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Comparison of the Contributions of Tetrahydrofurfuryl Alcohol and PEG to α-Chymotrypsin Renaturation with High Performance Hydrophobic Interaction Chromatography

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Abstract: The contributions of tetrahydrofurfuryl alcohol (THFA) and polyethylene glycol (PEG) to the renatured efficiency of α-chymotrypsin were investigated and compared with each other. The maximum increments of bioactivity recovery of α-Chy were found to be 25.1% for THFA, 10.4% for PEG, respectively. The experimental results indicated that the denaturant solution containing THFA contributed more to the renaturation of α-Chy in high performance hydrophobic interaction chromatography (HPHIC) than that containing PEG, when the concentration of THFA was 3.2%, the bioactivity recovery of α-Chy is the highest.

Keywords: High-perforamnce hydrophobic interaction chromatography, tetrahydrofurfuryl alcohol (THFA), polyethylene glycol (PEG), protein renaturation, α-chymotrypsin.

The investigation of protein renaturation, or refolding has become a very hot point in both liquid chromatography (LC) and life science fields. Besides usual dilution and dialysis methods, many new methods concern protein renaturation have been presented, such as reverse micelles[1], chaperones[2], polyethylene glycol (PEG)[3], surfactant[4] and antibodies[5] *etc*. One of the authors suggested high performance hydrophobic interaction chromatography (HPHIC) to be a new tool for protein renaturation[6]. A review on protein renaturation with liquid chromatography (LC) was recently reported also[7]. However, not all denatured proteins can be renatured partially or completely by LC. So study must be made to enhance the renaturation efficiency for the proteins which are difficult to be renatured. In this paper, a new type of protein cosolvent, tetrahydrofurfuryl alcohol, was first employed for the renaturation of one of the difficultly renatured proteins, α-chymotrypsin (α-Chy), originally denatured in guanidine hydrochloride solution, by high performance hydrophobic interaction chromatography (HPHIC). From the experimental results, it was found that the renaturation efficiency of α-Chy was indeed increased by adding THFA into the denaturing agent solution. Furthermore, based on the HPHIC method, the different contributions between tetrahydrofuryl alcohol (THFA) and PEG to the renaturation of α-Chy were studied and compared. THFA was found to be better than PEG in enhancing the renaturation

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efficiency of α-Chy.

Experimental

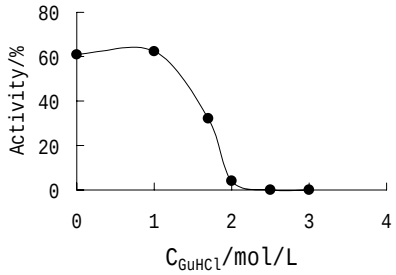
5.0 mg solid α-Chy (Shanghai Reagent Company, Shanghai, China) was dissolved in 1.0 mL of 1.7 mol/L guanidine hydrochloride (GuHCl) and 1.0 mL of 1.7 mol/L GuHCl with different concentrations of THFA and PEG respectively. The samples were then incubated at 25°C and 200 rpm for 24 hours. 200 μL of each sample was injected into the column and the eluate with the same retention time as that of the native α-Chy was collected. The bioactivity recoveries of α-Chy were determined according to reference 8.

A Shimadzu LC-10A system with LC-10Atvp pumps, SPD-10Avp UV-VIS detector monitoring at 280 nm and Class-vp 5.03 software was used. The HPHIC packings was synthesized and packed by ourselves. Mobile phases consisted of solutions A of 2.5 mol/L $(NH_4)_2SO_4$ +0.05 mol/L KH_2PO_4 (pH7.0) and B of 0.05 mol/L KH_2PO_4 (pH7.0). The linear gradient was 0~100%B in 25 min with 1.0 mL/min flow rate.

Results and Discussion

The bioactivity recovery of the renatured α-Chy by HPHIC under various concentrations of GuHCl is shown in **Figure 1**.

