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A thermodynamic study of the binding of linear and cyclic oligosaccharides to the maltodextrin-binding protein of *Escherichia coli*

James Thomson [a,b], Yufeng Liu [b], Julian M. Sturtevant [b,*], Florante A. Quiocho [c]

[a] *Agouron, San Diego, CA 92121, USA*
[b] *Departments of Chemistry and Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA*
[c] *Howard Hughes Medical Institute and Department of Biochemistry, Baylor College of Medicine, Houston, TX 77030, USA*

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Abstract

Isothermal titration calorimetric (ITC) studies over a range of temperatures of the binding of maltose, maltotriose, maltotetraose and β-cyclodextrin to the maltodextrin-binding protein (MBP) of *Escherichia coli* are reported. The binding constants of maltose, maltotriose and β-cyclodextrin are not very different, namely 8.7×10^5, 13.0×10^5 and 2.55×10^5 M^{-1}, respectively at 25°C. The calorimetric data obtained with maltotetraose cannot be interpreted in terms of a definite binding constant. The binding of maltose and maltotriose is endothermic with a large entropy increase while that of β-cyclodextrin is exothermic, with a smaller entropy increase. The binding of maltotetraose was endothermic or exothermic depending on the temperature. © 1998 Elsevier Science B.V.

Keywords: Escherichia coli; Maltose; Entropy

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1. Introduction

In this paper, we report isothermal titration calorimetric (ITC) studies of the binding to the maltodextrin-binding protein (MBP) of maltose, maltotriose, maltotetraose and β-cyclodextrin. MBP is a 40,600 Da protein which is involved in the active transport of maltodextrins and resulting chemotaxis in *Escherichia coli*. The binding constants for three of these ligands, namely maltose, maltotriose and β-cyclodextrin, lie within the range 2.6×10^5 M^{-1} to 13×10^5 M^{-1} at 25°C. The data obtained with maltotetraose were complex, and could not be interpreted in terms of a single binding constant.

The rates and binding equilibria of several of these ligands have been studied by fluorescence spectroscopy and ^{3}H NMR [1,2]. The three-dimensional structures of MBP alone and with various

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Abbreviations: DSC, differential scanning calorimetry; ITC, isothermal titration calorimetry; ABP, L-arabinose binding protein; MBP, maltodextrin binding protein; YHK, yeast hexokinase; ΔH_{cal}, calorimetric enthalpy change; ΔH_{vH}, van't Hoff enthalpy change; N, number of binding sites per molecule; K_B, binding constant

[*] Corresponding author. Department of Chemistry, Yale University, P.O. Box 208107, New Haven, CT 06520-8107, USA. Tel.: +1-203-432-3997; fax: +1-203-432-6144.

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bound α(1–4)-linked linear and cyclic maltodextrins have been determined by X-ray crystallography and analyzed in detail by Spurlino et al. [3] and Sharff et al. [4,5] [J.C. Spurlino, L.E. Rodseth and F.A. Quiocho, unpublished data]. The protein is composed of two globular domains of unequal size, separated by a deep cleft wherein the sugars are bound. The linear maltodextrins are buried in the cleft and held in place, with the reducing group at the bottom of the cleft, by extensive systems of hydrogen bonds and van der Waals interactions involving residues in both domains. There is evidence from ^{3}H NMR [2] that the linear ligands longer than maltose may also have an additional binding mode involving the middle of the sugar molecule at the bottom of the cleft. This 'middle binding mode' is not observed in any of the crystallographic analyses of the complexes of MBP with linear oligosaccharides. A hinge-bending motion between the two domains modulates access to and from the binding site cleft [4]. In the ligand-free 'open form' structure, the two domains are far apart and the cleft very much accessible, whereas, in the 'closed form' structures with bound linear maltodextrins, the two domains are much closer and engulf the saccharides almost completely.

MBP also binds cyclic ligands such as α-cyclodextrin (cyclomaltohexaose) and β-cyclodextrin (cyclomaltoheptaose) with binding constants similar to those of the linear ligands [1]. The binding of these cyclic molecules is also mediated by hydrogen bonds and van der Waals forces, but does not lead to closure of the binding cleft [5].

