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Note

Affinity of phycoerythrin chromopeptides to histidyl, tyrosyl and tryptophyl Sepharose gels

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Histidyl Sepharose results in specific separations of chromopeptides from phycocyanin, which could be a model for histidine–tetrapyrrole interactions in biliproteins[1]. If preferential interactions exist between phycobilin and histidine, histidyl Sepharose should also separate phycoerythrin chromopeptides whereas the parent gels tyrosyl and tryptophanyl Sepharose should show weaker interactions.

EXPERIMENTAL

Materials

Phycoerythrin from *Porphyridium cruentum* was a gift from Dr. H. P. Köst (Munich, F.R.G.) and was 30% pure. Pepsin was purchased from Sigma (St. Louis, MO, U.S.A.) and substituted histidyl, tyrosyl and tryptophanyl Sepharose gels were prepared according to ref. 2 from D,L-histidine, -tyrosine and -tryptophan. The degree of substitution determined by the micro-Kjeldahl method was within the range 0.4–0.6 mmol per gram of dried gel for each gel. Columns of I.D. 1 cm and with volumes adjustable with piston adapters were a gift from BMC (Uppsala, Sweden). Silica gel plates and other chemicals for high-performance thin-layer chromatography (HPTLC) were purchased from Merck (Darmstadt, F.R.G.). The absorbances of the chromatographic fractions were determined with a Hewlett-Packard HP 8450A UV–visible spectrophotometer.

Preparation of phycoerythrin peptides

A partially purified preparation of phycoerythrin was prepared according to Köst-Reyes and Köst[3]. A suspension of *Porphyridium cruentum* in 0.1 *M* Tris–HCl (pH 7.8) was frozen and thawed twice. The suspension was then centrifuged for 30 min at 30,000 *g*; the supernatant containing phycoerythrin was then adjusted to 40%

ammonium sulphate saturation and fractionated. The precipitated proteins were dialysed against distilled water, lyophilized and kept in the dark at −20°C. For digestion, samples of the lyophilized preparation (10 mg) were dissolved in 100 ml of distilled water and the solution was acidified with 5 ml of formic acid (final pH 1.5–2.0). The solution was incubated with 0.5 mg of pepsin for 1 h at 37°C (water-bath) and then immediately lyophilized. Samples of the digest (mixture of chromopeptides and colourless peptides) were dissolved in 0.5 ml of the desired buffer (see Figs. 1–3) for chromatography. All manipulations of the pigment were carried out in dim light as previously described[1]. The samples were concentrated by lyophilization before TLC using *n*-butanol–acetic acid–water (4:4:1) as the solvent system.

RESULTS AND DISCUSSION

A peptide–chromopeptide mixture from phycoerythrin was eluted on histidyl Sepharose at pH 5 (Fig. 1a), which was the optimal pH for the separation of phycocyanin chromopeptides[1]. The results were expressed in relative elution volume, *i.e.* the ratio of the elution volume (V_e) of the substance eluted to the total volume (V_t) of the column bed. The chromopeptides were more retarded than the colourless peptides and separated into two major peaks (V_e/V_t = 1.12 and 1.36). Analysis of the chromopeptide fractions by TLC showed a separation into two main groups of chromopeptides, which eluted in the same ordeRr on both silica gel plates and on histidyl Sepharose gel. The separation was similar for the chromopeptides from phycoerythrin and for those from phycocyanin. Moreover, the two major groups of chromopeptides eluted in each instance had very similar TLC R_F values for phycoerythrin and phycocyanin.

However, the resolution obtained with chromopeptides from phycoerythrin was poorer than that from phycocyanin. The chromopeptide fractions from phy-

