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Note

Calorimetric analysis of the interaction of calcium ions with galactose, *myo*-inositol, and lactose

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A number of investigations of complexing between metal ions and sugars in aqueous solution have been conducted, and approximate values of stability constants reported for some of these complexes[1–6]. However there appears to have been very few, if any, calorimetric studies on the energetics of these interactions. For this reason, we initiated a calorimetric investigation of the interactions of calcium ion with galactose, lactose, and *myo*-inositol in aqueous solution.

EXPERIMENTAL

Materials. — (+)-Lactose was obtained from the Mallinckrodt Chemical Works†; (Lot No. XCVO). *myo*-Inositol (Lot No. 1-9740, m.p. 225-227(C) and D(+)-galactose were products of the Baker Chemical Company. $CaBr_2 \cdot 2H_2O$ was purchased from Matheson Coleman and Bell (quality control No. 16), and $Ca(NO_3)_2 \cdot 4H_2O$ was a Mallinckrodt product. All materials were used as received, and solutions of the desired molarity were prepared in deionized, distilled water.

Methods. — A flow modification of a microcalorimeter previously described was used for the calorimetric measurements[7]. Equal volumes of solutions of sugar and calcium ions were allowed to flow into the calorimeter. The total volumes of solutions of sugar and calcium ions were the same in all calorimetric experiments.

Corrections for heats of dilutions of the reactants, and viscous heating effects, were applied to the experimental heats. In the experiments with $CaBr_2 \cdot 2H_2O$, these corrections were substantial, ranging from 50–70% of the total observed heat. Most of this correctional heat was attributable to dilution of the bromide. Correctional heats in the $Ca(NO_3) \cdot 4H_2O$ experiments were considerably lower, amounting to 10–15% of the total experimental heats. Although these correctional heats were unusually large, particularly in the bromide experiments, reproducibilities of the net heat-effects were good.

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*One of the facilities of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture.

†Mention of companies or commercial products does not imply recommendation by the U.S. Department of Agriculture over others not mentioned.

RESULTS AND DISCUSSION

Table I summarizes the results obtained with $CaBr_2 \cdot 2H_2O$. The increase in the corrected, experimental heat is nearly linear as the sugar concentrations are increased at constant salt-concentration. We have interpreted our results as being best represented in terms of 1:1 interactions. However, in order to check for the possible involvement of successive equilibria, the series nos. 8 and 9, 11 and 12, and 14 and 15 of Table I were performed. Thus, considering successive equilibria of the type $A+B=AB$, $AB+B=AB_2$, and so on, if, for example, the concentration of B is twice that of A in one experiment, and then, in another, the converse is true, there should be a difference in the experimental heats if successive equilibria are involved. As shown in Table I, there is no such difference.

TABLE I

HEATS OF INTERACTION OF D-GALACTOSE, *myo*-INOSITOL, AND LACTOSE WITH $CaBr_2 \cdot 2H_2O$ AT 25°

Series no.	*Sugar conc.* (M)	*Salt conc.* (M)	*No. of expts.*	$-Q$ *(expt. heat) (mcal)*
		D-*Galactose*		
1	0.100	0.100	5	6.95 ±0.04[a]
2	0.200	0.100	5	13.97 ±0.18
3	0.300	0.100	5	20.36 ±0.02
4	0.400	0.100	5	27.89 ±0.21
5	0.200	0.025	5	3.38 ±0.03
6	0.300	0.025	5	4.98 ±0.02
7	0.400	0.025	5	7.08 ±0.12
8	0.100	0.025	1	1.68
9	0.025	0.100	1	1.72
		myo-*Inositol*		
10	0.100	0.100	5	8.64 ±0.07
11	0.200	0.100	5	16.85 ±0.06
12	0.100	0.200	1	16.92
		Lactose		
13	0.100	0.100	5	10.80 ±0.04
14	0.200	0.100	5	21.85 ±0.08
15	0.100	0.200	1	21.70

[a] Standard error of the mean.

Another series of calorimetric experiments with the three sugars, using $Ca(NO_3)_2 \cdot 4H_2O$ as the reactant, was conducted to determine whether the anion might influence the results. The data in Table II indicate that this is indeed the case, as evidenced by the increased experimental heats. However, it is well established that solutions of calcium nitrate enter into ion-pair formation[8], and the increased experimental heats might be due in part to the dissocation of the $CaNO_3^+$ ion-pair when the calcium ion reacts with the sugars. (Insofar as is known, calcium bromide solutions do not take part in ion-pair formation.) Interestingly, the experimental heats of the nitrate reactions increase in the same, near-linear manner as the bromide reactions. Furthermore, as the nitrate anion probably competes with the sugars for calcium, calorimetry might prove to be a useful probe in ion-association studies in such weakly interacting systems as these.

TABLE II

HEATS OF INTERACTION OF D-GALACTOSE, *myo*-INOSITOL, AND LACTOSE WITH $Ca(NO_3)_2 \cdot 4H_2O$ AT 25°

Series no.	*Sugar conc.* (M)	*Salt conc.* (M)	*No. of expts.*	$-Q$ *(expt. heat) (mcal)*
		D-*Galactose*		
1	0.100	0.100	3	9.24 ±0.10[a]
2	0.200	0.100	3	18.08 ±0.14
3	0.300	0.100	3	27.03 ±0.15
		myo-*Inositol*		
4	0.100	0.100	4	10.79 ±0.11
5	0.200	0.100	5	21.72 ±0.08
		Lactose		
6	0.100	0.100	3	13.58 ±0.01 *f*
7	0.152	0.100	3	20.50 ±0.10
8	0.200	0.100	3	27.34 ±0.09

[a]Standard error of the mean.

A number of crystalline, calcium complexes of simple carbohydrates have been isolated from water[9]. In the specific instance of the three sugars studied in this investigation, the stoichiometry of the crystalline calcium complexes has been reported[10–12,13,14] to be 1:1. The calorimetric data rather markedly suggest that similar stoichoimetries exist in aqueous solutions.

The studies of Bugg and Cook[10–12] on the crystalline calcium complexes of lactose, galactose, and *myo*-inositol indicate that pairs of adjacent hydroxyl groups may serve as effective sites for chelation of calcium in these and other crystalline, calcium complexes of sugars. Moreover, as pointed out by Bugg and Cook, in view of the role that water molecules play in these calcium–sugar complexes, it seems reasonable to assume that these calcium–sugar interactions are typical of those that occur in aqueous systems. Thus, calcium binding in water may be attributed to a simple substitution of hydroxyl groups for water molecules in the hydration shell of calcium. However, previous studies in solution have indicated that simple carbohydrates bind calcium ions only weakly unless they can provide sites having three or more hydroxyl groups in a suitable geometric arrangement for coordination to calcium[1–3]. Our results in aqueous solutions are in complete accord with these studies as regards the weak interactions of calcium with lactose, galactose, and *myo*-inositol. Thus, in a reaction of the type $A+B=AB$ (where AB denotes the complex), simple mass-action considerations show that, for the experimental heats to have varied in the observed, nearly linear manner, the upper limit of the stability constants would have values less than 1. A necessary consequence is small, positive, standard free-energy changes and (in view of the experimental heats) negative entropy changes. Therefore, this observation indicates that these interactions in aqueous solution are enthalpic in origin. If, as suggested by Bugg and Cook, binding in aqueous solution may be attributed to simple substitution of hydroxyl groups for water molecules in the hydration shell of calcium, then the release of water molecules from that shell must be more than compensated by the decrease in degrees of freedom of the sugars on complexation.

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