2. Materials and methods

2.1. Materials

MBP was isolated and purified according to a previously described protocol [3]. Although purified MBP has been previously shown to be free of bound oligosaccharide [1], the preparation used in this study (150 mg MBP in 300 ml buffer) was extensively dialyzed against 4 l of 10 mM Tris pH 7.2, 0.02% azide over at least 10 days in the cold room, the dialysis solution being changed once a day. The MBP was concentrated to about 10 mg/ml and dialyzed for 4 days with three changes of 500 ml of buffer (15 mM phosphate plus 50 mM KCl at pH 7.20). The final MBP concentration was determined by quantitatively diluting the MBP and measuring the absorbance of the diluted solution at 280 nm. The extinction coefficient used was 1.93 mg/(ml cm). The sugar solutions were all made by weight with anhydrous sugars, and the sugar concentrations were approximately 15–20 times higher than that of the MBP. The MBP solution was placed in the cell of the titration calorimeter and the sugar solution in the syringe. D-maltose was purchased from Aldrich, maltotriose and β-cyclodextrin from Calbiochem, and maltotetraose from Boehringer Mannheim. These materials were used without further purification.

2.2. Calorimetry

All calorimetric titrations were carried out with the Omega calorimeter from MicroCal (Northampton, MA) [6,7]. In the titrations, the protein concentration in the calorimeter cell varied from 0.15 to 0.24 mM and the concentration of the sugar in the micropipette from 3.1 to 5.3 mM. The raw data, illustrated in Fig. 1, were analyzed by the software supplied with the calorimeter.

2.3. van't Hoff analysis

The binding constant data for maltose, maltotriose and β-cyclodextrin were analyzed in terms of the integrated van't Hoff equation:

$$\ln\frac{K_B}{K_r} = \frac{\Delta H_r - T_r \Delta C_p}{R} \times \left(\frac{1}{T_r} - \frac{1}{T}\right) + \frac{\Delta C_p}{R}\ln\frac{T}{T_r} \qquad (1)$$

including a temperature-independent ΔC_p. A reference temperature, T_r, was arbitrarily selected, and values for K_r and ΔH_r at that temperature and ΔC_p served as variable parameters with which to minimize the standard deviation between ln K_B (observed) and ln K_B (calculated). In all cases, it was possible to carry the calculation until the standard deviation in ln K_B did not change by more than 10^{-7} when the parameters were changed by $\pm 10^{-5}$

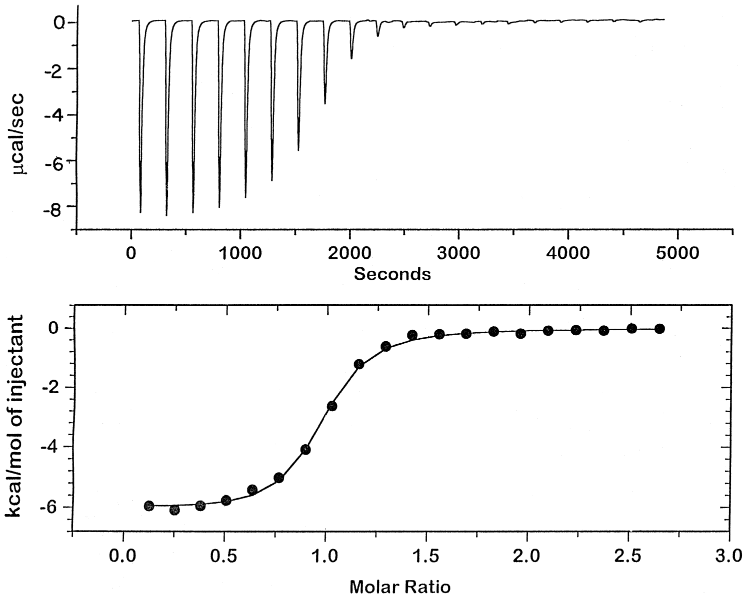

Fig. 1. Experimental data for the titration of 1.322 ml of 0.209 mM MBP with 5.27 mM β-cyclodextrin at 7.0°C. The upper panel shows the peaks generated by 20 injections of 7.00 μl each, and the lower panel shows the results of curve fitting to a single-site model by the software supplied with the calorimeter.

in ln K_B, ± 0.1 cal mol^{-1} in ΔH_{vH} and ± 0.01 cal K^{-1} mol^{-1} in ΔC_p.

3. Results and discussion

Fig. 1 shows the experimental data for a titration of MBP with β-cyclodextrin at 7°C. The upper panel shows the trace recorded for each of 20 7.00-μl injections made at 4-min intervals, and the lower panel shows the fit of each integrated enthalpy change to a titration curve calculated on the basis of one binding site per MBP molecule. The results obtained by this curve fitting were $N = 0.992 \pm 0.006$ binding sites per molecule, $K_B = (3.51 \pm 0.32) \times 10^5$ M^{-1} and $\Delta H_{cal} = -5.99 \pm 0.05$ kcal mol^{-1}.

