We previously presented the cloning, heterologous expression, and characterization of a novel multidomain endoxylanase from _Arthrobacter_ sp. GN16 isolated from the feces of _Grus nigricollis_. Molecular and biochemical characterization studies indicate that the glycoside hydrolase (GH) family 10 domain at the N-terminus of the multidomain xylanase (rXynAGN16L) is a low-temperature-active endoxylanase. Many low-temperature-active enzymes contain regions of high local flexibility related to their kinetic and thermodynamic properties compared with mesophilic and thermophilic enzymes. However, the thermodynamic property of low-temperature-active enzymes is typically characterized as a low-temperature-active enzyme.}

**Introduction**

Xylan is the most common hemicellulosic polysaccharide. The structure of xylan contains substitute groups of acetyl, 4-O-methyl-D-glucuronosyl, and α-arabinofuranosyl residues that are linked to the backbone of β-1,4-linked xylopyranose units. Endoxylanases (endo-β-1,4-xylanases; EC 3.2.1.8) are glycosidasases that can catalyze the endohydrolysis of the xylan backbone; therefore, these enzymes have been extensively applied in many industries, including food, feed, energy, textile, paper, and pulp.

More than 80% of the Earth’s biosphere, including oceans, alpine, polar regions, and caves, is permanently cold, and the remaining biosphere comprises seasonally cold environments. The highest global proportion of biomass is generated at low temperatures. Low-temperature-active enzymes exhibit high catalytic activity at low temperatures and can be potentially used in the cleaning, food, and feed industries; moreover, biotechnological processes are performed at cold temperatures to decrease economical cost and/or prevent product denaturation.

In recent years, low-temperature-active xylanases have elicited much attention. The use of low-temperature-active xylanases can effectively improve dough properties and final bread volume (up to 28%). Some studies have investigated the catalytic adaptation to low temperatures of xylanases. The majority of low-temperature-active xylanases belong to the glycoside hydrolase (GH) family 10.
Arthrobacter sp GN16 isolated from the feces of Grus nigricollis. In the previous study, the GH 10 catalytic domain at the N-terminus of the multidomain xylanase (rXynAGN16L) was expressed in Escherichia coli and the purified recombinant enzyme was characterized. Biochemical characterization indicates that rXynAGN16L plays a key role in catalysis of xylans and is active at low temperatures. Molecular characterization reveals that the structural adaptation of rXynAGN16L to low temperatures may be ascribed to the surface loop from A57 to Y77 and the decreased salt bridges. Many low-temperature-active enzymes contain regions of high local flexibility related to their kinetic and thermodynamic properties. However, the thermodynamic property of low-temperature-active xylanases, including rXynAGN16L, has rarely been reported. In the present study, the kinetic and thermodynamic properties of rXynAGN16L were determined using different substrates and temperature conditions to completely characterize its activity properties.

**Kinetic characterization**

$K_m$, $V_{max}$, and $k_{cat}$ values of the purified rXynAGN16L toward xylans were determined using our previously described method. The kinetic values of rXynAGN16L were calculated according to the double-reciprocal plots (Fig. 1), and the results are summarized in Table 1. $K_m$, $V_{max}$, $k_{cat}$, and $k_{cat}/K_m$ values of the purified rXynAGN16L toward beechwood xylan increased from 1.42 mg/mL to 1.81 mg/mL, 4.00 μmol/min/mg to 62.89 μmol/min/mg, 3.13/s to 49.17/s, and 2.20 mL/mg/s to 27.17 mL/mg/s, respectively, when the temperature increased from 0°C to 45°C. In addition, the $K_m$ value determined at 30°C was 2.21 mg/mL. $K_m$, $V_{max}$, $k_{cat}$, and $k_{cat}/K_m$ values of the purified rXynAGN16L toward birchwood xylan increased from 1.62 mg/mL to 2.61 mg/mL, 5.31 μmol/min/mg to 65.36 μmol/min/mg, 4.15/s to 51.10/s, and 2.56 mL/mg/s to 19.58 mL/mg/s, respectively, when the temperature increased from 0°C to 45°C. These results implied that rXynAGN16L

