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Short Communication

Adsorption chromatography on cellulose

VIII. The salting-out behaviour of some peptides with aromatic groups

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ABSTRACT

The adsorption behaviour of some aromatic peptides on cellulose thin layers was studied. It was found that tryptophanyltyrosine and ditryptophan exhibited a "double" salting-out effect. Similarly, it was shown that for the series tyrosine, dityrosine, trityrosine and hexatyrosine the slope of the plot of R_M *vs.* salt concentration increased with increase in the number of amino acid residues, indicating an increase in the salting-out effect.

INTRODUCTION

Relatively few organic substances adsorb on cellulose from water and those which do have usually aromatic groups and exhibit an increase in adsorption when salting-out agents are added to the solution (*e.g.*, ammonium sulphate).

In previous work it became evident that this salting-out effect is generally the same for most substances so that graphs of R_M *versus* salt concentration yield parallel curves [1]. There are two notable exceptions to this general behaviour. Leu-enkephalin and Met-enkephalin exhibited a "double" salting-out effect, and this was explained [2] as being due to two aromatic groups adsorbing "independently" on the cellulose surface. Peptides with only one aromatic amino acid did not show this "double effect". The situation is best shown in Fig. 1, where the steeper salting-out slope of the two enkephalins is evident. The other exception to the general rule is inorganic anions such as perrhenate, which have a lower salting-out "slope".

In this paper we report on the first exception for which we have more results with a wider range of peptides.

EXPERIMENTAL

All chromatograms were obtained with cellulose thin layers (Merck cellulose Art. No. 5577, made of microcrystalline cellulose), unless stated otherwise. They were developed with ammonium sulphate so-

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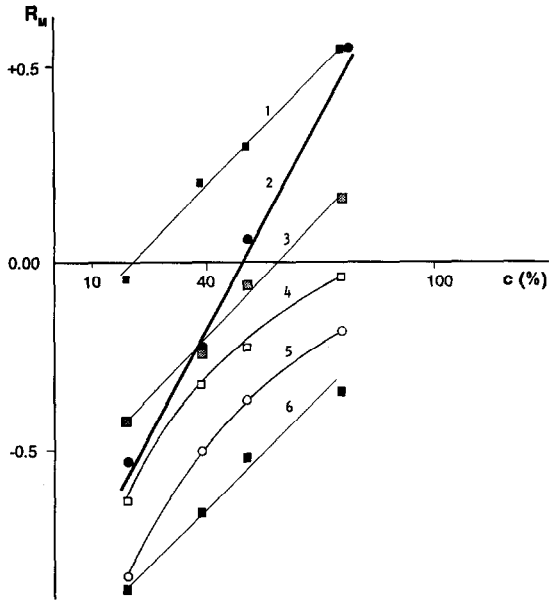

Fig. 1. R_M *versus* salt concentration plot for some data taken from ref. 2. Curves: 1 = L-tryptophan; 2 = Leu-enkephalin, Met-enkephalin; 3 = L-alanyl-L-tryptophan; 4 = L-tyrosine; 5 = L-phenylalanine; 6 = L-alanyl-L-tyrosine.

lutions by ascending development in small, well closed jars. We prepared a saturated solution of ammonium sulphate at room temperature (22°C) and diluted this with deionized water. The saturated solution is referred to in this work as 100% and subsequent dilutions are referred to as 10% or 20%, *i.e.*, 10% or 20% of the saturation concentration.

Spots of the peptides and amino acids were detected with iodine vapour or with 1% ninhydrin in acetone.

RESULTS

Tryptophan peptides

Table I and Fig. 2 show the R_F and R_M values of ditryptophan and tryptophanyltyrosine compared with tryptophan and tryptophanamide. Both dipeptides show a "double" salting-out effect, *i.e.*, a steeper slope of the R_M *vs.* ammonium sulphate concentration plot than tryptophan or tryptophanamide, while dipeptides formed of an aromatic and a non-aromatic amino acid give the usual slope, as shown in Fig. 1 for L-Ala-L-Trp and L-Ala-L-Tyr.

