# Enhancement of Monoclonal Antibody Production by Lysine-Containing Peptides

**František Franěk,*,† Tomáš Eckschlager,‡ and Hermann Katinger§**

Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Radiova 1, CZ-10227 Prague 10, Czech Republic, Second Medical Faculty, Charles University, V Uvalu 84, CZ-15006 Prague 5, Czech Republic, and Institute of Applied Microbiology, University of Agricultural Sciences, Muthgasse 18, A-1190 Vienna, Austria

In the search for peptides that could effectively enhance the monoclonal antibody production of a model hybridoma, the performance of five lysine-containing peptides was compared. The capacity of the peptides to enhance the monoclonal antibody yield correlated with their growth-suppressing activity. No correlation of the production-enhancing activity with the character of the distribution of cell-cycle phases could be found. All of the tested peptides, including the negative control peptide Gly-Phe-Gly, altered the cell-cycle phases distribution in favor of the proportion of the S phase.The peptides added to the hybridoma culture were found to be gradually decomposed into dipeptides and free amino acids. Among the set of tested lysine-containing di- to pentapeptides, the best results were obtained with the tripeptide Gly-Lys-Gly. The growth-suppressing and production-enhancing capacity of this peptide supplement was obviously associated with the temporary presence of the intact peptide molecule in the culture media, because the addition of a mixture of free amino acids constituting this peptide, i.e., glycine and lysine, displayed a different effect—a slight promotion of cell growth.

## Introduction

Increasing demands for large quantities of monoclonal antibodies (mAbs) and other protein products of cultured animal cells stimulate efforts at increasing volumetric productivity of cell cultures. Three factors are of decisive importance in the achievement of this goal: first, the genetic background of the production cell line (the nature of the genome may be substantially improved by various kinds of genetic manipulations, including transfection with foreign genes); second, optimization of nutrients supply; third, medium additives modulating the cell metabolism and, perhaps, also gene expression in favor of enhanced proteosynthesis.

Our previous work led us to suggest not only that protein hydrolysates, frequently used as beneficial medium supplements (*1*−*4*), contribute to optimization of nutrition but also that they may act as a source of bioactive peptides of signal character (*5*). This idea prompted us to start an investigation of the effects of synthetic peptides that were likely to be similar to putative peptides contained in the protein hydrolysates (*6*, *7*). Using a series of oligoglycines and oligoalanines, we were able to show that the growth- and production-enhancing effects increased with the peptide chain length up to the length of pentapeptides and manifested themselves at higher than millimolar peptides concentration. Higher viability at the decline phase of batch cultures pointed to antiapoptotic activity of those peptides. It was also shown that oligoglycines and oligoalanines were relatively stable during the course of the culture and that the amino acids liberated from them were utilized only marginally.

The assay of the effects of various tripeptides reported in the previous paper (*7*) showed that peptides containing a basic amino acid, lysine or histidine, suppressed cell growth and enhanced the mAb yield. In the present work we focused our attention to a more detailed analysis of the effects of a set of lysine-containing peptides in laboratory-scale experiments. An altered character of the distribution of the cell-cycle phases pointed to a complex nature of the action of the peptides.

## Materials and Methods

**Materials.** Synthetic peptides of L-configuration were purchased from Bachem (Bubendorf, Switzerland). Cell culture media and supplements were from Sigma-Aldrich (St. Louis, MO).

**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 (*8*), 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 (*9*). 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 0.25 mL of a feeding mixture (DMEM fortified with 10x BME amino acids, 10x BME vitamins, and 20 mM glutamine) was added daily starting from day 1. The kinetics of mAb production was determined in a series of 25 $cm^2$ T-flasks. Two control flasks and two flasks with peptide-supplemented medium were taken for evaluation each day.

* To whom correspondence should be addressed. Phone: (420) 2 6700 8469. Fax: (420) 2 6700 8329. E-mail: franek@biomed.cas.cz.
† Academy of Sciences of the Czech Republic.
‡ Charles University.
§ University of Agricultural Sciences.

