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Proteinase N-catalysed transesterifications in DMSO–water and DMF–water: preparation of sucrose monomethacrylate

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Abstract

The selective acylation of sucrose was catalysed by proteinase N (from *Bacillus subtilis*) in DMF suspensions, providing 1′-monoesters, and notably, methacryloyl esters. Presence of water up to 7% (vol.) led to faster reactions. Alternatively, a slurry of the enzyme with a minimum volume of DMSO could also be used, despite its ability to denature enzymes. © 2000 Elsevier Science Ltd. All rights reserved.

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Proteases, just like lipases, can be active catalysts in organic solvents.[1–3] In the case of carbohydrates, the efficiency of enzyme catalysed esterifications depends on many factors, and notably, for a given solvent, on the compatibility between the stability of the enzyme and the solubility of the substrates.[4–7] Short-chain esters of sucrose were prepared using crude proteases such as protease N (Amano) in dimethylformamide.[8,9] Using this method, sucrose methacrylates, which are interesting polymerisable derivatives in the context of the use of sucrose as a chemical raw material,[10–12] were obtained in a non-regioselective reaction, whereas a regioselective acylation was observed using the purified protease *subtilisin Carlsberg*.[13]

In this communication, we report the results of our study of the acylation of sucrose catalysed by another crude protease (proteinase N, Fluka), also isolated from *Bacillus subtilis.* The importance of the hydration of the enzyme being a crucial factor when organic solvents are used,[14–16] the effect of water was examined, as well as the possibility to use DMSO as alternative solvent.

Since sucrose monomethacrylates (MMAS) were our main target, we first evaluated proteinase N as a catalyst for the transesterification of trifluoroethyl short esters (butyrate (**1a**), isobutyrate (**1b**), transcrotonate (**1c**), and methacrylate (**1d**), Scheme 1, Table 1) in conditions similar to those used by Riva et al.[8] (DMF suspensions).

The crude enzyme (freeze-dried powder prepared from an aqueous solution adjusted at pH 7.8 with 0.1 M KOH) was added to a sucrose solution in DMF containing the acylating agent (2 equiv.). Preparing

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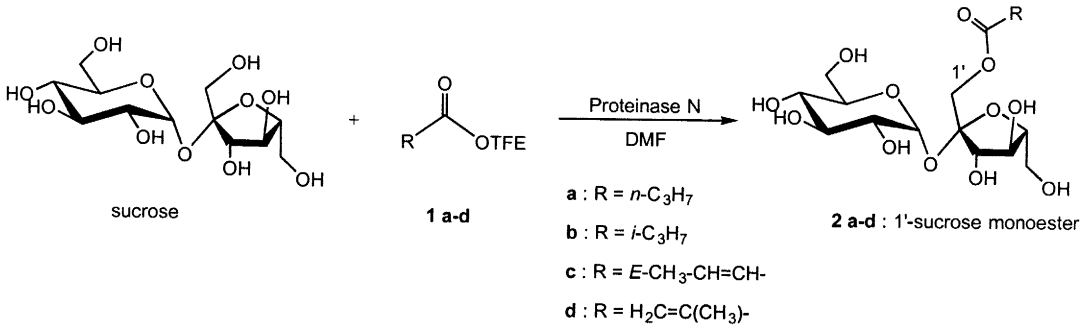


Scheme 1.

Table 1

Sucrose conversion and yield of monoester **2a–d**[a] after 24 h at 45°C for the proteinase N-catalysed reaction of sucrose with trifluoroethyl C_4-esters **1a–d** (2 equiv.) in anhydrous DMF[b]

Acylating agent	**1a**	**1b**	**1c**	**1d**
Conversion/yield (%)[c]	80/76	43/43	11/6	20/15

a. All new compounds gave satisfactory analytical data.
b. Reaction conditions: sucrose : 68 mg ; PN (7.2 U/mg) : 30 mg ; DMF : 0.5 mL ; stirring : 250 rpm.
c. Analytical yields: HPLC Lichrosorb diol column (250x4.6 mm), CH_3CN/H_2O 80/20, 0.8 mL/mn, 30°C.

the enzyme from a phosphate buffer 0.1 M led to less- or non-regioselective acylations, only due to the presence of phosphate salts (more substituted esters were also formed via this competitive non-enzymatic reaction). Faster reaction rates were observed for aliphatic esters **1a–b**, confirming their better reactivity compared to α,β-unsaturated esters with respect to the acylation of carbohydrates. The reactions were very regioselective towards the formation of 1′-*O*-monoacylated sucrose derivatives **2a–d**, as already observed in the case of other protease-catalysed sucrose acylations with short acids. The regioisomeric purity was over 90% for the ester at OH-1′ (^{1}H NMR),[17] the remaining, less than 10%, being esters at OH-6 or OH-6′ (HPLC). Compounds were fully characterised and identified by 2D NMR spectroscopy (one-bond ^{1}H–^{13}C correlation (HSQC) and ^{1}H–^{13}C multiple bond correlation (HMBC) showing correlation between the CO peak of the ester and the hydrogen atoms at C-1′).