Tyrosine peptides

Table II and Fig. 3 show the R_F and R_M values of the series tyrosine (both D- and L-), dityrosine, trityrosine and hexatyrosine. Here the slope increases with each additional tyrosine residue and thus the salting-out effect really increases with increase in the number of amino acids in the peptide.

We repeated this work with 40-cm long thin layers of cellulose MN 400 (microcrystalline cellulose) and cellulose MN 300-50 (native cellulose). These yielded the same results as above for mono-, di- and trityrosine, but hexatyrosine yielded elongated

TABLE I

R_F AND R_M VALUES OF SOME TRYPTOPHAN COMPOUNDS ON CELLULOSE THIN LAYERS (MERCK ART. NO. 5577) DEVELOPED WITH AQUEOUS AMMONIUM SULPHATE SOLUTIONS

Compound	Parameter	Ammonium sulphate concentration (%)				
		10	20	40	60	80
D-Tryptophan	R_F	0.59	0.53	0.43	0.34	0.27
	R_M	−0.158	−0.052	0.122	0.288	0.43
DL-Tryptophanamide	R_F	0.68	0.60	0.47	0.42	0.31
	R_M	−0.327	−0.176	0.052	0.14	0.347
L-Tryptophanyl-L-tyrosine	R_F	0.62	0.51	0.36	0.26	0.15
	R_M	−0.212	−0.017	0.25	0.454	0.75
L-Tryptophanyl-L-tryptophan	R_F	0.44	0.35	0.20	0.125	0.06
	R_M	0.105	0.269	0.60	0.87	1.2

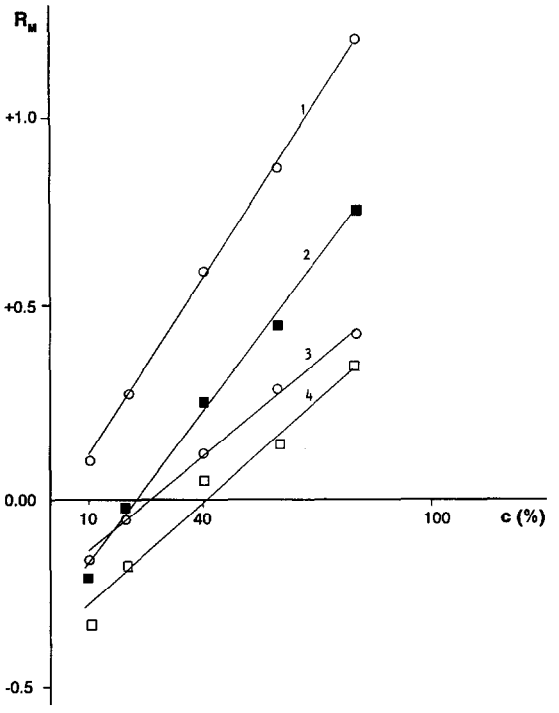

Fig. 2. R_M *versus* salt concentration plot for tryptophan and peptides reported in Table I. Curves: 1 = L-tryptophanyl-L-tryptophan; 2 = L-tryptophanyl-L-tyrosine; 3 = D-tryptophan; 4 = DL-tryptophanamide.

spots and could not be detected at low concentrations. This may be due to an unpredictable interaction between irregular cellulose adsorption sites and the six independently adsorbing aromatic groups of the hexapeptide.

Glycine peptides

Di-, tri-, tetra-, penta- and hexaglycine were all found to move just below the liquid front at all concentrations of ammonium sulphate. Hence there is no increased adsorption in this series of peptides.

