Journal of Chromatography, 513 (1990) 367–369
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 426

Note

Effect of type and concentration of coupling buffer on coupling yield in the coupling of proteins to a tresyl-activated support for affinity chromatography

KOJI NAKAMURA, TSUTOMU HASHIMOTO and YOSHIO KATO*
Central Research Laboratory, TOSOH Corporation, Tonda, Shinnanyo, Yamaguchi 746 (Japan)
and
KIYOHITO SHIMURA and KEN-ICHI KASAI
Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01 (Japan)
(First received November 23rd, 1989; revised manuscript received February 27th, 1990)

Affinity chromatography is a very powerful technique for separating biological substances such as proteins, owing to its selectivity. However, a different support coupled with a ligand that is complementary to the sample to be separated must be prepared for a different kind of sample. To simplify this, it is desirable to have good activated supports to which many ligands can be coupled easily under mild conditions. Various activation methods were examined in the past, and a method with tresyl chloride[1] was found to be useful. Subsequently some tresyl-activated supports based on agarose or silica became commercially available but they had unsatisfactory mechanical or chemical stability of the base material. However, new tresyl-activated supports based on synthetic hydrophilic resin have become commercially available recently under the trade-names TSKgel Tresyl-Toyopearl 650M (ref. 2) and Tresyl-5PW (ref. 3). We have been evaluating these supports for the coupling of proteins and have reported results of the study of coupling conditions, pH of the coupling buffer, temperature, reaction time and amount of reacted ligand[2]. We have now examined the effects of the type and concentration of the coupling buffer on the coupling yield and the results are reported here.

EXPERIMENTAL

Proteins were dissolved in 4 ml of coupling buffer and mixed with 0.4 g of dried Tresyl-Toyopearl 650M powder, which gives a volume of 2.0 ml in the swollen state. After the mixture had been left to stand with gentle shaking at a constant temperature for a certain period of time, 36 ml of distilled water were added and the diluted mixture was filtered with a sintered-glass funnel (this dilution was necessary to prevent adsorption of proteins on the support without covalent bonding in some instances). The protein coupling yield was calculated from the UV absorption at 280 nm of protein solutions before and after the coupling reaction. The coupling buffers

0021-9673/90/$03.50 © 1990 Elsevier Science Publishers B.V.

examined were 0.1–1 *M* carbonate buffers (pH 8.0), 0.1–1 *M* N-(2-hydroxyethyl)piperazine-N'-2-ethanesulphonic acid (HEPES) buffers (pH 8.0), 0.1–1 *M* phosphate buffers (pH 8.0) and 0.1 *M* carbonate buffer (pH 8.0) containing 0.5–2.0 *M* sodium chloride. The proteins employed were purchased from Sigma (St. Louis, MO, U.S.A.).

RESULTS AND DISCUSSION

The coupling yields observed for bovine serum albumin in the various buffers are summarized in Table I. When the buffer concentration increased from 0.1 to 1 *M*, the coupling yield remained almost unchanged with carbonate buffer, but it increased with HEPES and phosphate buffers. It also increased with increase in the sodium chloride concentration in 0.1 *M* carbonate buffer. In particular, it increased to a great extent when the concentration of phosphate buffer was raised to 1 *M*. We postulate that this great increase in coupling yield occurred because the bovine serum albumin was forced to approach near the surface of the support owing to the salting-out effect of 1 *M* phosphate buffer and the coupling reaction between bovine serum albumin and tresyl groups then became easier. We also examined if 1 *M* phosphate buffer is effective for the coupling of other proteins. Some proteins were coupled in 1 *M* phosphate buffer (pH 8.0) and in 0.1 *M* carbonate buffer (pH 8.0) containing 0.5 *M* sodium chloride. Proteins for which low coupling yields were observed in carbonate buffer were selectively used in this experiment. The results are summarized in Table II. Much higher coupling yields were obtained in 1 *M* phosphate buffer than in carbonate buffer for all the proteins examined except cytochrome C. In particular, almost quantitative coupling yields were achieved for α-1-antitrypsin, bovine serum albumin, myoglobin and α-chymotrypsin, although the coupling reaction was performed at 4°C for 4 h. The coupling yield did not change for cytochrome *c* with the two buffers, probably because phosphate buffer of 1 *M* is not concentrated enough to salt-out cytochrome *c* as the latter is very hydrophilic. The coupling yield may be increased by further increasing the phosphate buffer concentration, *e.g.*, up to 1.5 *M*.

It is concluded that 1 *M* phosphate buffer is very effective in general for the coupling of protein ligands to Tresyl-Toyopearl 650M. It is better than buffers which have been commonly employed in the coupling of ligands to various types of activated

TABLE I

EFFECTS OF TYPE AND CONCENTRATION OF COUPLING BUFFER ON COUPLING YIELD

Bovine serum albumin (30 mg) was reacted with TSKgel Tresyl-Toyopearl 650M (0.4 g) in 4 ml of various buffers (pH 8.0) at 25°C for 5 h.

Coupling buffer	*Coupling yield (%)*	*Coupling buffer*	*Coupling yield (%)*
0.1 *M* carbonate buffer	16	0.1 *M* phosphate buffer	17
0.5 *M* carbonate buffer	15	0.5 *M* phosphate buffer	41
1.0 *M* carbonate buffer	12	1.0 *M* phosphate buffer	88
0.1 *M* HEPES buffer	16	0.1 *M* carbonate buffer containing 0.5 *M* NaCl	28
0.5 *M* HEPES buffer	35	0.1 *M* carbonate buffer containing 1.0 *M* NaCl	29
1.0 *M* HEPES buffer	32	0.1 *M* carbonate buffer containing 2.0 *M* NaCl	36

TABLE II

COUPLING OF VARIOUS PROTEINS TO TSKgel Tresyl-Toyopearl 650M

Proteins (20 mg) were reacted with Tresyl-Toyopearl 650M (0.4 g) in 4 ml of (A) 0.1 *M* carbonate buffer (pH 8.0) containing 0.5 *M* NaCl and (B) 1 *M* phosphate buffer (pH 8.0) at 4°C for 4 h.

Protein	*pI*	*Coupling yield (%)*	
		A	B
α_1-Acid glycoprotein	1.8–2.8	2	58
α-1-Antitrypsin	4.0	25	98
Bovine serum albumin	4.9	31	99
Myoglobin	6.8–7.8	55	99
α-Chymotrypsin	9.1	70	100
Cytochrome *c*	10.1	43	46

supports, such as 0.1 *M* carbonate buffer (pH 8.0) containing 0.5 *M* sodium chloride. When 1 *M* phosphate buffer is not effective in achieving a high coupling yield, higher concentrations, *e.g.*, 1.5–2 *M*, may be useful for increasing the coupling yield. It is considered that other buffers having similar salting-out effects to 1 *M* or more concentrated phosphate buffer would also be effective.

REFERENCES

1 K. Nilsson and K. Mosbach, *Biochem. Biophys. Res. Commun.*, 102 (1981) 449.
2 K. Nakamura, K. Toyoda, Y. Kato, K. Shimura and K. Kasai, *J. Chromatogr.*, 478 (1989) 159.
3 K. Nakamura, T. Hashimoto, Y. Kato, K. Shimura and K. Kasai, *J. Chromatogr.*, 510 (1990) 101.