The results of the titration of MBP with maltose, maltotriose and β-cyclodextrin are listed in Tables 1–3, respectively. The raw titration data for maltotetraose could not be reduced to results of this sort by

Table 1
The binding of maltose to MBP

Temp. (°C)	N	$K_B \times 10^{-5}$ (M^{-1})	ΔH_{cal} (kcal mol^{-1})	$\Delta G°$ (kcal mol^{-1})	$\Delta S°$ (cal K^{-1} mol^{-1})
7.0	1.11	3.36	6.24	−7.08	47.5
7.5	1.06	3.08	6.35	−7.05	47.7
15.0	1.10	5.16	4.78	−7.53	42.7
15.0	1.07	5.30	4.82	−7.55	42.9
20.0	–	2.6[a]	–	–	–
25.0	1.07	8.10	2.82	−8.06	36.5
25.0	1.07	9.30	2.75	−8.14	36.5
35.0	1.06	17.0	0.534	−8.78	30.2
35.0	1.06	22.0	0.573	−8.94	30.9
Mean	1.07 ± 0.01				

$\Delta H_{cal} = 7.86 - 0.207\ t(°C) \pm 0.097$ kcal mol^{-1}.

[a] Miller et al. [1].

Table 2
The binding of maltotriose to MBP

Temp. (°C)	N	$K_B \times 10^{-5}$ (M^{-1})	ΔH_{cal} (kcal mol^{-1})	$\Delta G°$ (kcal mol^{-1})	$\Delta S°$ (cal K^{-1} mol^{-1})
6.8	1.13	4.37	4.87	−7.23	43.2
10.0	1.10	4.93	4.65	−7.37	42.5
15.0	1.09	6.78	4.17	−7.69	41.2
20.0	–	29[a]	–	–	–
25.0	1.09	13.0	2.33	−8.34	35.8
Mean	1.10 ± 0.01				

$\Delta H_{cal} = 6.03 - 0.143$ t(°C) ± 0.213 kcal mol^{-1}.
[a] Miller et al. [1].

the software supplied with the Omega calorimeter. These data will be discussed in a later section.

In each of the tables the first column gives the temperature of the ITC experiment. The number of binding sites, N, per protein molecule is listed in the second column, with the mean value listed at the bottom of the column, and the value of the binding constant, K_B in M^{-1}, in the third column. Also included are binding constants reported by Miller et al. [1] based on measurements at 20°C and pH 7.4 by stopped-flow fluorescence spectroscopy of the binding and dissociation rates of maltose, maltotriose and β-cyclodextrin. There is reasonable agreement with our data for maltose (Table 1) and β-cyclodextrin (Table 3) considering the very different time scales involved. The fluorescence measurements involved binding rate constants of approximately 10^{-7} M^{-1} s^{-1} whereas the ITC procedure allowed 240 s for the signal caused by each injection of titrant to return to the baseline. The disagreement seen for maltotriose (Table 2) reflects a dissociation rate indicated by the fluorescence measurements less than one-tenth those found for maltose and β-cyclodextrin.

The calorimetrically determined values for the enthalpy of binding, ΔH_{cal}, in kcal mol^{-1} are listed in the fourth column of each table, and the calculated values for the standard free energies of binding and the standard entropies of binding in the fifth and sixth columns of the tables. The least squared expression for ΔH_{cal} is given at the bottom of each table, with the standard deviation of the individual

Table 3
The binding of β-cyclodextrin to MBP

Temp. (°C)	N	$K_B \times 10^{-5}$ (M^{-1})	ΔH_{cal} (kcal mol^{-1})	$\Delta G°$ (kcal mol^{-1})	$\Delta S°$ cal (K^{-1} mol^{-1})
7.0	0.99	3.51	−5.99	−7.11	4.0
10.0	1.00	3.13	−5.57	−7.12	5.5
10.0	1.00	3.39	−5.56	−7.16	5.7
15.0	0.94	3.03	−4.65	−7.23	8.9
15.0	0.99	3.00	−4.63	−7.22	9.0
20.0	–	4.8[1]	–	–	–
25.0	0.93	2.49	−2.99	−7.36	14.7
25.0	0.96	2.52	−2.98	−7.37	14.7
25.0	0.92	2.63	−2.76	−7.39	15.5
35.0	0.86	2.37	−1.54	−7.58	19.6
35.0	0.85	2.36	−1.59	−7.58	19.4
Mean	0.94 ± 0.02				

$\Delta H_{cal} = -7.09 + 0.162$ t(°C) ± 0.128 kcal mol^{-1}.
[a] Miller et al. [1].

points. Data for maltotriose at 30°C and above were subject to high uncertainty because of the small value for ΔH_{cal}.