10.1021/bp020077m CCC: $25.00 
Published on Web 12/04/2002

**Table 1. Batch Cultures of Hybridoma ME-750 Supplemented with Various Peptides[a]**

| | day 3 | | day 6 | | |
|---|---|---|---|---|---|
| peptide | viable cells ($\times 10^{-3}$ cells mL$^{-1}$) | viability (%) | viable cells ($\times 10^{-3}$ cells mL$^{-1}$) | viability (%) | mAb (mg L$^{-1}$) |
| none (control) | 1590 | 87 | 1060 | 55 | 31 |
| Gly-Lys-Gly | 990 | 91 | 930 | 62 | 49 |
| Gly-Gly-Lys-Ala-Ala | 920 | 92 | 1120 | 75 | 44 |
| Lys-Lys-Lys | 1010 | 90 | 1130 | 56 | 39 |
| Gly-His-Lys | 1350 | 89 | 1110 | 54 | 38 |
| Gly-Lys | 1620 | 87 | 1290 | 70 | 37 |
| Gly-Phe-Gly | 1760 | 88 | 1350 | 65 | 29 |

[a] The concentration of peptides at inoculation was 0.2% (w/v).

**Table 2. Fed-Batch Cultures of Hybridoma ME−750 Supplemented with Various Peptides[a]**

| | day 3 | | day 6 | | day 10 | | |
|---|---|---|---|---|---|---|---|
| peptide | viable cells ($\times 10^{-3}$ cells mL$^{-1}$) | viability (%) | viable cells ($\times 10^{-3}$ cells mL$^{-1}$) | viability (%) | viable cells ($\times 10^{-3}$ cells mL$^{-1}$) | viability (%) | mAb (mg L$^{-1}$) |
| none (control) | 1840 | 90 | 1560 | 72 | 1070 | 45 | 74 |
| Gly-Lys-Gly | 1280 | 91 | 1620 | 83 | 1770 | 54 | 118 |
| Lys-Lys-Lys | 1310 | 88 | 1780 | 73 | 1350 | 53 | 90 |
| Gly-Phe-Gly | 2210 | 86 | 1750 | 67 | 1110 | 40 | 73 |

[a] The concentration of peptides at inoculation was 0.2% (w/v).

**Assays of Peptide Activity.** The cultures were inoculated at a density of (250 ± 50) × $10^3$ cells mL$^{-1}$ and incubated until the decline phase, i.e., for 6 or 7 days (batch cultures) or up to 14 days (fed-batch cultures). The starting concentration of tested peptides was 0.2% (w/v). 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 mAb concentration in culture supernatants was determined by immunoturbidimetry (*10*). Briefly, aliquots of media were diluted by 5% solution of poly(ethylene glycol) and incubated for 1 h with porcine anti-mouse immunoglobulin affinity-purified antibody. The turbidity was measured at 340 nm. The mAb concentrations were determined using a calibration curve. The experimental error associated with the estimation of mAb concentration was ±5%.

**Determination of Peptides and Amino Acids Concentrations.** The culture supernatants were freed of high-molecular weight components by ultrafiltration (Ultrafree-CL centrifugal filter UFC4LTK25, MilliporeCorp., Bedford, MA). The concentrations of peptides and amino acids in ultrafiltered culture supernatants were determined on an automatic analyzer Biochrom 20 (Amersham Pharmacia Biotech, Freiburg, Germany) using peptide standards. The experimental error involved in peptide and amino acid analyses was ±10%.

**Cell-cycle Phases Profiles.** The proportion of cell-cycle phases in cell populations was determined upon permeabilization and staining with propidium iodide using DNA Prep kit (Coulter Immunology, Hialeah, FL). The samples were measured with FACSCalibur flow cytometer 20−120 min after staining, and the data were analyzed by ModFit software. The proportion of apoptotic cells was determined in separate aliquots. The cells were fixed in ice-cold 70% ethanol for at least 24 h, DNA fragments were extracted with phosphate-citrate buffer, and the cells were stained with propidium iodide (*11*).

