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A DIFFERENTIAL SCANNING CALORIMETRIC STUDY OF THE CONFORMATIONAL TRANSITIONS OF SEVERAL KINDS OF (1→6) BRANCHED (1→3)-β-D-GLUCANS IN A MIXTURE OF WATER AND DIMETHYLSULFOXIDE

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SUMMARY

The thermal conformational transitions of seven kinds of (1→6) branched (1→3)-β-D-Glucans were comparatively studied in a water-dimethylsulfoxide mixture containing 16.47 weight % of water by high-sensitivity differential calorimetry(DSC). The DSC curves for schizophyllan, scleroglucan, and PS-1426 glucan, showed two endothermic peaks which were assigned to an internal change of triple helix and a triple helix-single coil transition, respectively. The differences in transition temperature and enthalpy of the transitions between these glucans suggested the variation of the thermal stability of triple helix of this type of glucans. It was found that the transition temperatures are correlated to the degree of branching for these glucans, suggesting that the side chain glucose contributes to the thermal stability of triple helix of (1→6) branched (1→3)-β-D-glucans. The glucans(T-4-N and T-5-N) isolated from fruit bodies of Dictyophora indusiata yield multiple peaks in the DSC curves, suggesting the heterogeneity in composition of the samples.

INTRODUCTION

In the earlier work[1], we have shown that schizophyllan, a (1→6) branched (1→3)-β-D-glucan produced by fungus Schizophyllum commune, undergoes two conformational transitions, which are observed as two endothermic peaks by differential scanning calorimetry(DSC), in water-dimethylsulfoxide(DMSO) mixtures over most of the range of solvent compositions. These transitions are assigned to an internal change of triple helix[2] and triple helix-single coil transition[3], respectively. In the present study we extend our DSC study to include the other six kinds of branched (1→3)-β-D-glucans whose glucose residues of the main chain are substituted by single, O-6-linked, D-glucosyl residues to various extent.

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MATERIALS AND METHODS

Samples

Table 1 summarizes the sample designation, sources of isolation, degree of branching, and molecular weight of fungal (1→6) branched (1→3) glucans used in this study. The degree of branching was determined from the molar ratio of glucose and gentiobiose which were released by hydrolysis with exo-(1→3)-β-D-glucanase of the glucans.

Schizophyllan(M-2)[4] was provided by Taito Co.Ltd. Low molecular weight scleroglucan samples(5913-1 and SCL-5) were prepared by sonication from a native sample[5] kindly donated by Prof. M. Takagi of University of Osaka Prefecture, and from a commercial product(Actigum CS 6). PS-1426 glucan(PN-35)[6] was provided by Takara Shuzo Co. Ltd. The isolation of T-4-N and T-5-N from fruit bodies of *Dictyophora indusiata* Fisch. are described previously[7,8]. T-4-N was used for DSC measurements after partial depolimerization by sonication. H-3-B is isolated from hot water extract of the fruit bodies of *Cryptoporus volvatus*. A weighed amount of the polysaccharide was dissolved in DMSO containing 16.47 weight % of water at 25°C and the solution was left standing for at least 1 day before measurements.

TABLE 1. Samples used in this study

Sample	Fungus	Degree of Branching[a]	$M_w^b/10^4$
Schizophyllan			
M-2	*Shizophyllum commune*	0.355	43.7
Scleroglucan			
5913-1	*Sclerotinia sclerotiorum*	0.425	22.6
SCL-5	*Sclerotium rolfsii*	0.311	21.9
PS-1426			
PN-35	*Pseudoplectania nigrella*	0.415	34.7
T-4-N	*Dictyophora indusiata*	0.375	83.3
T-5-N	*Dictyophora indusiata*	0.30	100
H-3-B	*Cryptoporus volvatus*	0.274	—

[a]Defined as (mole of side chain glucose/mole of mainchain glucose)

[b]Molecular weight.

