NOTES

Specific Effects of Synthetic Oligopeptides on Cultured Animal Cells†

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Synthetic oligopeptides, tri- to pentaglycine and tri- and tetraalanine, were found to enhance viable cell density and culture viability when applied at concentrations higher than milllimolar to the cultures of a model hybridoma line. Oligoalanines, in addition, enhanced monoclonal antibody yields. Oligoglycines promoted solely the cell growth, unless the batch culture was fed with a medium concentrate. Examination of the effects of various tripeptides composed of glycine, alanine, serine, threonine, lysine, and histidine showed that some of the peptides promoted the growth of the culture, while other peptides suppressed the growth and enhanced the monoclonal antibody yield. Determination of the levels of amino acids and peptides in culture media indicated that the observed changes of culture parameters were caused by intact peptide molecules, rather than by amino acids liberated from the peptides by enzymic cleavage.

Introduction

The beneficial effect of protein hydrolysates on the growth of animal cell lines employed for production of biologicals has been mostly interpreted in terms of improved nutrition (*1*−*4*). Our previous study (*5*) evaluating the effects of chromatography fractions of enzymic hydrolysates from soy and from wheat gluten revealed substantial differences in the activities among individual fractions. We proposed that protein hydrolysates did not serve only as a source of utilizable amino acids but that they also provided peptides exerting specific effects on cell growth and productivity. A report on growth stimulating activity of some synthetic di- to tetrapeptides can be found in an early study carried out on a human cell line (*6*).

In the present work we chose a novel approach: instead of laborious isolation and identification of individual peptides from complex hydrolysates, we investigated possible activities of available synthetic peptides. The peptides subjected to screening were likely to be similar, by amino acid composition and molecular mass, to putative most active peptides in plant protein hydrolysates (*5*). We report here for the first time that some defined oligopeptides can selectively modulate various parameters of animal cell cultures.

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Materials and Methods

Materials. Synthetic peptides of L-configuration were purchased from Bachem (Bubendorf, Switzerland). Cell culture media and supplements were from Life Technologies (Paisley, United Kingdom).

Cell Culture. Mouse hybridoma ME-750 was cultured in DMEM/F12/RPMI 1640 (3:1:1) medium supplemented with BME amino acids, 2.0 mM glutamine, 0.4 mM each of alanine, serine, asparagine, and proline (*7*), 15 mM HEPES, and 2.0 g L^{-1} sodium bicarbonate and with the iron-rich protein-free growth-promoting mixture containing 0.4 mM ferric citrate (*8*). The cultures in 25 cm^2 T-flasks were kept at 37 °C in a humidified atmosphere with 5% CO_2. The culture volume was 6.0 mL. In fed-batch cultures, a volume of 0.2 mL of medium, containing concentrated essential amino acids, vitamins, and glucose, was added daily starting from day 1.

Assays of Peptide Activity. The cultures were inoculated at a density of (300 ± 50) × 10^3 cells mL^{-1} and incubated until the decline phase, i.e., for 6 days (batch cultures) or 8 days (fed-batch cultures). The concentration of tested peptides was 0.2% (w/v) if not stated otherwise. The assays were conducted at least in duplicate. Viable cells and dead cells were counted in a hemocytometer using the trypan blue exclusion test. The experimental error involved in the estimation of cell density, and viability was ±10%. The monoclonal antibody (MAb) concentration was determined by immunoturbidimetry (*9*). The experimental error associated with the estimation of MAb concentrations was ±5%.

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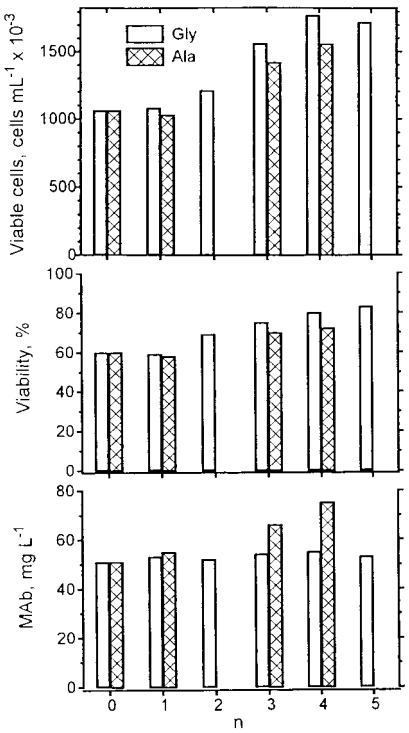

Figure 1. Effect of glycine, L-alanine, and of the corresponding *n*-mers on growth, viability, and MAb yield in cultures of hybridoma ME-750. All added substances were present at 0.2% (w/v) concentrations. Data of day 6 of batch cultures are presented. The degree of oligomerization is designated "*n*". Control culture without peptides is designated "0".