## Results and Discussion

**Effects of Lysine-Containing Peptides on Cell Growth and mAb Production.** A set of five lysine-containing peptides was tested at standard concentrations of 0.2% (w/v) (Table 1). The peptide Gly-Phe-Gly was included in the set as a negative control. Supplementation of the media with the peptides, except for the dipeptide Gly-Lys and the negative control peptide, led to a certain suppression of viable cell density observed at the end of the exponential phase, i.e., on day 3. In contrast, at the decline phase on day 6 the viable cell density was higher than in the control experiment in most cultures, except for the culture containing the peptide Gly-Lys-Gly. Largest differences could be seen in the values of the final mAb yields. MAb concentration values in cultures supplemented with lysine-containing peptides were significantly higher than in the control. The value of 158% of control was achieved with the peptide Gly-Lys-Gly and 126% with the peptide Lys-Lys-Lys. The addition of the dipeptide Gly-Lys led to slightly enhanced viable cell density, as well as to slightly enhanced mAb yield. In the culture supplemented with the negative control peptide the mAb concentration was even lower than in the control, but the viable cell number exceeded markedly the cell number in the control, particularly on day 6.

Promising results were obtained also with the pentapeptide Gly-Gly-Lys-Ala-Ala. However, because the final goal of our research was an enhancement of volumetric productivity in large-scale cultures, the application of this pentapeptide was discontinued, with respect to the high cost of synthetic peptides, increasing progressively with the peptide length. The peptides concentration of 0.2% (w/v), applied in the reported experiments, was selected on the basis of previous experience (*6*, *7*). When the peptides were added at 0.1% (w/v) concentrations, the observed effects were of the same character, but quantitatively lower (data not shown).

Two lysine-containing peptides and the negative control peptide Gly-Phe-Gly were applied to cultures in the fed batch mode. Again, the values of viable cell densities on day 3 were lower than the control value, but higher than the control value on days 6 and 10 (Table 2). Conceivably, the final mAb yields were more than two times higher than in the batch experiment. The enhancement of the mAb yields, relative to control, was very similar to that found in the batch experiment (159% for Gly-Lys-Gly and 122% for Lys-Lys-Lys). No enhancement of the product yield was found with the negative control peptide Gly-Phe-Gly.

**Table 3. Specific mAb Production Rate in the Hybridoma Culture with Gly-Lys-Gly**

| | control | | | 0.2% (w/v) Gly-Lys-Gly | | |
|---|---|---|---|---|---|---|
| culture duration (days) | viable cells ($\times 10^{-3}$ cells mL$^{-1}$) | mAb (mg L$^{-1}$) | $q_{mAb}$[a] (pg cell$^{-1}$ day$^{-1}$) | viable cells ($\times 10^{-3}$ cells mL$^{-1}$) | mAb (mg L$^{-1}$) | $q_{mAb}$[a] (pg cell$^{-1}$ day$^{-1}$) |
| 0 | 210 | 2 | | 210 | 2 | |
| | | | 3.2 | | | 7.3 |
| 1 | 410 | 3 | | 340 | 4 | |
| | | | 3.3 | | | 7.3 |
| 2 | 1390 | 6 | | 760 | 8 | |
| | | | 4.5 | | | 7.5 |
| 3 | 1740 | 13 | | 1390 | 16 | |
| | | | 4.9 | | | 7.1 |
| 4 | 1500 | 21 | | 1420 | 26 | |
| | | | 3.8 | | | 6.3 |
| 5 | 1160 | 26 | | 1110 | 34 | |
| | | | 2.8 | | | 4.7 |
| 6 | 1010 | 29 | | 1000 | 39 | |
| | | | 2.1 | | | 3.3 |
| 7 | 860 | 31 | | 800 | 41 | |

[a] Specific mAb production rate.