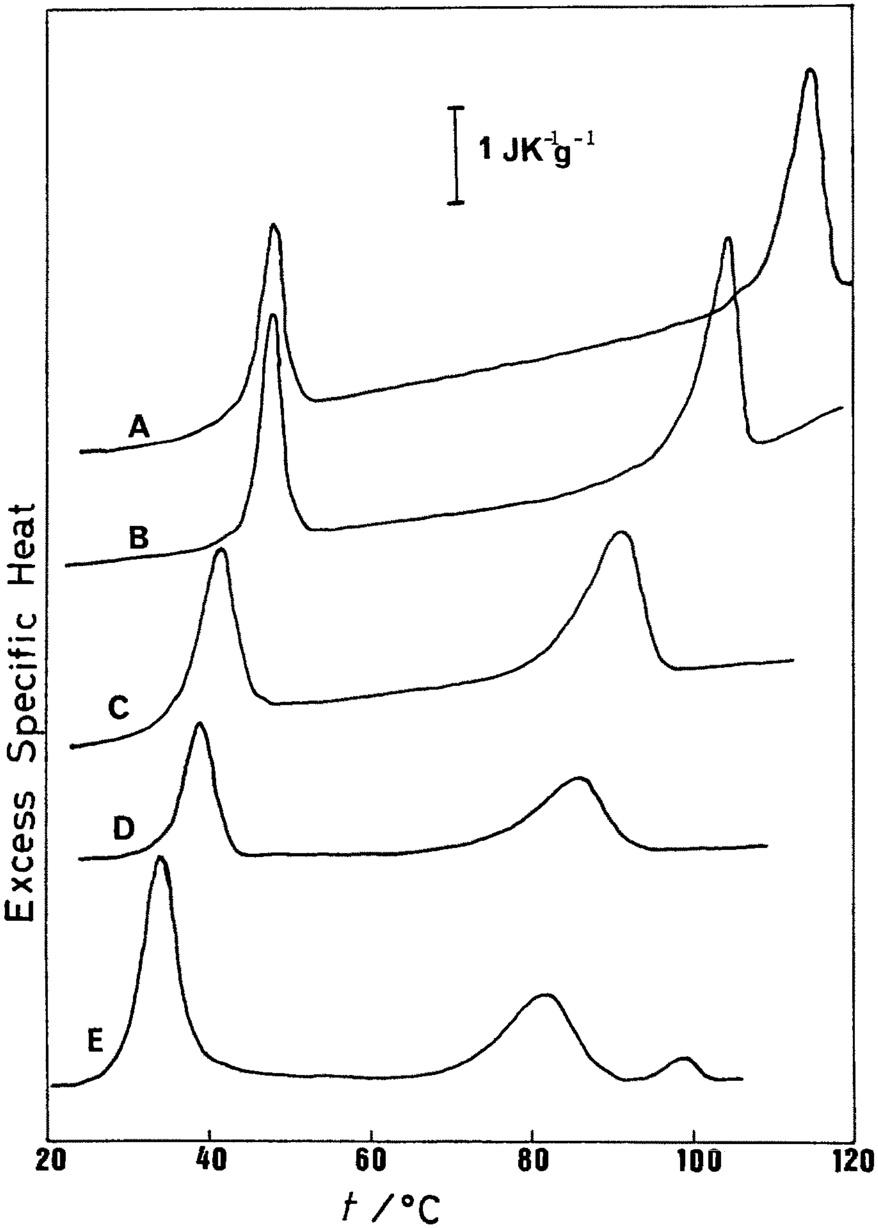

Fig. 1. Tracing of DSC curves for 5913-1(A), PN-35(B), M-2(C), SCL-5(D) and H-3-B(E) in the mixture of DMSO-water containing 16.47 weight % of water. No significant noise was visible in the original recordings.

DSC Measurements

DSC measurements were performed with DASM-4 scanning microcalorimeters at a scan rate of 0.5 K /min. The calorimetric enthalpy ,Δh, was evaluated by planimeter integration of the DSC curves. Chemical baselines were drown according to the method of Takahashi and Sturtevant[9].

RESULTS AND DISCUSSION

Fig. 1 shows the DSC curves for the conformational transitions of scleroglucan(5913-1). PS-1426(PN-35), schizophyllan(M-2), scleroglucan(SCL-5) ,and H-3-B. Except for the case of H-3-B, two transitions are observed. As established in the earlier study[1] on schizophyllan, the transition at lower temperature can be assigned to an internal change of the triple helix associated with a change in side chain orientation involving surrounding solvent molecules[2]. The transition at higher temperature is due to a triple helix-single coil transition[3].