In a recent publication primarily devoted to differential scanning calorimetry (DSC) study of MBP alone and in the presence of maltose, Novokhatny and Ingham [8] include data obtained at pH 7.4 by mixing flow calorimetry for the enthalpy of binding of maltose to MBP at 25°C. The mean of nine experiments was -1.0 kcal mol^{-1} with a standard deviation of ± 0.6 kcal mol^{-1}; each experiment involved evaluation of the small difference between two relatively very large enthalpy vs. time curves.

In the cases of maltose and maltotriose, the values for N are slightly higher than one, for reason(s) we do not understand. Deviations of this magnitude, with decreases as the temperature rises, have been observed in many of our previous titrations of proteins with ligands. With the cyclic ligand, N starts at the expected value of unity and also decreases with rising temperature.

The binding constants for all three ligands start at similar values at low temperature, but those for the linear ligands *increase* rapidly with increasing temperature while those for the cyclic ligand *decrease* more slowly with increasing temperature. A corresponding striking difference is seen in the values for the enthalpy of binding, those for the linear ligands being endothermic and those for β-cyclodextrin being exothermic. It is also surprising that the heat capacity changes for the two types of ligands have opposite signs. All the enthalpies are equal to zero at 40° ± 4°C.

It is evident, in the temperature range studied, that the binding of both maltose and maltotriose is entropy driven with unfavorable enthalpy changes. In contrast, the binding of β-cyclodextrin is accompanied by both favorable entropy and favorable enthalpy changes, with the former exceeding the latter at temperatures above 20°C.

Sharff et al. [5] concluded on the basis of structures determined by X-ray crystallography that there are 64 ordered water molecules in the MBP cleft when β-cyclodextrin is bound as compared to only 21 in unliganded MBP. This change would be expected to be accompanied by a large decrease in entropy. Furthermore, according to Sharff et al. [4], there are 35 ordered water molecules in the cleft when maltose is bound. It is evident that the binding of maltose and other maltodextrins to MBP leads to large, at present unexplained, increases in entropy.

The existence of a binding cleft seems to be a characteristic of sugar binding proteins. X-ray crystallography has shown similar structures for yeast hexokinase (YHK) [9] and for the L-arabinose binding protein (ABP) of *E. coli* [10]. Despite this similarity in apparent mechanism of binding, these proteins all have very different changes in thermodynamic properties associated with sugar binding. This is shown in Table 4 with data for 25°C. The binding of glucose to YHK is evidently the weakest binding and it is entirely entropy driven. The bindings to ABP are strongly enthalpy driven with unfavorable entropy changes. As noted above, the binding of maltose to MBP is entirely entropy driven, while that of β-cyclodextrin has both favorable entropy and favorable enthalpy changes. We are not able at present to give useful rationalizations of these confusing thermodynamic data, especially in view of the apparent similarity of the mechanisms involved.

In recent papers, we have noted and discussed significant discrepancies between enthalpies derived

Table 4
Comparison of the thermodynamics of sugar binding to MBP, L-arabinose binding protein (ABP) and yeast hexokinase (YHK)

Protein	Sugar	$K_{\mathrm{B}} \times 10^{-5}$ M^{-1}	$\Delta G°$ (kcal mol^{-1})	ΔH_{cal} (kcal mol^{-1})	ΔC_{p} (cal K^{-1} mol^{-1})	$\Delta S°$ (cal K^{-1} mol^{-1})
MBP	Maltose	8.70 ± 1.45	-8.10 ± 0.09	$+2.79 \pm 0.10$	-207 ± 24	36.5 ± 0.2
MBP	β-Cyclodextrin	2.55 ± 0.09	-7.37 ± 0.03	-2.91 ± 0.13	$+162 \pm 18$	15.0 ± 0.2
ABP	L-Arabinose[a]	24.0 ± 2.0	-8.70 ± 0.05	-15.3 ± 0.5	-436 ± 47	-22.1 ± 0.5
ABP	D-Galactose[a]	17.1 ± 2.0	-8.50 ± 0.07	-15.0 ± 0.2	-376 ± 14	-21.8 ± 0.2
YHK	D-Glucose[b]	0.057 ± 0.004	-5.12 ± 0.04	$+0.18 \pm 0.83$	$+50 \pm 110$	$+14.7 \pm 0.8$