**Table 4. Decomposition of the Peptide Gly-Lys-Gly in the Hybridoma Culture**

| | day 0 | day 3 | |
|---|---|---|---|
| peptide or amino acid | concn found (mM) | concn found (mM) | concn calcd (mM) |
| Gly-Lys-Gly intact | 5.2 | 1.9 | na[b] |
| Gly-Lys-Gly decomposed | na | na | 3.3 |
| Gly-Lys-or Lys-Gly | 0 | 2.5 | 2.3 |
| Gly[a] | 0 | 3.5 | 3.3 |
| Lys[a] | 0 | 0.3 | 0.5 |

[a] Increment over the value found in the control culture. [b] Not applicable.

The kinetics of the effect of a production-enhancing peptide was studied with the peptide Gly-Lys-Gly (Table 3). The values of the mAb concentrations and of the specific mAb production rates were markedly higher, relative to control, for the whole duration of the batch culture. The values of the specific production rate in the first 2 days were apparently more than two times higher than the values obtained in the control. This enhancement of the specific production rate is striking even if we take the values of the first 2 days with caution, because the absolute values of the mAb concentrations are very low.

We have shown in our previous paper (*7*) that tetraglycine promoted solely the cell growth when applied to a batch culture, while in the fed-batch culture, under a sufficient supply of nutrients, a higher mAb yield could be found with this peptide, as well. The present results with the peptide Gly-Phe-Gly revealed another dimension of diversity of peptide effects. Solely the cell growth was promoted in the presence of this peptide even in the culture conducted in the fed-batch mode.

**Consumption and Decomposition of the Peptides in the Culture.** Changes of the concentrations of the added peptides were determined on an amino acid analyzer. Ultrafiltered samples of culture supernatants taken after inoculation and on day 3 were analyzed. During the 3 day period the levels of the peptides were found to decrease substantially. The concentration of the peptide Gly-Lys-Gly decreased by 63%, that of the peptide Gly-His-Gly by 67%, and that of the peptide Gly-Phe-Gly by 35%. Analogous data on the peptide Lys-Lys-Lys could not be obtained due to technical difficulties associated with the extremely basic character of this peptide.

The analyses of culture supernatants collected on day 3 revealed a general phenomenon, namely, that the concentrations of free amino acids, constituting the respective peptides, increased in comparison with levels on day 0. New peaks of peptides, most likely dipeptides arising by partial cleavage of the tripeptides, emerged in the analyzer records of the culture supernatants. An attempt at a more profound analysis of the fate of the added peptide was done with the tripeptide Gly-Lys-Gly. The mode of decomposition of the 63% fraction of the peptide Gly-Lys-Gly was analyzed on an assumption that (1) a new peak emerging in the analyzer record of