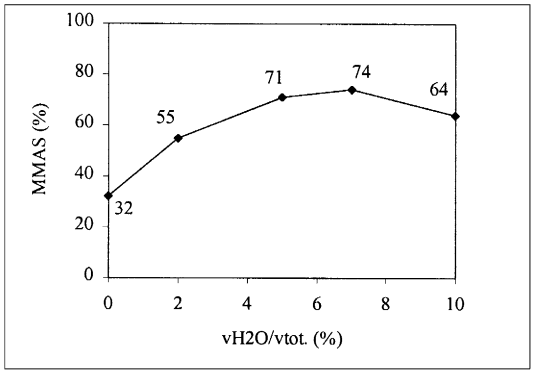
In order to compare also the importance of the leaving group part of the acylating agent, vinyl methacrylate (**3**) was used and faster reactions were observed (Table 2). But the acetaldehyde produced in the medium is prejudicial to the stability of the enzyme (appearance of a red colour) and therefore to the yield of monomethacryloyl sucrose (MMAS).[18] All other studies were thus achieved using trifluoroethyl methacrylate as the acylating agent.

Next, the effect of water on the reaction was studied. Faster reactions were obtained with up to 7% (vol.) of water in DMF, probably significant of an optimised conformation of the enzyme. Above this amount, lower yields were obtained as water becomes a competitive nucleophilic species in the medium (Fig. 1, curve A). In 7% aqueous DMF, a very high conversion of sucrose to its methacryloyl ester **2d** was obtained after 2 d at 45°C (Fig. 1, curve C). A typical procedure for the aqueous DMF reaction was to mix sucrose (0.5 g, 1.46 mmol), trifluoroethyl methacrylate (1.05 mL, 5 equiv.) and the freeze-dried proteinase N (7.2 U/mg, 220 mg) in 7% (vol.) aqueous DMF (3.7 mL) at 250 rpm (the volume of the enzyme solution to be freeze-dried can be considered as the only limitation to the scaling-up of the method). After 3 d at 45°C, the suspension was placed directly on a silica gel column which was eluted with a 56/20/20/4 CH_2Cl_2/MeOH/Me_2CO/H_2O mixture. Evaporation and freeze drying provided MMAS as a white foam (515 mg, 86%) containing 90% of 1′-MMAS (NMR).

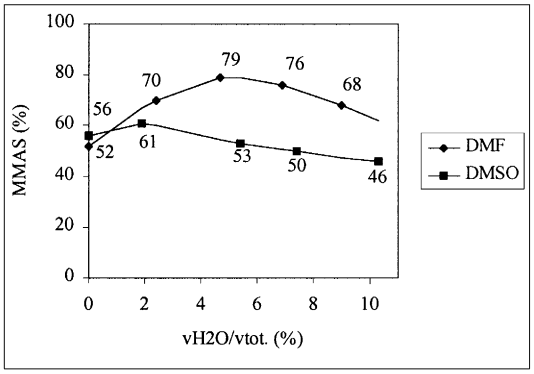
Under the same conditions but using DMSO, a much less toxic solvent, instead of DMF, no reaction

Table 2
Sucrose conversion and yield of MMAS at 45°C for proteinase N-catalysed reaction of sucrose with trifluoroethyl or vinyl methacrylate (**1d** or **3**) in anhydrous DMF (same conditions as for Table 1)

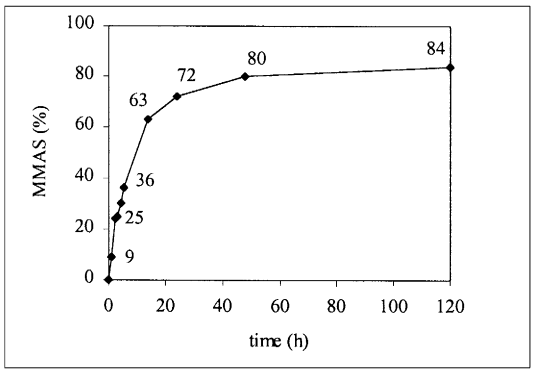
Conditions		Reaction with **1d**		Reaction with **3**	
Acylating agent excess (equiv)	Time (d)	Sucrose conversion (%)	MMAS yield (%)	Sucrose conversion (%)	MMAS yield (%)
1.2	1			22	18
"	2	20	15	31	28
"	6	22	17		
2	1	20	15	27	25
"	3	30	23	31	27
5	1	38	35	33	27
"	3	55	55	41	41



