes, 1955).

As has been stated above, no evidence was found for the existence of complexes between Ca^{2+} ions and HG1-P$^-$. Davies & Hoyle (1953) have, however, found evidence for the existence of the two complexes $CaHPO_4$ and $CaH_2PO_4^+$, the $-\log K$ values for the dissociations of which are 2·699 and 1·076 respectively at 25°. The complex $CaH_2PO_4^+$ is thus 42 times less stable than $CaHPO_4$. As with the magnesium salts the complex CaG1-P is less stable than $CaHPO_4$, and the absence of any detectable complex between Ca^{2+} and HG1-P$^-$ ions may be due to the large size of the anion compared with that of $H_2PO_4^-$. The possibility that the undetected presence of such a complex affected the results reported here was minimized by keeping the concentration of G1-P^{2-} greater than that of HG1-P$^-$ in the solutions studied.

SUMMARY

1. The thermodynamic dissociation constants of calcium glucose 1-phosphate have been measured at 10° intervals from 10° to 50° by using hydrogen and silver–silver chloride electrodes in cells without liquid junction.

2. From these measurements have been calculated the changes in standard free energy, heat content, entropy and heat capacity for the dissociation at 25° and 37°.

3. Sufficient information has been given for the dissociation of this salt to be considered quantitatively in any kinetic or other study of glucose 1-phosphate in the presence of Ca^{2+} ions.

The authors thank the Central Research Fund of the University of London for a grant to purchase potentiometric equipment; they also thank Mr V. L. Bengeri for invaluable technical assistance. One of the authors (H.B.C.) thanks the Department of Scientific and Industrial Research for a personal grant.

REFERENCES

Ashby, J. H., Crook, E. M. & Datta, S. P. (1954). *Biochem. J.* **56**, 190.

Clarke, H. B., Cusworth, D. C. & Datta, S. P. (1954). *Biochem. J.* **58**, 146.

Clarke, H. B., Datta, S. P. & Rabin, B. R. (1955). *Biochem. J.* **59**, 209.

Davies, C. W. & Hoyle, B. E. (1953). *J. chem. Soc.* p. 4134.

Greenwald, I., Redish, J. & Kibrick, A. C. (1940). *J. biol. Chem.* **135**, 65.

Harned, H. S. & Robinson, R. A. (1940). *Trans. Faraday Soc.* **36**, 973.

McLean, F. C. & Urist, M. R. (1955). *Bone*, p. 59. Chicago: The University of Chicago Press.

Martell, A. E. & Calvin, M. (1952). *The Chemistry of the Metal Chelate Compounds*, p. 194. New York: Prentice-Hall Inc.

Pauling, L. (1945). *The Nature of the Chemical Bond*, p. 346. Ithaca, N.Y.: Cornell University Press.

Please, N. W. (1954). *Biochem. J.* **56**, 196.

Robinson, R. A. & Stokes, R. H. (1955). *Electrolyte Solutions*, p. 121. London: Butterworths Scientific Publications.