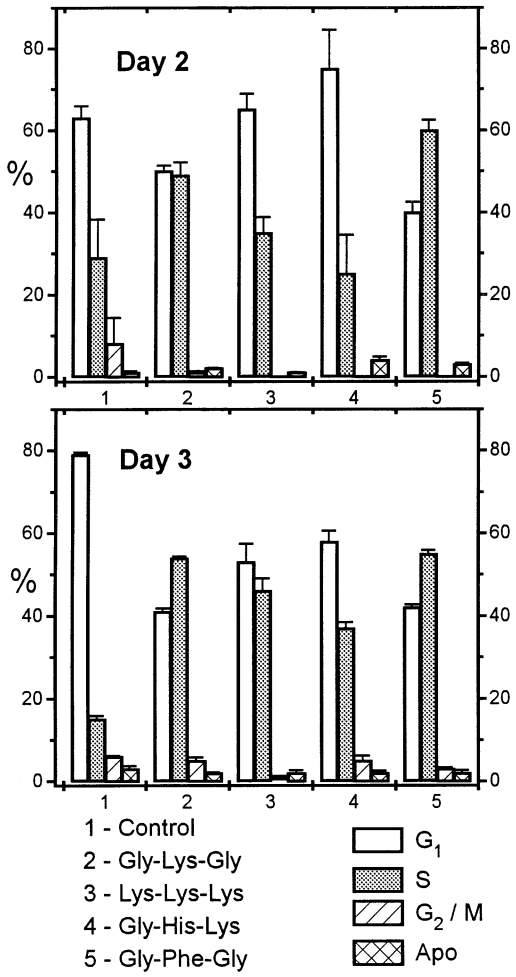


**Figure 1.** Cell-cycle-phase distribution of hybridoma ME-750 cells in media supplemented with various peptides. The error bars represent standard deviations. The concentration of peptides was 0.2% (w/v).

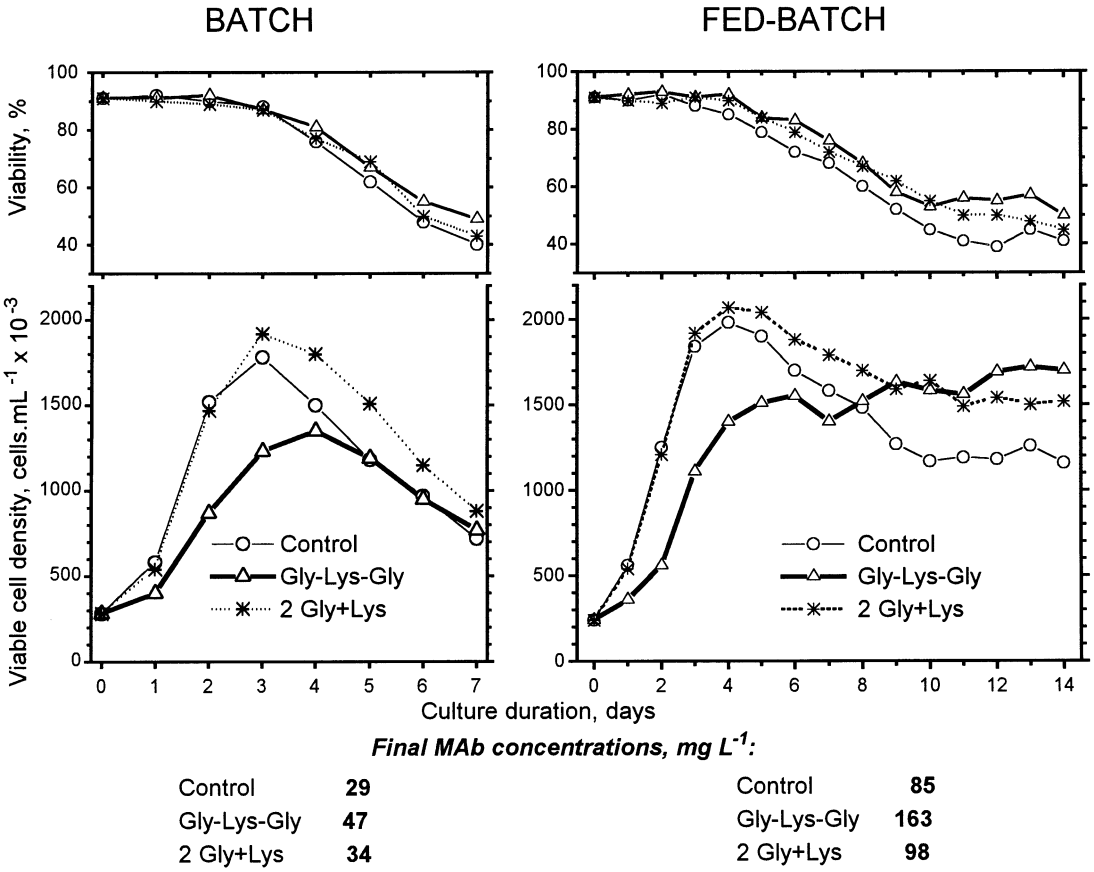


**Figure 2.** Batch culture and fed-batch culture of hybridoma ME-750 in the presence of the tripeptide Gly-Lys-Gly and of amino acids glycine and lysine. The culture volume was 6.0 mL. The concentration of the peptide was 0.2% (w/v). The amino acids glycine and lysine in a total concentration 0.2% (w/v) were present in a molar ratio of 2:1. A volume of 0.25 mL of a feeding mixture (DMEM fortified with 10x BME amino acids, 10x BME vitamins, and 20 mM glutamine) was added daily starting from day 1.

