

TABLE 1

Comparison of first order rate constants for hydrolysis (K_h) and aminolysis (K_{am}) of SC-PEG and SS-PEG ^a					
pH	Temp. (°C.)	Hydrolysis: K_h^b (min ⁻¹) × 10 ³ and [h ₁ (min)]		Aminolysis: K_{am}^c (min ⁻¹) × 10 ³ and [K_{am}/K_h]	
		SC-PEG	SS-PEG	SC-PEG	SS-PEG
7.0	4	0.87 [793]	1.84 [376]	2.64 [3.0]	3.74 [2.0]
	27	6.05 [115]	10.4 [67]	26.4 [4.4]	41.4 [4.0]
	37	14.2 [49]	25.9 [27]	81.7 [5.8]	104 [4.0]
7.4	22	5.37 [129]	10.7 [65]	29.1 [5.4]	42.7 [4.0]
	27	9.0 [77]	16.0 [43]	48.6 [5.4]	73.6 [4.6]
	37	19.3 [36]	37.6 [18]	145 [7.5]	193 [5.1]
7.8	4	1.37 [505]	2.58 [268]	12.4 [9.1]	15.0 [5.8]
	27	10.3 [67]	21.6 [32]	130 [12.6]	152 [7.0]
	37	21.8 [32]	48.8 [14]	226 [10.6]	267 [5.5]

^aAll the measurements were performed by following the appearance of N-hydroxysuccinimide anion (-OSu) at 260 nm in 0.008 M sodium phosphate; concentration of PEG-bound succinimidyl active acyl at time zero [SX-PEG]₀ was 0.1 mM; in aminolysis experiments concentration of N^ε-acetyl-lysine at time zero [NAL]₀ was 3 mM.

^b $K_h = \text{Rate}_h / [\text{SX-PEG}]_0$, where $\text{Rate}_h = dA_{260}/dt \times 1/E_{260} \times 1F$; $\epsilon_{260} = 8500 \text{ M}^{-1} \text{ cm}^{-1}$ is an extinction coefficient of -OSu; and $F = [-\text{OSu}]/([\text{HOSu}] + [-\text{OSu}]) = (1 + 10^{6.0-\text{pH}})^{-1}$.

^c $K_{am} = \text{Total Rate}/[\text{SX-PEG}]_0 - K_h$. The Total Rate in aminolysis experiments was calculated the same way as Rate_h in hydrolysis experiments.

TABLE 2

SUMMARY OF MODIFICATION, ESTEROLYTIC ACTIVITY, AND AMIDOLYTIC ACTIVITY DATA FOR TRYPSIN AND ITS mPEG DERIVATIVES							
Trypsin Derivatives ^a	Modifi ^b (%)	BAEE ^c (u/mg)	% Native	BAPA ^d (u/mg)	% Native	ZAPA ^d (u/mg)	% Native
Native Trypsin	0	92.4	100	1.26	100	7.81	100
SC-PEG _M -Trypsin							
N = 6	42.3	103	112	2.26	179	15.3	196
N = 7	45.8	87.9	95.1	2.38	188	17.5	224
N = 9	58.8	90.1	97.5	2.67	212	18.9	242
N = 12	77.9	85.1	92.2	3.83	304	25.5	326
SS-PEG _H -Trypsin							
N = 7	44.8	102	110	3.25	258	18.8	241
N = 12	77.0	94.3	102	4.34	344	24.7	316

^aFor SX-PEG_H-Trypsin, N = 15 × (% Modifi)/100 and is rounded to the nearest integer.

^bThe percent of amino groups modified was determined by the fluorescamine assay [Stocks, et al. (1986) Anal. Biochem. 154, 232].

^cThe BAEE (Nα-benzoyl-L-arginine ethyl ester) trypsin assay was done at pH 7.8, 37° C. w/ a substrate conc'n of 0.5 mM. The extinction coefficient was $\epsilon_{285} = 808 \text{ M}^{-1} \text{ cm}^{-1}$ [Kedzy, et al. (1965) Biochemistry 4, 99].

^dThe BAPA (Nα-benzoyl-DL-arginine-p-nitroanilide) and ZAPA (Nα-CBZ-L-arginine-p-nitroanilide) amidolytic assays were done w/ a substrate conc'n of 1 mM in 50 mM Tris-HCl pH 8.1, 10 mM CaCl₂, at 37° C. The extinction coefficient for p-nitroaniline, $\epsilon_{410} = 8800 \text{ M}^{-1} \text{ cm}^{-1}$, was used in both assays.

TABLE 3

MICHAELIS-MENTEN CONSTANTS FOR THE AMIDOLYTIC ACTIVITY OF NATIVE TRYPSIN AND ITS mPEG DERIVATIVES ^a				
Trypsin Derivatives	K _m (mM)	V _{max} (μM/min)	K _{cat} (min ⁻¹)	K _{cat} /K _m (min ⁻¹)
Native Trypsin	1.08	15.7	378	349
SC-PEG _H -Trypsin				
N = 7	0.29	19.6	470	1626
N = 9	0.21	20.2	484	2290
N = 12	0.11	22.9	549	4973
SS-PEG _H -Trypsin				
N = 7	0.21	18.6	447	2172
N = 12	0.13	22.5	539	4159

^aThe measurements took place at 37° C. with a constant trypsin protein concentration of 1.0 μg/ml (via Bluret assay). Nα-carboxy-L-arginine-p-nitroanilide (ZAPA) was used as a substrate in concentrations varying from 0.02 to 1.71 mM in 50 mM Tris-HCl pH 7.8, 10 mM calcium chloride. The constants were calculated from Lineweaver-Burk plots of the initial rates of the appearance of p-nitroaniline ($\epsilon_{410} = 8800 \text{ M}^{-1} \text{ cm}^{-1}$).

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	EFFECT OF Nα-Ac-L-Tyr (NAT) ON THE EXTENT OF REACTION OF SS-PEG AND SC-PEG WITH Nα-Ac-L-Lys (NAL)		
	NAT/NAL	% of NAL Reacted with ^a	SS-PEG
45	1.0	77.15 (98.2)	55.25 (103)
50	2.5	74.50 (94.8)	52.55 (97.8)
	5.0	68.50 (87.2)	48.60 (90.4)

^aTo triethanolamine-borate buffer (0.3 M, pH 8.1) the following was added: a solution of NAL (50 mM, 0.1 ml) and a solution of NAT (100 mM, volume corresponding to the ratios given in the table and bringing the combined volume to 1.1 ml) both in the same buffer, and lastly the appropriate activated PEG in CH₃CN (50 mM active acyl, 0.1 ml). The resultant solution was vortexed and incubated at 28° C. for 1 hr. A mixture of the same components but leaving out SX-PEG was used as a control. The TNBS assay version of Snyder, et al. [(1975) Anal. Biochem. 64, 284] was used to determine the unreacted NAL.

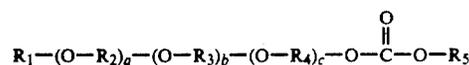
^bThe numbers given in the parentheses represent the values of percent of NAL reaction divided by the percent of NAL reaction when NAT/NAL = 0.

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I claim:

1. A compound having the structure:

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wherein R₁ is H—, H₃C—, or an oxycarbonyl-N-dicarboximide group;

EFFECT OF Nα-Ac-L-Tyr (NAT) ON THE EXTENT OF REACTION OF SS-PEG AND SC-PEG WITH Nα-Ac-L-Lys (NAL)

NAT/NAL	% of NAL Reacted with ^a	
	SS-PEG	SC-PEG
0	78.55 (100) ^b	53.75 (100)