![Figure 1. Lineweaver–Burk plots of the purified rXynAGN16L determined using 0.5–10.0 mg/mL xylans as substrates in McIlvaine buffer (pH 5.5).](image-url)

**Table 1. Kinetic characterization of rXynAGN16L.**

| Kinetic parameters | Beechwood xylan | Birchwood xylan |
|--------------------|-----------------|-----------------|
| $K_m$ (mg/mL)      | 0°C  | 10°C  | 20°C  | 30°C  | 45°C  | 0°C  | 10°C  | 20°C  | 30°C  | 45°C  |
|                    | 1.42 | 1.46  | 1.92  | 2.21  | 1.81  | 1.62 | 1.93  | 2.06  | 2.51  | 2.61  |
| $V_{max}$ (μmol/min/mg) | 4.00 | 6.97  | 13.72 | 20.66 | 62.89 | 5.31 | 6.39  | 12.23 | 19.12 | 65.36 |
| $k_{cat}$ (s)      | 3.13 | 5.45  | 10.73 | 16.15 | 49.17 | 4.15 | 5.00  | 9.56  | 14.95 | 51.10 |
| $k_{cat}/K_m$ (mL/mg/s) | 2.20 | 3.73  | 5.59  | 7.31  | 27.17 | 2.56 | 2.59  | 4.64  | 5.96  | 19.58 |
exhibits higher affinity and catalytic efficiency toward beechwood xylan than toward birchwood xylan. The low-temperature-active GH 10 endoxylanases from *Glaciecola mesophila* KMM 241, *Flavobacterium johnsoniae*, and goat rumen contents also exhibit similar phenomenon. 5,11,12 Many low-temperature-active enzymes present higher *Km* values than their more thermostable homologs. 3 However, the *Km* values of the low-temperature-active endoxylanases isolated from *G. mesophila* KMM 241, *Flavobacterium* sp. MSY2, and goat rumen contents toward beechwood xylan at 30°C are 1.22, 1.8, and 1.8 mg/mL, respectively; 8,11,12 these results are similar to the values of rXynAGN16L but lower than the values of many thermostable xylanases reviewed in a previous study. 5,15 In addition, the *Km* values of rXynAGN16L determined at low temperatures were higher than those determined at intermediate temperatures; this finding indicated that rXynAGN16L exhibits a high affinity toward xylan at low temperatures. The low-temperature-active endoxylanase from *G. KMM 241 also exhibits similar phenomenon. 12 However, the *Km* values determined at 4°C of low-temperature-active endoxylanases from *F. johnsoniae* and goat rumen contents toward xylans are higher than those determined at 30°C. 5,11

Many low-temperature-active enzymes have higher *kcat* values than their more thermostable homologs. 3 However, *kcat* values of the low-temperature-active endoxylanases isolated from *G. mesophila* KMM 241 and *F. johnsoniae* toward beechwood xylan at 30°C are 69 and 10.70/s, respectively; 5,11,12 in addition, the value of rXynAGN16L is 16.15/s. The *kcat* values of the 3 low-temperature-active endoxylanases are lower than the values of many thermostable xylanases reviewed in a previous study. 5,15