the supernatant collected on day 3 was the dipeptide Gly-Lys or Lys-Gly and (2) that the decomposed 3.3 mM Gly-Lys-Gly (i.e., the 63% of the value found on day 0) was cleaved into 2.3 mM dipeptide(s) (Gly-Lys or Lys-Gly) and free amino acids. The data presented in Table 4 have to be considered as an approximation, because the correct constants for the putative dipeptides were not available. With this reservation, the differences between the found and calculated concentrations of glycine and lysine indicated that a fraction of the liberated lysine, but not that of glycine, was most likely consumed by the cells during the 3 day culture period.

Our previous study dealing with the application of homooligomeric peptides tetraglycine and tetraalanine to hybridoma cultures led to a conclusion that the molecules of these peptides preserved largely their integrity during a 4 day culture period (*7*). The lysine-containing peptides investigated in the present work were found to be more accessible to proteolytic enzymes. Nevertheless, the impact of a single dose, applied on day 0, could be observed not only during 6 or 7 days of the batch culture (Table 3), but also in a prolonged fed-batch culture (see below). It seems to be necessary to consider seriously a hypothesis that a single peptide dose, if large enough, may provoke long-lasting shifts in the metabolism, such as a switch to another steady state (*13*), and possibly alterations in gene expression.

**Distribution of Cell-cycle Phases in Peptide-Supplemented Batch Cultures.** The cell-cycle-phase profiles of the cultures were investigated in the course (day 2) and on the end of the exponential phase (day 3) of the peptide-supplemented cultures (Figure 1). The profiles obtained on day 3 were characterized by an appreciable increase of the S-phase fraction in cultures supplemented with any of the tested peptides, including the negative control peptide Gly-Phe-Gly. This finding pointed to a complex mechanism of the action of peptides under investigation. Increase of the S-phase fraction was found both with the lysine-containing peptides, which suppress the cell growth, and with the peptide Gly-Phe-Gly, which stimulates the cell growth. No meaningful correlation could be seen between the values of the mAb product yield (Table 1) and the proportions of the cell-cycle phases. This finding is in agreement with the conclusion of other authors (*12*), namely, that product formation is not restricted to any particular cell-cycle phase.

**Effect of Peptide versus the Effect of Constituting Amino Acids.** Batch and fed-batch cultures were set up, in which single doses of either the tripeptide Gly-Lys-Gly or of the constituting amino acids glycine and lysine in free form were applied upon culture inoculation. The substances were given on an equivalent mass basis, i.e., 0.2% (w/v) peptide or 0.2% (total w/v) of the mixture glycine + lysine at a molar ratio of 2:1.

The batch cultures showed striking differences between parameters of the culture with the peptide, on one hand, and those of the culture with glycine/lysine mixture, on the other hand (Figure 2). While the viable cell density in the presence of the peptide was lower than in the control, higher viable cell density values than in the control were reached upon addition of the amino acid mixture. With the glycine/lysine mixture a substantially lower mAb yield was obtained than that reached with

the peptide. This was true both in the batch cultures and in the fed-batch cultures. The fed-batch cultures could be continued for 14 days before the viability of the peptide-supplemented culture dropped below 50%.

At the end of the fed-batch culture the distribution of the cell-cycle phases in individual cultures was analyzed. No significant differences could be observed more between the peptide-supplemented culture and the control. In all cell populations the proportion of the $G_0/G_1$ phase was dominant, i.e., 67−70%, while the S-phase cells represented 21−25% and the $G_2$/M phase cells 7−9%. As expected, the apoptotic cell fraction of the total cell count was quite high, 45% in the amino acid-supplemented culture, 47% in the peptide-supplemented culture, and 53% in the control.

