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Note

Interaction of arylsulphatase-A with SP-Sephadex

A note on the isolation of arylsulphatase-A from human urine

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In a study intended to improve the isolation procedure of arylsulphatase-A (EC 3.1.6.1) from human urine, following the procedure described by Stevens *et al.*[1], we encountered unexpectedly low yields in the separation step with SP-Sephadex. In column experiments we found generally 40–80% of the enzyme activity in the void volume of the adsorption buffer and only 0–20% immediately after the breakthrough volume of the desorption buffer. Experiments with arylsulphatase-A in dialysed urine concentrates and with arylsulphatase from *Helix pomatia* gave analogous results.

Therefore this separation step, described by Stevens *et al.*[1] as a biospecific adsorption of the enzyme by the sulphopropyl groups on the SP-Sephadex, was studied in more detail.

EXPERIMENTAL, RESULTS AND DISCUSSION

Extracts in 0.02 *M* Tris–HCl*, pH 7.5, of acetone-washed ammonium sulphate precipitates of human urine were prepared according to Stevens *et al.*[1]. Aliquots (10 ml) of this extract, containing *ca.* 700 Baum-units** of arylsulphatase-A activity and *ca.* 2–3 mg of protein*** per ml, were dialysed against adsorption buffer. 0.5 ml portions of this dialysate were rotated in centrifuge tubes at 4 or 22° for 4–72 h, with 0.1 or 0.5 ml of a slurry of 0.5 g of SP-Sephadex C50 (Pharmacia, Uppsala, Sweden) in 50 ml of adsorption buffer. The tubes were centrifuged and the activity was measured in the supernatant. By comparing this activity with that found in the dialysate the percentage of bound enzyme was calculated. The ionic strength and the pH of the adsorption buffer (10 m*M* sodium acetate) were varied systematically. In Fig. 1 the influence of the ionic strength is given, together with that of incubation time, temperature and gel volume. In Fig. 2 the effect of pH is shown. (In the experiments at pH 4.25 the dialysate was turbid; at pH $\leqslant$ 4.00 a precipitate formed.)

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* All solutions of the enzyme contained 0.02% of sodium azide.

** Enzyme activity was determined according to Baum *et al.*[2]. One Baum-unit represents the amount of enzyme liberating 1 μg of nitrocatechol per hour under the prescribed test conditions.

*** Protein content was determined according to Lowry *et al.*[3].

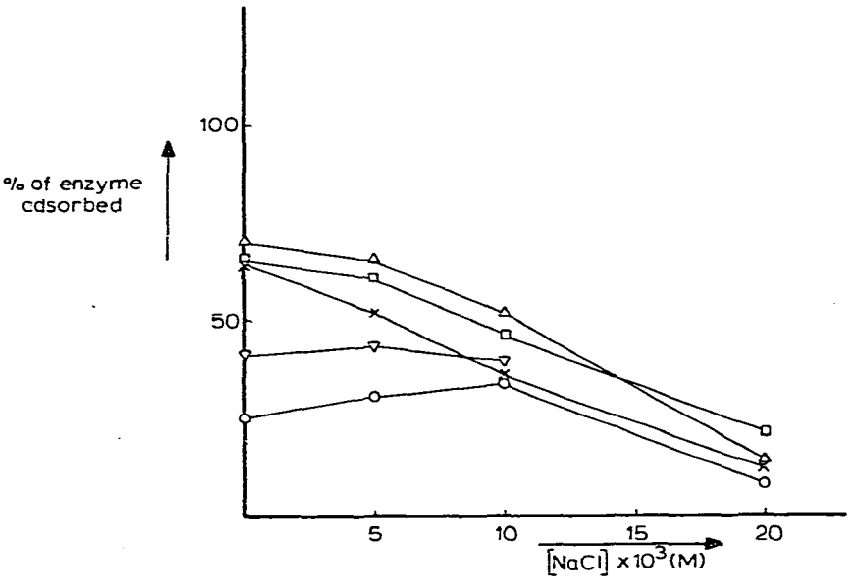

Fig. 1. Percentage of enzyme adsorbed versus sodium chloride content of the adsorption buffer: 10 m*M* sodium acetate, pH 5.10. Conditions (gel volume, temperature, incubation time): 0.1 ml, 4°, 4 h (○); 0.1 ml, 4°, 24 h (▽); 0.1 ml, 4°, 48 h (×); 0.1 ml, 22°, 24 h (□) and 0.5 ml, 4°, 48 h (△).