### Thermodynamic characterization

Activation energy (*Ea*), free energy of activation (Δ*G*), enthalpy of activation (Δ*H*), entropy of activation (Δ*S*), and temperature coefficient (*Q* ~ 0) of rXynAGN16L were calculated using the equations described in a previous study. 16 The Arrhenius plots (Fig. 2) show that the *Ea* values for the hydrolysis of beechwood and birchwood xylans by rXynAGN16L were 27.08 and 29.74 kJ/mol, respectively. *Q* ~ 0 values (35°C and 45°C) for the hydrolysis of beechwood and birchwood xylans in McIlvaine buffer (pH 5.5) by rXynAGN16L were 1.39 and 1.44, respectively. Other thermodynamic parameters, including Δ*G* ~ 0, Δ*H*, and Δ*S* at 0°C to 45°C in McIlvaine buffer (pH 5.5) for rXynAGN16L are summarized in Table 2. The Δ*G* ~ 0 and Δ*S* values of the purified rXynAGN16L toward beechwood xylan increased from 64.07 kJ/mol to 67.75 kJ/mol and from −143.80 J/mol/K to −136.21 J/mol/K, respectively, when the temperature increased from 0°C to 45°C; however, the Δ*H* ~ 0 values decreased from 24.81 kJ/mol to 24.44 kJ/mol. The Δ*G* ~ 0 and Δ*S* ~ 0 values of the purified rXynAGN16L toward birchwood xylan increased from 63.43 kJ/mol to 67.65 kJ/mol and from −134.32 J/mol/K to −127.53 J/mol/K, respectively, when the temperature increased from 0°C to 45°C; conversely, the Δ*H* ~ 0 values decreased from 27.47 kJ/mol to 27.09 kJ/mol. In addition, the Δ*S* ~ 0 value for rXynAGN16L toward birchwood xylan was −131.72 at 0°C.

Many low-temperature-active enzymes present lower *Ea* values than thermostable homologs to allow easier conformational changes during catalysis at low temperatures. 3 The *Ea* of the thermostophilic GH 10 endoxylanase from *Bacillus halodurans* TSEV1 for birchwood xylan hydrolysis is 30.51 kJ/mol, 17 which is higher than that required for rXynAGN16L. Furthermore, transition-state theory indicates that an equilibrium exists between the ground-state and transition- or activated-state reactants affected by the magnitude of the

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**Table 2. Thermodynamic characterization of rXynAGN16L**

| Thermodynamic parameters | Beechwood xylan | Birchwood xylan |
|--------------------------|----------------|----------------|
| *Ea* (kJ/mol)            | 27.08          | 29.74          |
| *Q* ~ 0 (35–45°C)        | 1.39           | 1.44           |
| Δ*G* (kJ/mol; 0°C)       | 64.07          | 63.43          |
| Δ*H* (kJ/mol; 0°C)       | 24.81          | 27.44          |
| Δ*S* (kJ/mol/K; 0°C)     | −143.80        | −131.72        |
| Δ*G* (kJ/mol; 10°C)      | 65.19          | 65.40          |
| Δ*H* (kJ/mol; 10°C)      | 24.73          | 27.38          |
| Δ*S* (kJ/mol/K; 10°C)    | −143.00        | −134.32        |
| Δ*G* (kJ/mol; 20°C)      | 65.93          | 66.21          |
| Δ*H* (kJ/mol; 20°C)      | 24.64          | 27.30          |
| Δ*S* (kJ/mol/K; 20°C)    | −140.92        | −132.81        |
| Δ*G* (kJ/mol; 30°C)      | 67.24          | 67.43          |
| Δ*H* (kJ/mol; 30°C)      | 24.56          | 27.22          |
| Δ*S* (kJ/mol/K; 30°C)    | −140.85        | −132.72        |
| Δ*G* (kJ/mol; 45°C)      | 67.75          | 67.65          |
| Δ*H* (kJ/mol; 45°C)      | 24.44          | 27.09          |
| Δ*S* (kJ/mol/K; 45°C)    | −136.21        | −127.53        |
ΔG° barrier between them. The ΔG° of the thermophilic GH 10 endoxylanase from *B. halodurans* TSEV1 for birchwood xylan hydrolysis is 197.65 kJ/mol, the result indicates that more energy is needed for the thermophilic endoxylanase to form the activated complex compared with rXynAGN16L.

**Conclusion**

This study presented the kinetic and thermodynamic properties of the GH 10 endoxylanase rXynAGN16L from *Arthrobacter* sp GN16 isolated from the feces of *G. nigricollis*. The kinetic property of rXynAGN16L is similar to that of some low-temperature-active GH 10 endoxylanases. Moreover, the thermodynamic property indicates that rXynAGN16L is typically characterized as a low-temperature-active enzyme.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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