TABLE 2. The temperature of maximal excess heat capacity (t_p) and specific enthalpy (Δh) for the conformational transitions of (1→6) branched (1→3)-β-D-glucans in the water-DMSO mixture containing 16.47 % of water.

Sample	C_0^a	1st transition t_p (°C)	1st transition Δh (J/g)	2nd transition t_p (°C)	2nd transition Δh (J/g)
M-2	9.05	41.4	10.4	92.0	12.3
5913-1	7.85	47.8	5.60	114.0	10.2
SCL-5	7.58	38.0	6.43	86.0	7.20
PN-35	7.45	47.8	7.70	104.3	14.1
H-3-B	8.08	33.9	8.28	81.3 98.6	5.50 0.78
T-4-N	5.64	32.5 51.4	1.02 5.47	72.5 106.9	0.58 5.76
T-5-N	8.04	32.0 37.4 43.5 50.2	Total 9.38	72.6 84.0 94.9 106.8	Total 6.71

[a]Polysaccharide concentration.

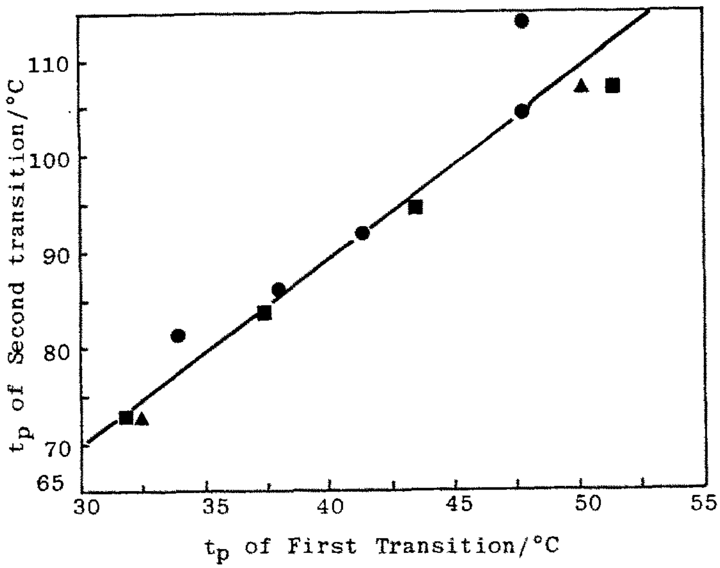

Fig. 2. Correlation between t_ps, the temperatures of maximal excess heat capacity, of first and second transitions: (●) M-2, 5913-1, SCL-5, PN-35 and H-3-B, (▲) T-4-N, (■) T-5-N. The solid line was obtained by linear regression analysis using all of the plots.

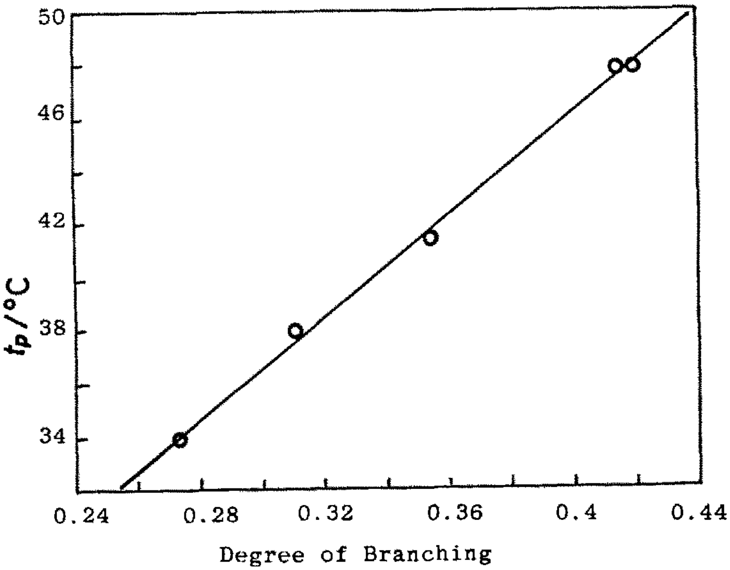

Fig. 3. Effect of the degree of branching on the transition temperature(t_p) of the conformational transition at lower temperature of (1→6) branched (1→3)- β-D-glucans in the mixture of DMSO-water containg 16.47 % of water. The solid line was obtained by linear regression analysis.