[a] Fukada et al. [14].
[b] Takahashi et al. [15].

from the temperature variation of equilibrium constants and calorimetrically measured enthalpies [11–13]. It has turned out that in nearly all cases of reversible reactions in solution, the usual application of the van't Hoff equation:

$$\frac{d \ln K}{dT} = \frac{\Delta H_{vH}}{RT^2} \tag{2}$$

in which ΔH_{vH} has been assumed to be independent of temperature, is incorrect. Nonlinear least squaring in terms of the integrated van't Hoff equation including a temperature-independent ΔC_p leads in these cases to enthalpy values showing a significant variation with temperature. Analysis in terms of temperature-dependent van't Hoff enthalpies is described in the section on experimental procedures.

In our three previous publications on discrepancies between ΔH_{vH} and ΔH_{cal}, we have been unable to account for the discrepancies in any quantitative manner, concluding that rapid processes not explicitly included in the simple chemical equation for the reaction must be involved. Processes such as changes in the binding of solvent or buffer ions or molecules might well make significant contributions which vary with temperature. We could see no basis for a definitive selection of the 'true' enthalpy value for a given reaction.

The application of this van't Hoff analysis to the data in Tables 1–3 leads to further striking differences between the linear and cyclic ligands, as shown in Table 5. For maltose, the ratio $\Delta H_{vH}/\Delta H_{cal}$ ranges from 1.17 at 6.8°C to 25.1 at 35°C, but is approximately constant at 0.65 for β-cyclodextrin. The van't Hoff enthalpies for the linear ligands increase with increasing temperature whereas the calorimetric enthalpies decrease with increasing temperature; for the cyclic ligand both enthalpies increase with increasing temperature. The van't Hoff analysis gives calculated values of the binding constant, K_B, which differ from the observed values by the percentage standard deviations listed at the bottom of the table.

Table 5
Differences between the linear and cyclic ligands as shown by van't Hoff analysis

Temp. (°C)	$\Delta H_{vH}/\Delta H_{cal}$		
	Maltose	Maltotriose	β-Cyclodextrin
7[a]	1.17	1.51	0.634
10	–	1.79	0.629
15	1.95	2.36	0.647
25	4.28	5.55	0.691
35	25.1	–	0.645
ΔH_{vH} at 0°C (kcal mol^{-1})	5.52	5.25	-4.50
ΔC_p (vH) (kcal K^{-1} mol^{-1})	0.256	0.307	0.100
ΔC_p (cal) (kcal K^{-1} mol^{-1})	-0.207	-0.143	$+0.162$
S.d. in $K_B \times 10^{-5}$ (M^{-1})	± 1.45	± 1.60	± 0.09

[a] Actually 6.8°C for maltotriose.

In the present cases, there are two known equilibria which might contribute to the simple binding equilibrium:

$$P + L \rightleftarrows PL;\ K_1 = \frac{[PL]}{[P][L]} \tag{3}$$

where P is the protein and L is the ligand, namely the conformational equilibrium

$$P\,(\text{open cleft}) = P\,(\text{closed cleft});\ K_2 = \frac{[P_c]}{[P_o]} \tag{4}$$

and the anomeric equilibrium:

$$L_\alpha \rightleftarrows L_\beta;\ K_2 = \frac{[L_\alpha]}{[L_\beta]} \tag{5}$$

The approximately normal behavior of β-cyclodextrin in the titrations [e.g., ΔC_p (vH) and ΔC_p (cal) both positive], could then stem from the facts that the binding of β-cyclodextrin does not cause cleft closure, and that it does not have an anomeric equilibrium.

Gehring et al. [2], on the basis of ^{3}H NMR measurements, estimate that the $t_{1/2}$ for the conversion of α-maltose to β-maltose is approximately 3.5 h at 10°C, pH 7.4. With maltose to MBP ratios less than one, $t_{1/2}$ is, according to their estimates, of the order of several days. Since the titrations with maltose and maltotriose showed no abnormalities either above or below molar ratios of one, we can conclude that the anomeric equilibria per se make no obvious contribution in these titrations. The unusual behavior noted in the maltotetraose titrations might involve the anomeric equilibrium.