The reported experiment demonstrates that the effect of the tripeptide Gly-Lys-Gly on the hybridoma culture is not equivalent to the effect of supplementation of the culture with free amino acids constituting the peptide. According to analyzes performed in this work, the half-life time of the lysine peptides in the culture can be estimated to about 2 days. Consequently, it seems to be necessary to accept the view that peptide molecules hit specific targets in the cell, and long-lasting alterations of the cell metabolism and the cell proliferation mechanism follow. The alterations induced by the peptide probably last for several days, and then gradually disappear. The findings of distinct differences in the cell-cycle-phase profiles obtained on day 3 in peptide-supplemented cultures (Figure 1) and the absence of the differences at the end of the fed-batch cultures support this view.

Data supporting the hypothesis of the crucial role of intact peptide molecules can be found in the Table 3. The specific mAb production rate in the peptide-supplemented culture is obviously markedly higher than the rate in the control experiment, even in the first days of the batch culture. At this time period the major fraction of the peptide is still in intact form, and the supply of all free amino acids in the medium is far from being exhausted (*14*), so that a production-stimulating effect of liberated additional glycine and lysine is highly improbable. Moreover, no data exist indicating that a relatively slight excess of glycine or lysine, over the standard media composition, could cause such a marked initial suppression of the cell proliferation that is evident from data in Tables 1−3 and in Figure 2. Thus, the suppression of cell proliferation, associated with the presence of the peptide in the media, might represent the primary step resulting in preferential channeling of nutrients to the synthesis of the secreted mAb molecules.

Systemic determination of culture parameters, including the dynamics of the growth rate, of the production rate, and of peptides and amino acid concentrations, will be necessary to elucidate conclusively all features of peptide-supplemented cultures. Feeding the culture not only with nutrients but also with the peptide is another promising possibility of increasing the mAb yields.

## Conclusions

Basic phenomena associated with the application of mAb production-enhancing lysine-containing peptides to a hybridoma culture may be characterized by the following points:

(1) Peptides of the randomly chosen set of three tripeptides and one pentapeptide containing at least one lysine residue suppressed hybridoma cell growth and concomitantly enhanced the mAb yield in batch cultures. With two selected tripeptides, Lys-Lys-Lys and Gly-Lys-Gly, the phenomena could be confirmed also in fed-batch cultures. A lysine-containing dipeptide, Gly-Lys, stimulated moderately both the cell growth and the mAb production.

(2) The specific character of lysine-containing peptides was confirmed by an application of a negative control peptide, Gly-Phe-Gly, whose presence in the culture resulted, in contrast, solely in an enhancement of cell growth.

(3) The cell-cycle-phase profiles of peptide-supplemented hybridoma cultures displayed a markedly increased proportion of the S-phase cells, irrespective of whether the peptide suppressed the cell growth or if it stimulated the cell growth.

(4) A significant fraction of lysine-containing tripeptides was found to be cleaved in the culture into dipeptides and free amino acids in the course of 3 days.

(5) The growth-suppressing effect of the tripeptide Gly-Lys-Gly was found to be associated with the intact peptide molecule. The peptide-constituting amino acids in free form displayed an opposite effect, namely, the promotion of cell growth.

This pilot study will hopefully pave the way to the identification of new lead peptide compounds, enabling rational manipulation with the balance growth versus production in animal cell technological processes.

## Acknowledgment

This work was supported by Grants OC 844.10 and 111300005 from the Ministry for Education, Youth, and Sports of the Czech Republic. The authors thank Ludmila Bordeová for excellent technical assistance. The expertise of Jaroslav Zbrožek in performing amino acid analyses is highly appreciated.

Received for review May 30, 2002.