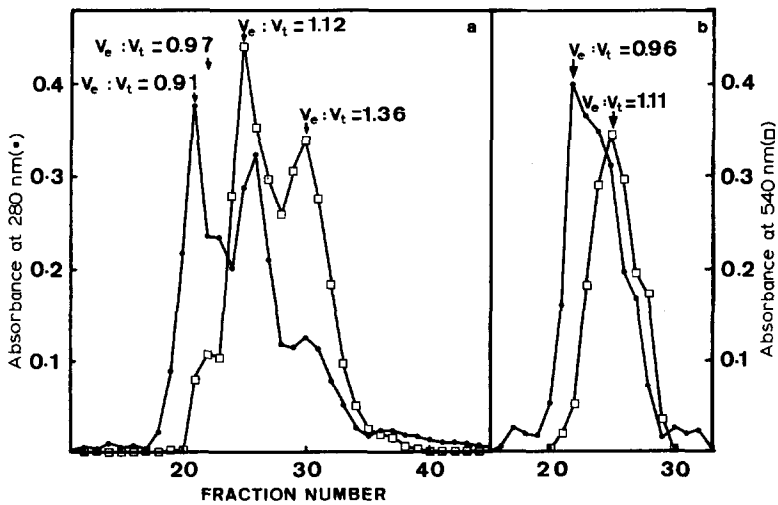

Fig. 1. Elution pattern of phycoerythrin digest on a histidyl Sepharose 4B column (length 22.6 cm). Absorbance at □, 540 nm and ●, 280 nm. (a) 0.1 *M* ammonium acetate buffer at pH 5.0, sample volume 0.5 ml, fraction volume 0.87 ml; (b) 0.1 *M* imidazole · HCl buffer at pH 5.0, sample volume 0.5 ml, fraction volume 0.87 ml.

coerythrin so purified still contained colourleess peptides. TLC analysis of these fractions revealed that the ninhydrin coloration corresponded to colourless peptides which co-migrated with the chromopeptides. We are currently investigating whether the difference in the interactions was due to the conjugated double bonds of the chromophores or the ionic state of the chromopeptides. The two conjugated double bonds less for the chromophore of phycoerythrin could explain its weaker interaction with the gel than the chromophore of phycocyanin. Another explanation could be the difference in the ionic states of these chromopeptides, reflected by the difference in the isoelectric points of the chromopeptides obtained from phycocyanin and phycoerythrin. We are looking for chromopeptides with structures similar to those of these two pigments in order to clarify these hypotheses.

If phycoerythrin and phycocyanin chromopeptides show different strengths of interactions with histidyl Sepharose, the separations obtained for both demonstrate that the model of the histidine–tetrapyrrole interaction with biliproteins is valid for both phycocyanin and phycoerythrin.

To test the specificity of the amino acid partner in the interaction we eluted phycoerythrin chromopeptides on Sepharose gels linked with amino acids with analogous structures. Tyrosyl and tryptophanyl Sepharose gave similar figures with interactions due to both charge transfer and —hydrophobic and ionic forces[4].

On tyrosyl Sepharose the chromopeptides were best separated at pH 5 (Fig. 2a, Table I), and eluted separately from the colourless peptide fractions. However, the chromopeptides were not well separated from each other and only one peak appeared, at $V_e/V_t = 1.15$; the second fraction eluted as a shoulder of the main peak at $V_e/V_t = 1.3$. With a lower or higher pH of the elution buffer, some colourless peptides were more retarded without improving the separation of the chromopeptides. No separation of the chromopeptides was obtained at pH 3 or 6 (Table I).

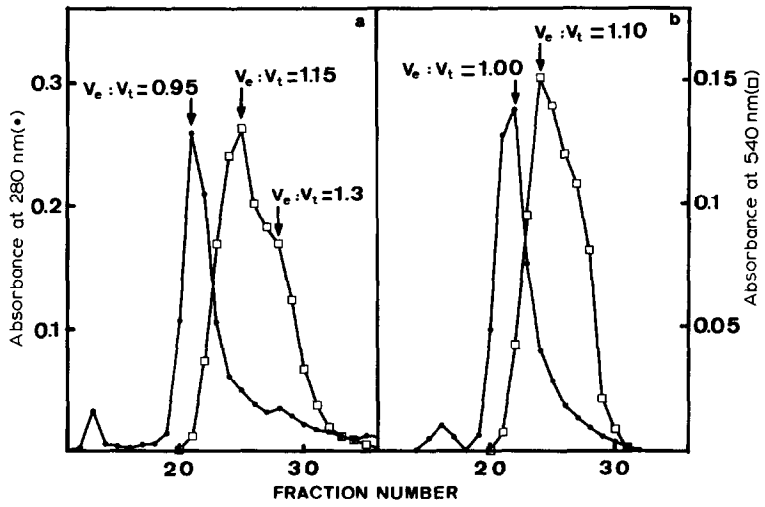

Fig. 2. Elution pattern of phycoerythrin digest on a tyrosyl Sepharose 4B column (length 21.8 cm). Absorbance at □, 540 nm and ●, 280 nm. (a) 0.1 *M* ammonium acetate buffer at pH 5.0, sample volume 0.5 ml, fraction volume 0.87 ml; (b) 0.1 *M* imidazole · HCl buffer at pH 5.0, sample volume 0.5 ml, fraction volume 0.87 ml.