The calorimetric results for maltotetraose at 7°C and 25°C are shown in Fig. 2. The upper curve shows the heat absorbed in each of twenty 8 μl

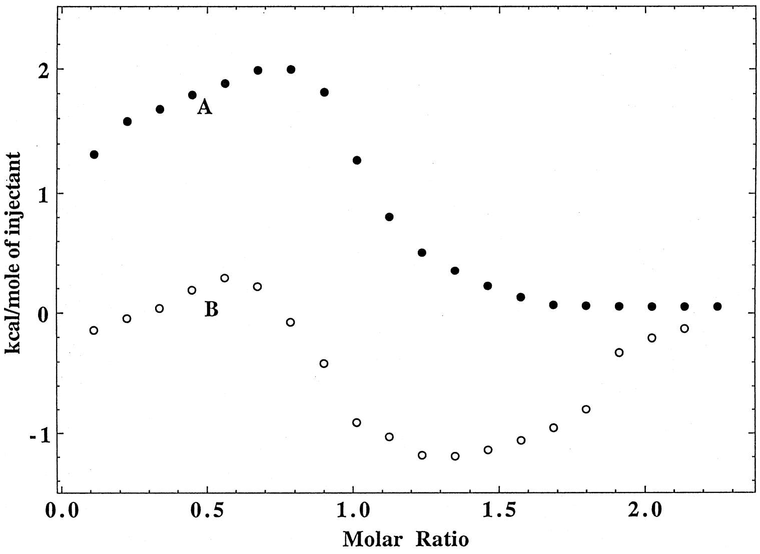

Fig. 2. Calorimetric data observed in the titration of 0.241 mM MBP with 4.47 mM maltotetraose at (A) 10°C and at (B) 25°C. The kcal per mole of tetraose produced by each 8 μl injection of the sugar is plotted as a function of the ratio of moles of injectant to moles of protein in the cell of volume 1.322 ml. The injections were made at 4-min intervals, and there was no indication of any abnormal delay of the temperature of the cell in returning to the baseline.

injections at 7°C of 4.47 mM ligand into 1.322 ml of 0.241 mM MBP, with 4-min intervals between the injections. The lower curve shows the results of nineteen 8 μl injections at 25°C with the same ligand and protein concentrations. It is immediately evident that these binding processes are quite complex. Fig. 3 presents the same data in the form of total enthalpy change per mole of MBP as a function

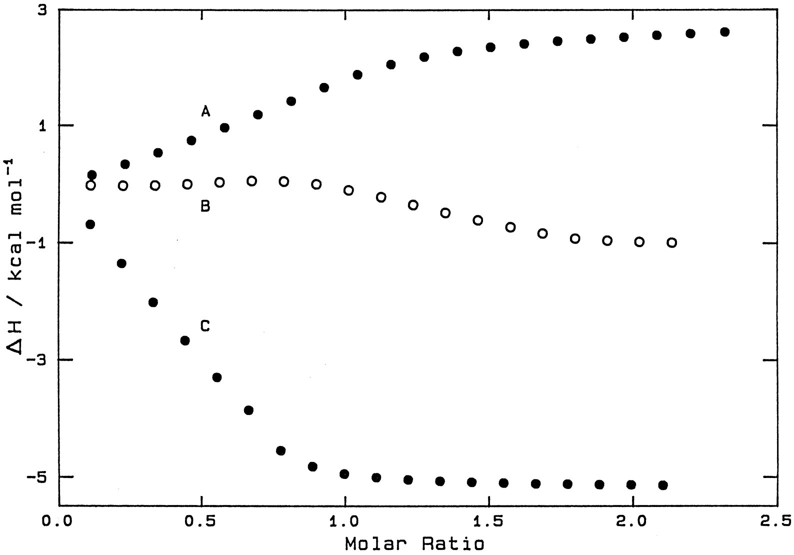

Fig. 3. Plots of the total enthalpy change per mole of MBP protein produced by each injection of sugar into the protein. Curve A, maltotetraose at 10°C, curve B, maltotetraose at 25°C, curve C, β-cyclodextrin at 7°C. The concentrations and volumes were as listed for Fig. 1 except that the concentration of the β-cyclodextrin was 4.40 mM.

of the molar ratio, and includes data for the titration with β-cyclodextrin at 7°C (cf. Fig. 1, lower panel). The fact that the titration of the cyclic ligand reaches its endpoint at a molar ratio slightly greater than one, as is to be expected with a normal titration, while those with maltotetraose continue well beyond unit molar ratio further emphasizes the abnormality of these titrations.

Acknowledgements

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