## References and Notes

(1) Jan, D. C. H.; Jones, S. J.; Emery, A. N.; Al-Rubeai, M. Peptone, a Low-Cost Growth-Promoting Nutrient for Intensive Animal Cell Culture. *Cytotechnology* **1994**, *16*, 17−26.

(2) Schlaeger, E.-J. The Protein Hydrolysate, Primaton RL, Is a Cost-Effective Multiple Growth-Promoter of Mammalian Cell Culture in Serum-Containing and Serum-Free Media and Displays Anti-Apoptotic Properties. *J. Immunol. Methods* **1996**, *194*, 191−199.

(3) Nyberg, G. B.; Balcarcel, R. R.; Follstad, B. D.; Stephanopoulos, G.; Wang, D. I. C. Metabolism of Peptide Amino Acids by Chinese Hamster Ovary Cells Grown in a Complex Medium. *Biotechnol. Bioeng.* **1999**, *62*, 324−335.

(4) Heidemann, R.; Zhang, C.; Qi, H.; Rule, J. L.; Rozales, C.; Park, S.; Chuppa, S.; Ray, M.; Michaels, J.; Konstantinov, K.; Naveh, D. The Use of Peptones as Medium Additives for the Production of a Recombinant Therapeutic Protein in High-Density Perfusion Cultures of Mammalian Cells. *Cytotechnology* **2000**, *32*, 157−167.

(5) Franěk, F.; Hohenwarter, O.; Katinger, H. Plant Protein Hydrolysates: Preparation of Defined Peptide Fractions Promoting Growth and Production in Animal Cell Cultures. *Biotechnol. Prog.* **2000**, *16*, 688−692.

(6) Franěk, F.; Katinger, H. Specific Effects of Synthetic Oligopeptides in Animal Cell Culture. In *Animal Cell Technology: From Target to Market*; Lindner-Olsson, E., Chatzissavidou, N., Lullau, E., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2001; pp 164−167.

(7) Franěk, F.; Katinger, H. Effects of Synthetic Oligopeptides on Cultured Animal Cells. *Biotechnol. Prog.* **2002**, *18*, 155−158.

(8) Franěk, F.; Šrámková, K. Cell Suicide in Starving Hybridoma Culture: Survival-Signal Effect of Some Amino Acids. *Cytotechnology* **1996**, *21*, 81−89.

(9) Franěk, F.; Vomastek, T.; Dolníková J. Fragmented DNA and Apoptotic Bodies Document the Programmed Way of Cell Death in Hybridoma Cultures. *Cytotechnology* **1992**, *9*, 117−123.
(10) Fenge, C.; Fraune, E.; Freitag, R., Scheper, T.; Schugerl, K. On-line Monitoring of Monoclonal Antibody Formation in High-Density Perfusion Culture Using FIA. *Cytotechnology* **1991**, *6*, 55−63.
(11) Darzynkiewicz, Z.; Bruno, S.; Del Bino, G.; Gorczyca, W.; Hotz, M. A.; Lassota, P.; Traganos, F. Features of Apoptotic Cells Measured by Flow Cytometry. *Cytometry* **1992**, *13*, 795−808.
(12) Lloyd, D. R.; Holmes, P.; Jackson, L. P.; Emery, A. N.; Al-Rubeai, M. Relationship between Cell Size, Cell Cycle and Specific Recombinant Protein Productivity. *Cytotechnology* **2000**, *34*, 59−70.
(13) Europa, A. F.; Gambhir, A.; Fu, P.-C.; Hu, W.-S. Multiple Steady States with Distinct Cellular Metabolism in Continuous Culture of Mammalian Cells. *Biotechnol. Bioeng*. **2000**, *67*, 25−34.
(14) Franěk, F.; Šrámková, K. Protection of B Lymphocyte Hybridoma against Starvation-Induced Apoptosis: Survival-Signal Role of Some Amino Acids. *Immunol. Lett*. **1996**, *52*, 139−144.

Accepted for publication September 20, 2002.

BP020077M