Amino Acid Analysis. The concentration of amino acids and peptides in ultrafiltered spent media was determined on an automatic analyzer Biochrom 20 (Amersham Pharmacia Biotech, D-79111 Freiburg, Germany).

Results and Discussion

Peptide Chain Length. While single amino acids or dipeptides did not significantly alter the culture parameters, tri-, tetra-, and pentaglycine as well as tri- and tetraalanine enhanced markedly the values of viable cell density and viability (Figure 1). In contrast to oligoalanines which stimulated both the cell growth and the MAb production, oligoglycines in batch cultures promoted solely the cell growth. However, under a sufficient supply of nutrients in the fed-batch mode of culture the tetraglycine-supplemented culture yielded 1.345 times more MAb than the fed-batch culture without tetraglycine (Table 1). Thus, the reason for low MAb productivity in batch cultures with tetraglycine was obviously not a suppression of secreted protein synthesis, but preferential channeling of the limited supply of nutrients to the synthesis of new biomass.

Peptide Concentration. The growth-promoting effect of tetraglycine manifested itself at peptide concentrations higher than millimolar (Table 2). Similar values of active concentrations were observed with tetraalanine (data not shown).

Integrity of the Peptides in the Culture. Tetraglycine and tetraalanine concentrations were found to decrease slightly during a 4 day culture period: tetraglycine to 92% and tetraalanine to 70% of the starting values, respectively (Table 3). In parallel, the concentrations of the corresponding monomeric amino acids substantially increased. We might assume that fractions of the tetrapeptides were cleaved by peptidases into amino acids. Under this assumption the balance of alanine was near zero, the balance of glycine indicated a certain consumption (see the last lines in Table 3).

It is unlikely that alanine and glycine liberated from the tetrapeptides are responsible for the observed growth-promoting effect, because the same amino acids in their monomeric form do not exert any effect (Figure 1). Moreover, alanine is known to be produced by hybridoma cells, even under conditions of limited starvation (*10*, *11*).

Diverse Effects of Tripeptides Composed of Various Amino Acids. Investigation of the activity of various tripeptides, present at 0.2% concentrations, demonstrated the relative independence of the two activities: stimulation of cell growth (biomass production) and stimulation of secreted protein production (Figure 2). The effects of the tripeptides tested at 0.1% concentrations were of the same character, but lower than the effects achieved at 0.2% concentrations.

The peptides which suppressed cell growth showed the highest MAb yields. The presence of any of the peptides tested resulted in an increased viability. On day 6 the

Table 1. Batch and Fed-Batch Cultures of Hybridoma ME-750 in the Presence of Tetraglycine

	day 4		day 8		
	viable cells, $\times 10^{-3}$ cells mL^{-1}	viability, %	viable cells, $\times 10^{-3}$ cells mL^{-1}	viability, %	MAb, mg L^{-1}
standard medium					
batch	1520	87	980	49	51
fed-batch	1850	90	1720	60	110
medium + 0.2% tetraglycine					
batch	2250	88	1650	58	48
fed-batch	2940	87	2050	52	148

Table 2. Concentration Dependence of Tetraglycine Growth-Promoting Activity

	peptide concn		day 4		day 6	
peptide	% (w/v)	mM	viable cells, $\times 10^{-3}$ cells mL^{-1}	viability, %	viable cells, $\times 10^{-3}$ cells mL^{-1}	viability, %
none (control)			1460	80	1140	64
tetraglycine	0.1	4.0	2170	88	1750	76
	0.2	8.1	2330	88	2120	75
	0.3	12.2	2450	80	2240	78