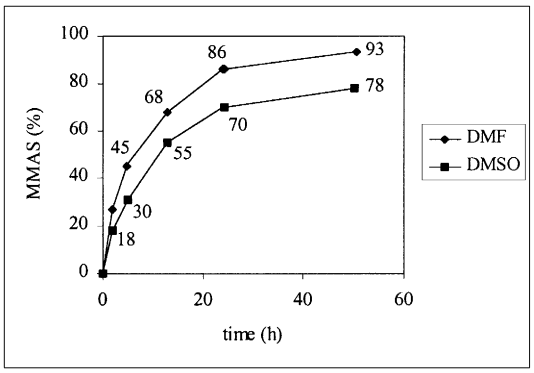
A. Suspension (sucrose : 25 mg ; **1d** : 5 equiv ; PN (5.6 U/mg) : 15 mg ; DMF-water : 0.18 mL ; stirring at 250 rpm, 1 d).



B. Slurries (sucrose : 25 mg ; **1d** : 5 equiv ; PN (5.6 U/mg) : 25 mg ; solvent-water (35-150 µL); stirring at 250 rpm, 17 h).



C. Suspension (7 % aq DMF).



D. Slurries (4.7 % aq. DMF or 2.2 % aq. DMSO).

Fig. 1. Influence of the amount of water on the yield of sucrose monomethacrylates (MMAS) for the PN catalysed reaction at 45°C (A, B) and their evolution at the optimum water amount (C, D)

could be observed as DMSO is known to denature enzymes.[19–21] However, it was possible to retain the activity of the enzyme and to get sucrose esters when a minimum amount of an aqueous DMSO solution of sucrose was used to give a slurry of the enzyme (Fig. 1, curves B and D), offering a useful alternative as there are only very few solvents in which sucrose is reasonably soluble (this is the first example of

a protease-catalysed sucrose acylation in DMSO). DMF can also be used under these conditions, with slightly higher rates. A typical procedure was to prepare a slurry by mixing sucrose (25 mg, 73 μmol) and a minimum amount of DMSO (35 μL) then to add water (0–10 μL) [or DMF (150 μL) then water (0–20 μL)], then trifluoroethyl methacrylate (52 μL, 5 equiv.), then the proteinase N (5.6 U/mg, 25 mg) with magnetic stirring at 250 rpm at 45°C, and using the usual work-up (vide supra).

In conclusion, we have shown that the crude protease proteinase N (Fluka) can be used in enzyme-catalysed selective acylations of sucrose, in DMF and even in DMSO, although this latter solvent is known to denature enzymes. High conversion of activated esters to 1′-sucrose esters of short acids, and notably 1′-sucrose monomethacrylate, were obtained upon adjusting the water content. The optimisation of the conditions and the application of this reaction to other carbohydrates and other acylating agents are currently studied in our laboratory.

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17. 1′-*O*-Butyryl sucrose (**2a**): $[\alpha]_D^{20}$ +62 (*c* 1, MeOH); ^{1}H NMR (CD_3OD, 300 MHz) δ (ppm) 5.44 (d, 1H, $J_{1,2}$=3.5 Hz, H-1), 4.43 (d, 1H, $J_{1'a,b}$=12.1 Hz, H-1′a), 4.25 (d, 1H, $J_{1'a,b}$=11.9 Hz, H-1′b), 4.15 (d, 1H, $J_{3'-4'}$=8.7 Hz, H-3′), 4.09 (t, 1H, $J_{4',5'}$=8.4 Hz, H-4′), 3.9–3.6 (m, 7H, H-3, H-5, H-6ab, H-5′, H-6′ab), 3.4 (dd, 1H, $J_{2,1}$=3.5 Hz, $J_{2,3}$=9.8 Hz, H-2), 3.3 (t, 1H, $J_{3,4}$=9.3 Hz, H-4), 2.4 (t, 2H, *J*=7.35 Hz, CH_2), 1.7 (m, 2H, CH_2), 1.0 (t, 3H, *J*=7.35 Hz, CH_3); ^{13}C NMR (CD_3OD, 75 MHz) δ (ppm) 173.6 (CO), 104.5 (C-2′), 93.1 (C-1), 82.7 (C-5′), 77.7 (C-3′), 73.9 (C-4′), 73.5 (C-5), 73.4 (C-3), 72.0 (C-2), 70.0 (C-4), 62.7 (C-1′), 62.2 (C-6′), 61.2 (C-6), 35.9, 18.4 (2 CH_2), 13.0 (1 CH_3). Anal. calcd for $C_{16}H_{28}O_{12}$+1.1 H_2O: C, 44.5; H, 7.1; found: C, 44.4; H, 7.3. HRMS (FAB+) *m/z* calcd: 435.1478, found: 435.1481 (^{13}C NMR, see Ref. 8).
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