DISCUSSION

The adsorption of organic compounds on cellulose from aqueous solutions has been considered to be akin to liquid–liquid chromatography in which the stationary phase (the cellulose–water complex [3]) is the less polar phase. Satisfactory ΔR_M values have been obtained for numerous functional groups [2] to support this assumption.

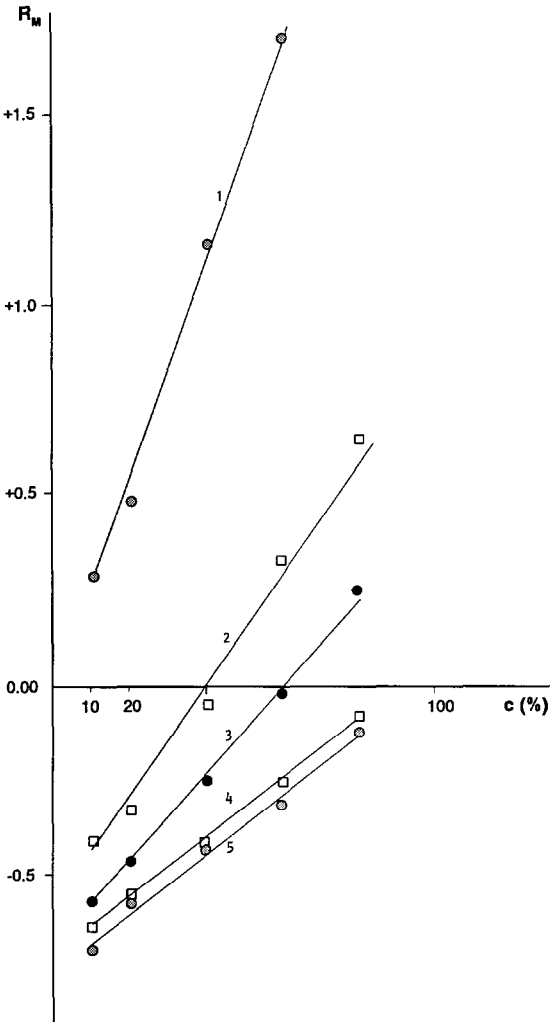

Fig. 3. R_M *versus* salt concentration plot for tyrosine peptides reported in Table II. Curves: 1 = hexatyrosine; 2 = trityrosine; 3 = dityrosine; 4 = D-tyrosine; 5 = L-tyrosine.

Aromatic compounds with two or more "hydrophobic" centres were found to give an unexpected behaviour. Usually ΔR_M values for functional groups such as CH_3 and OH vary, but not greatly, on increasing the ammonium sulphate concentration in the eluent [2], but an exponential behaviour, as observed here, is only known in ion-exchange equilibria (where there is desorption with increasing salt concentration, not greater adsorption). This unexpected behaviour may stimulate peptide chemists to explore further peptides in cellulose adsorption chromatography.

TABLE II

R_F AND R_M VALUES OF TYROSINE PEPTIDES ON CELLULOSE THIN LAYERS (MERCK ART NO. 5577) DEVELOPED WITH AQUEOUS AMMONIUM SULPHATE SOLUTIONS

Compound	Parameter	Ammonium sulphate concentration (%)				
		10	20	40	60	80
L-Tyrosine	R_F	0.83	0.79	0.73	0.67	0.57
	R_M	−0.69	−0.575	−0.43	−0.31	−0.12
D-Tyrosine	R_F	0.81	0.78	0.72	0.64	0.55
	R_M	−0.63	−0.55	−0.41	−0.25	−0.08
Dityrosine	R_F	0.79	0.75	0.66	0.51	0.36
	R_M	−0.57	−0.477	−0.25	−0.02	0.25
Trityrosine	R_F	0.72	0.68	0.47	0.32	0.18
	R_M	−0.41	−0.327	0.05	0.33	0.65
Hexatyrosine	R_F	0.34	0.25	0.06	0.019	0.006
	R_M	0.29	0.48	1.16	1.70	2.2

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