Table 3. Concentrations of Amino Acids and Peptides in Culture Media of Hybridoma ME-750

amino acid or peptide	amino acid or peptide concn, mM					
	tetraglycine-supplemented culture			tetraalanine-supplemented culture		
	day 0	day 4	diff	day 0	day 4	diff
Lys	0.43	0.28	−0.15	0.47	0.36	−0.11
His	0.18	0.14	−0.04	0.18	0.12	−0.06
Arg	0.48	0.34	−0.14	0.45	0.36	−0.09
Asp	0.06	0.02	−0.04	0.10	0.05	−0.05
Thr + Asn	0.43	0.42	−0.01	0.47	0.42	−0.05
Ser + Gln	2.94	0.91	−2.03	2.38	0.85	−1.53
Glu	0.08	0.02	−0.06	0.08	0.01	−0.07
Pro	0.37	0.49	+0.12	0.39	0.43	+0.04
Gly	0.59	1.62	+1.03	0.80	0.77	−0.03
Ala	0.63	1.92	+1.29	0.48	2.68	+2.20
Val	0.62	0.46	−0.16	0.47	0.35	−0.12
Met	0.24	0.17	−0.07	0.18	0.11	−0.07
Ile	0.34	0.05	−0.29	0.54	0.22	−0.32
Leu	0.28	0.04	−0.24	0.35	0.12	−0.23
Tyr	0.45	0.41	−0.04	0.25	0.11	−0.14
Phe	0.33	0.28	−0.05	0.25	0.21	−0.04
Trp	0.05	0	−0.05	0.05	0.01	−0.04
tetraglycine	7.14	6.65	−0.49			
equiv Gly[a]	*28.56*	*26.60*	*−1.96*			
diff Gly[b]			*+1.03*			
tetraalanine				1.84	1.28	−0.56
equiv Ala[a]				*7.36*	*5.12*	*−2.24*
diff Ala[b]						*+2.20*

[a] The values of tetrapeptide × 4. [b] For easier comparison the values from above amino acid lines are repeated.

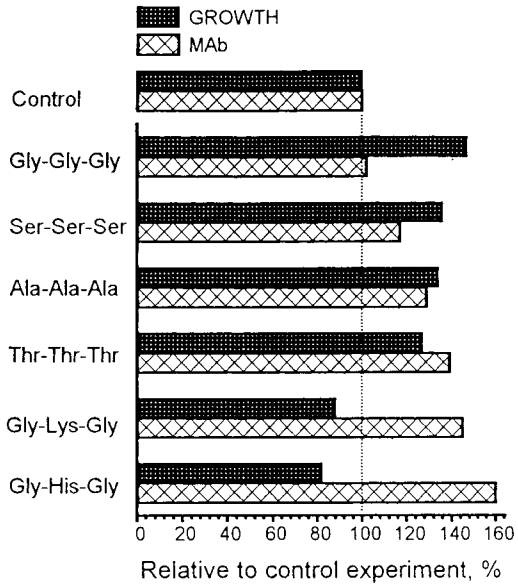

Figure 2. Growth-promoting and production-enhancing effects of various tripeptides in cultures of hybridoma ME-750. The peptides were present at 0.2% (w/v) concentrations. Data of day 6 of batch cultures are presented relative to the values of the control culture.

viability in the control experiment was 58%. The viability of peptide-supplemented cultures was between 65 and 72%.

Conclusions

The new phenomena revealed upon application of pure and homogeneous oligopeptides to a model hybridoma culture may be characterized by the following points:

(1) The growth- and production-enhancing effects of peptides increase with peptide chain length up to the length of pentapeptides and manifest themselves at peptides concentrations > 1 mM.

(2) Higher viability at the decline phase of batch cultures in peptide-supplemented media points to anti-apoptotic activity of the peptides.

(3) Some peptides promote the growth of the culture, while other peptides suppress the growth and enhance the product yield. Supplementation of the culture with a growth-promoting peptide and feeding with a medium concentrate act synergistically to increase the product yield.

(4) The peptides are relatively stable during the course of the culture; utilization of amino acids liberated from the peptides is at most marginal.

Experiments with other cell lines frequently used in the production of biologicals are in progress. It can be expected that synthetic oligopeptides will qualify as a new class of media additives serving to process control in animal cell culture.

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