Figure 1 The bioactive recovery of α-Chy denatured in different concentrations of guanidine hydrochloride



From **Figure 1**, the denatured α-Chy by 1.70 mol/L GuHCl can only be partially (about 30%) renatured by usual HPHIC. This concentration of 1.70 mol/L GuHCl was selected to be the denatured condition of α-Chy, because it would be sensitive to detect any changes in the bioactivity recovery of α-Chy due to the presence of THFA or PEG. To avoid the possible appearance of the aggregation and/or precipitation of the denatured α-Chy, THFA or PEG was added in the denaturing solution GuHCl during the α-Chy denaturing for 24 hrs. The bioactivity recovery of the renatured α-Chy by HPHIC under various concentrations of THFA and PEG were listed in **Table 1**.

From R_1 and ΔR_1 shown in **Table 1**, when THFA was added into 1.7 mol/L GuHCl

in the concentration range from 1.92% to 4.0% (V/V), the bioactivity recoveries of the renatured α-Chy were higher. When the concentration of THFA is 3.2%, the bioactivity recovery of α-Chy from 34.2% approaches to maximum, 59.3%. However, with the continuously increasing the concentration of THFA, *i.e*, 8.0% ~ 16.0%, the bioactivity recovery of the renatured α-Chy decreased.

Table 1 The bioactivity recoveries of the renatured α-Chy in the presence of various concentrations of THFA and PEG by HPHIC

THFA(v/v, %)*	R_1 (%)	ΔR_1(%)	PEG (v/v, %)*	R_2 (%)	ΔR_2 (%)
0	34.2	0	0	34.2	0
1.92	45.0	10.8	0.24	28.4	-5.8
2.4	55.0	20.8	0.48	33.5	-0.7
3.2	59.3	25.1	0.88	44.6	10.4
4.0	37.7	3.5	1.28	37.4	3.2
6.0	30.2	-4.0	2.08	35.8	1.6
8.0	21.7	-12.5	3.68	31.9	-2.3

*: The ratio of THFA and PEG added to 1.70 mol/L GuHCl solution. R_1 and R_2 are the bioactivity recoveries of the renatured α-Chy by HPHIC, respectively. ΔR_1 and ΔR_2 are the increments (positive sign) or decrements (negative sign) of the bioactivity recoveries after addition of THFA and PEG into 1.70 mol/L GuHCl, respectively.

The experiment result showed that the bioactivity recovery of the renatured α-Chy with PEG was the similar as that with THFA. However, some differences between them still exist. When the concentration of PEG was in the range from 0.88% to 2.08%, the bioactivity recovery of α-Chy was increseaed than that in the absence of PEG. The maximum bioactivity recovery was found at 0.88% PEG (V/V). When the concentration of PEG increased continuously from 1.78% to 3.68%, the bioactivity recovery of the renatured α-Chy decreased.

The maximum increments of bioactivity recovery of α-Chy for THFA and PEG are 25.1%, 10.4%, respectively. The difference of maximum even approached to 14.7%. In conclusion, the experimental results indicated that the renaturing efficiency of α-Chy was higher in the presence of THFA than that of PEG, when the concentration of THFA was lower than 6%.

Two alcohols interacted with α-Chy give the positive contributions to the α-Chy renaturation. But the stationary phase of HPHIC could absorb the alcohols, when the concentration reached appropreate value. The negative contribution to the α-Chy renaturation was obtained. That is why when the concentration of THFA was higher than 3.2%, the positive contribution to α-Chy renaturation decreased. THFA and PEG have both hydrophilic and hydrophobic groups, but the THFA has stronger hydrophobicity. Thus, α-Chy not only can interact with THFA, even more strongly, as proteins interact with PEG and lipid[3]. The hydrophobic groups in THFA and PEG associated with protein intermediates by hydrophobic interactions and the hydrophilic groups in these alcohols faced to the solution to avoid aggregation of denatured α-Chy. In addition, these two alcohols also increased the viscosity of the solution and limited the movement of denatured α-Chy molecules and also partially inhibited the aggregation

and/or precipitation. When the concentrations of THFA and PEG were too high, the amounts of adsorbtion of these two alcohols on the HPHIC stationary phase would greatly increase and occupied more and more surface of the stationary phase with the continuous increase of concentration. This fact would decrease the HPHIC stationary phase to protein renaturation[9]. The conclusion when the concentrations of THFA and PEG were 3.2% and 0.88%, respectively, the highest renaturation efficiency of α-Chy could be obtained.

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