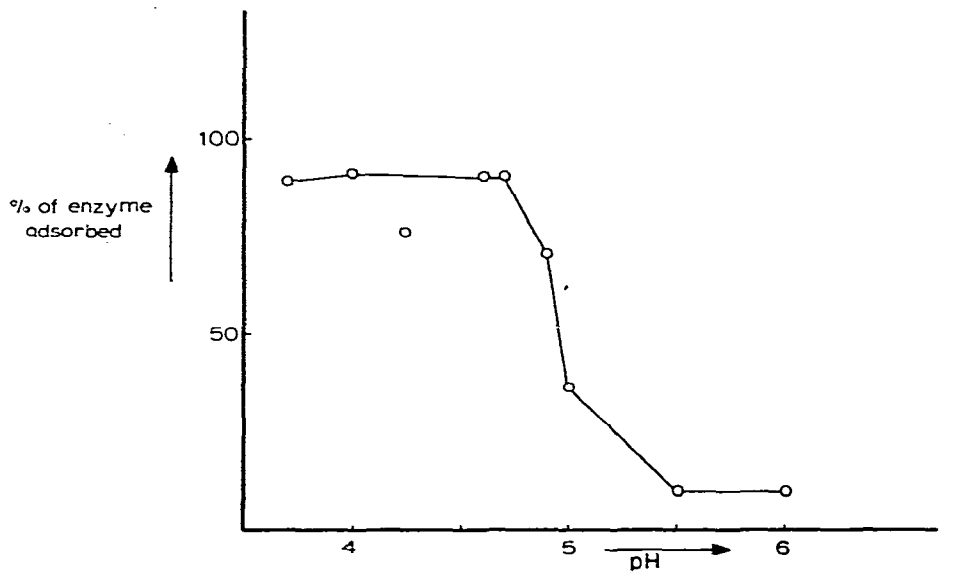

Fig. 2. Percentage of enzyme adsorbed versus pH of the adsorption buffer: 10 m*M* sodium acetate + 10 m*M* sodium chloride. Conditions: 0.1 ml gel, temperature 4°, incubation time 48 h. (Increasing the incubation time to 72 h did not influence the results appreciably).

The results of these batch experiments corroborate the above-mentioned findings with column experiments. Moreover, they indicate that the adsorption conditions employed by Stevens *et al.*[1] (buffer: 10 m*M* sodium acetate + 20 m*M* sodium chloride, pH 5.0) are not ideal: at lower ionic strength (Fig. 1) and lower pH (Fig. 2) the degree of adsorption is significantly enhanced.

In order to find better conditions for the purification of the enzyme by this separation step, some adsorption/desorption experiments were performed.

After coupling (4°, 48 h) of the enzyme to SP-Sephadex by the procedure outlined above, the gel was repeatedly washed with adsorption buffer until no activity could be detected in the supernatant. Subsequently the gel was treated several times at 4° with desorption buffer (10 m*M* sodium acetate of variable pH and ionic strength) until no activity was desorbed from the gel. Enzyme activity and protein content were determined in the original dialysate and in the combined washings with adsorption and desorption buffer. The results are given in Table I.

TABLE I

RESULTS OF ADSORPTION/DESORPTION EXPERIMENTS OF ARYLSULPHATASE-A ON SP-SEPHADEX

Exp. No.	*Adsorption conditions*					*Desorption conditions*			
	Volume of dialysate (ml)	*Volume of gel slurry (ml)*	*pH*	*[NaCl] (mM)*	*Enzyme activity adsorbed* (%)*	*pH*	*[NaCl] (mM)*	*Enzyme activity desorbed* (%)*	*Purification factor*
1	1.0	0.5	5.10	0	61	5.10	50	35	5.1
2	1.0	0.5	4.70	10	92	5.10	50	64	3.7
3	1.0	0.5	4.70	10	92	5.54	10	69	5.0
4	2.0	0.5	4.70	10	90	6.00	10	62	3.2

* Relative to the activity in the original dialysate.

If the adsorption is performed at pH 5.10 without addition of sodium chloride (contrary to the prescriptions of Stevens, to enhance the adsorption) and the desorption is performed at the same pH by 50 m*M* sodium chloride, a high purification factor but a low yield is obtained (exp. 1).

If the adsorption is performed at pH 4.70 to enhance the yield, the purification factor decreases (exp. 2).

Desorption by raising the pH instead of the sodium chloride concentration results in a high yield as well as a high purification factor (exp. 3).

An attempt to obtain a still higher yield by a further increase of the pH in the desorption step results in a decreased purification factor (exp. 4).

It is evident that the conditions of exp. 3 are the best: they give a high yield (69%) and a high purification factor (5 times). These figures compare favorably with those of Stevens *et al.*[1] (74% and 3.4 times), but are obtained, as stated, under different conditions.

Some qualitative features of the behaviour of the enzyme on SP-Sephadex seem to be characteristic for an ion-exchange interaction. Thus, the increasing adsorption with decreasing ionic strength at constant pH (Fig. 1) and the decreasing adsorption with increasing pH at constant ionic strength (Fig. 2) are in accordance with ion-exchange phenomena on a strongly acidic cation exchanger. From a quantitative point of view an ion-exchange mechanism is doubtful, however. The isoelectric point of human urinary arylsulphatase-A has been reported[4] to be at pH 4.7. There-

fore the strong adsorption at pH 4.7–5, where the protein should be predominantly negatively charged, is difficult to reconcile with an ion-exchange mechanism. To investigate whether a biospecific interaction mechanism occurs, the following desorption experiments were performed. After coupling (4°, 48 h) of the enzyme to SP-Sephadex in adsorption buffer (10 m*M* sodium acetate + 10 m*M* sodium chloride, pH 4.70) and subsequent washing of the gel as described above, the gel was treated (4°, 1 h) with desorption buffer (10 m*M* sodium acetate, pH 4.70) containing different additives, as indicated in Table II. The enzyme activity was determined in the original dialysate and in the desorption buffer.

TABLE II

RESULTS OF DESORPTION EXPERIMENTS

Exp. No.	*Additives to desorption buffer (10 mM sodium acetate, pH 4.70)*	*Enzyme activity desorbed* (%)*
1	10 m*M* dipotassium *p*-nitrocatechol sulphate	14
2	5 m*M* dipotassium *p*-nitrocatechol sulphate	11
3	20 m*M* potassium chloride	0
4	10 m*M* 5-sulphosalicylic acid + 20 m*M* potassium hydroxide	0.5

* Relative to the activity in the original dialysate.

Table II shows that *p*-nitrocatechol sulphate (a substrate-analogue to arylsulphatase-A) is able to desorb a substantial amount of the enzyme activity under conditions where a salt solution of comparable concentration or a related aromatic compound have no desorbing ability. These findings support the original interpretation by Stevens *et al.*[1] that the adsorption of arylsulphatase-A on SP-Sephadex is caused by a biospecific reaction.

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