Table 2 summarizes the DSC data obtained in this study. As shown in Fig.2, plots of the temperature of maximal excess heat capacity, t_p, of the first transition versus of the second transition give a linear relation, suggesting that the same structural origin determines these two conformational transitions. In Fig. 3, the t_p of the first transition is plotted against the degree of branching. The transition temperature increases with increasing the branching. It is evident that the t_p of the second transition is correlated to the degree of branching as well. Thus it is considered that the side chain glucose contributes to the thermal stability of triple helix of (1→6) branched (1→3)-β-D-glucans at the solvent condition used in this study. Since these transitions are strongly affected by the solvent compositions[1], we should investigate the thermal stability of these glucans in water-DMSO mixtures over the entire composition range.

The specific enthalpies of both transitions vary between the glucans. Although this variation should be attributed to some structural difference of the glucans, no significant correlation between Δh and the degree of branching has been found.

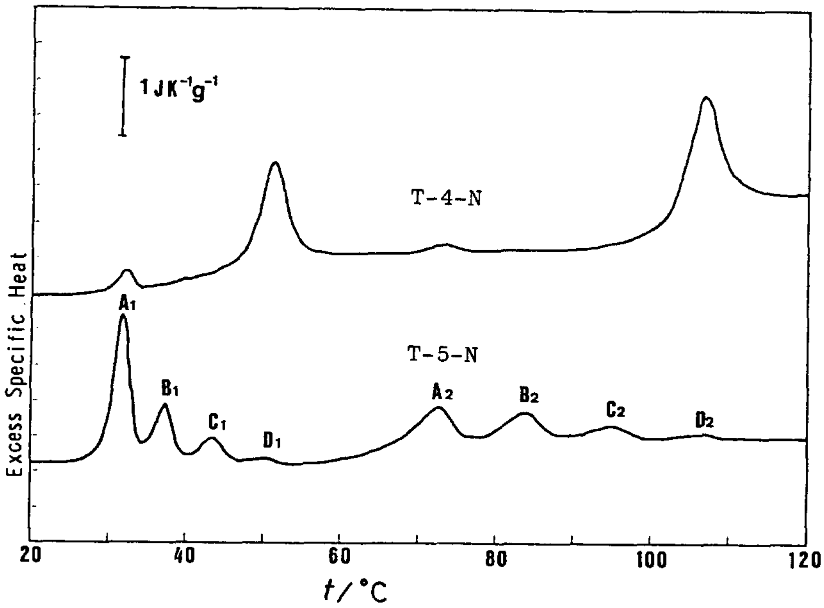

Fig. 4. Tracing of DSC curves for T-4-N and T-5-N in the mixture of DMSO-water containg 16.47 weight of water. No significant noise was visible in the original recordings.

The DSC curves obtained for T-4-N and T-5-N show multiple peaks in the curves (Fig. 4). It can be considered that each one of the transitions (A1, B1, C1 and D1) at low temperature for T-5-N corresponds to the transition(A2, B2, C2 and D2) at higher temperature, respectively. In the case of T-4-N, two sets of transitions(A1 and A2, and D1 and D2) are seen in the DSC curve. As shown in Fig. 2, four plots for T-5-N and two plots for T-4-N fall on a straight line in the figure. Thus the multiple transitions may be interpreted in terms of heterogeneity in composition of the samples. On the contrast to extracellular polysaccharides such as schizophyllan, scleroglucan and PS-1462, T-4-N and T-5-N are samples isolated from the cell wall of the fruit bodies in which branched (1-3)-βD-glucans construct a number of complex polymeric structure in combination with other material. The result obtained for T-4-N and T-5-N implies the presence of four components of (1→3)-β-D-glucans having different degree of branching in the cell wall and that these four components might be successively extracted in different proportion by 2 % sodium carbonate and 1 mol/dm^3 sodium hydroxide. It can be said that DSC study gives information about not only thermodynamic aspects of the conformational transitions but also homogeneity in primary structure of (1→6) branched(1→3)-β-D-glucans.

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