TABLE I

INFLUENCE OF pH ON THE ELUTION OF A PHYCOERYTHRIN DIGEST ON TYROSYL SEPHAROSE

Values in parentheses represent shoulders on the major peak.

pH	*Chromopeptides*		*Colourless peptides*	
	V_e/V_t	A_{540}/A_{280}	V_e/V_t	A_{540}/A_{280}
3	1.25	1.26	0.90	0
4	1.153	1.37	0.848	0
	(1.35)	(0.73)		
5	1.153	2.64	0.95	0.02
	(1.30)	(2.361)		
6	1.148	0.995	1.03	0.247

Elution chromatography on tryptophyl Sepharose separated only a colourless fraction from one chromopeptide peak. The optimum separation was obtained at pH 4, but some colourless peptides were also retarded (Fig. 3). The separation between peptides and chromopeptides was worse than for the two other gels and only one chromopeptide peak was observed.

Hence the interactions are weaker than would be expected from the structure of the indole ring and the high retentions of, *e.g.*, tyrosyl residues and the di- and tripeptides on the same gel[2,4]. This emphasizes the specificity of the interaction of the histidyl partner *versus* other aromatic amino residues.

Moreover, when the elution is carried out on histidyl Sepharose at pH 5 but

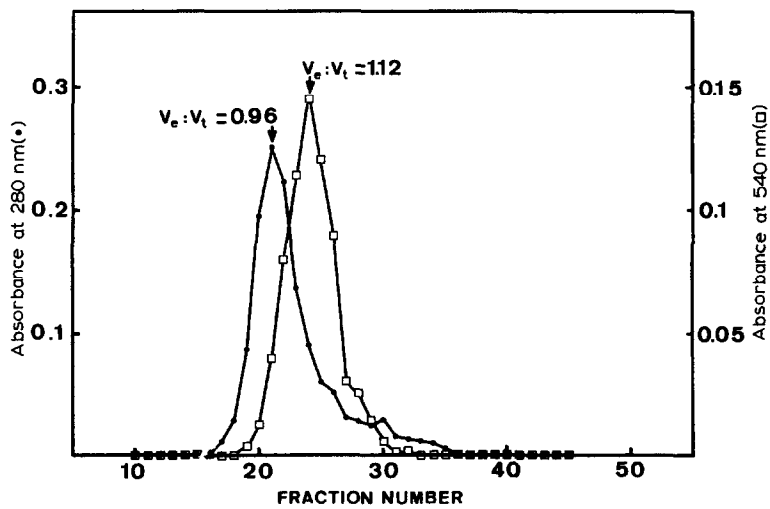

Fig. 3. Elution pattern of phycoerythrin digest on a tryptophyl Sepharose 4B column (length 21.5 cm). Absorbance at □, 540 nm and ●, 280 nm. 0.1 *M* ammonium acetate buffer at pH 4.0, sample volume 0.5 ml, fraction volume 0.87 ml.

with an imidazole buffer as a competitor for histidyl the separation is completely suppressed (Fig. (b). The same inhibition occurs for the separation of these chromopeptides on tyrosyl Sepharose.

This demonstrate the specific character of the interaction of the imidazole ring of the histidyl ligand with the chromopeptides. It can be concluded from these results that the interactions of phycobilin chromophores with histidine are stronger than for other amino acids. For practical purposes or for comparison of amino acid–chromophore interactions for biliproteins, the results show that chromopeptides from different sources can be separated on histidyl Sepharose.

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REFERENCES

1 J. Rabier, M. A. Vijayalakshmi and W. Rüdiger, *Z. Naturforsch.*, 38 (1983) 230.
2 M. A. Vijayalakshmi and J. Porath, *J. Chromatogr.*, 177 (1979) 201.
3 E. Köst-Reyes and H. P. Köst, *Eur. J. Biochem.*, 102 (1979) 83.
4 M. A. Vijayalakshmi, *Thesis, Docteur en Science*, University of Compiègne, Compiègne, 1980.