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Combination of polymer-bound charged groups and affinity ligands for extraction of enzymes by partitioning in aqueous two-phase systems[a]

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ABSTRACT

The partition of glucose-6-phosphate dehydrogenase in a water–dextran–polyethylene glycol two-phase system was influenced by the mutual addition of polymers carrying charged groups and affinity ligands. These polymers included DEAE-dextran, dextran sulphate, ligand–dextran, ligand–polyethylene glycol, ligand–Ficoll and ligand–DEAE-dextran, where the ligands used were the dyes Cibacron Blue F3G-A and Procion Yellow HE-3G. Some of these two-phase systems were also used for the counter-current distribution of yeast proteins, demonstrating the separation of several enzymes.

INTRODUCTION

It is well known that the partition of proteins within aqueous two-phase systems can be strongly influenced by either charged groups or affinity ligands restricted to one of the phases [1–8]. This location is achieved by binding these chemical groups to one of the phase-forming polymers. The most popular two-phase system is that containing dextran and polyethylene glycol (PEG). In this work a combination of both affinity and charged groups was tested on the partitioning of glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49, from baker's yeast) and of a protein extract from baker's yeast.

EXPERIMENTAL

Chemicals

Dextran T-500 and T-2000, DEAE-dextran 500 (2.5% nitrogen), dextran sulphate 500 (27% sulphur) and Ficoll 400 were purchased from Pharmacia (Uppsala, Sweden). PEGs with M_r 3400 and 8000 were obtained from BP Chemicals (Hythe, U.K.). Procion Yellow HE-3G–dextran 2000 (46 μmol dye g^{-1}), Procion Yellow HE-3G–PEG 8000 (106 μmol dye g^{-1}), Cibacron Blue F-3GA–PEG 8000 (127 μmol

[a] This paper is dedicated to Professor Per-Åke Albertsson on the occasion of his 60th birthday.

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dye g^{-1}), and Procion Yellow HE-3G–Ficoll 400 (6 μmol dye g^{-1}) were synthesized as described elsewere [9–11]. Fresh baker's yeast (*Saccharomyces cerevisiae*) was purchased from Jästbolaget (Sollentuna, Sweden). Glucose-6-phosphate dehydrogenase and 3-phosphoglycerate kinase, from yeast, together with biochemicals for enzyme assays were purchased from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical-reagent grade. The distilled water used was first passed through a mixed ion exchanger.

Synthesis of dye–DEAE-dextran 500

A 1-g amount DEAE-dextran was dissolved in 20 ml of water together with 0.2 g of Procion Yellow HE-3G. To start the reaction, 2 ml of 1 *M* sodium hydroxide solution containing 0.5 *M* sodium sulphate were added. The mixture was kept at 70°C for 45 min and then 0.25 ml acetic acid was added to stop the reaction. The polymer was precipitated with 30 ml of ethanol and the precipitate was dissolved in 20 ml of water. The precipitation procedure was repeated twice. The yellow DEAE-dextran was dissolved in 10 ml of water and dialysed overnight against water, filtered and the dye content (by measurement of the absorbance at 400 nm) as well as the dextran content (measured polarimetrically [12]) were determined. The polymer contained 50 μmol dye g^{-1}.

Extract of baker's yeast

Baker's yeast (100 g) was suspended in 100 ml of 100 m*M* triethanolamine (TEA)–HCl buffer (pH 7.6) and sonicated in an ice-bath in two portions with a Benson B50 sonifier for 20 min using 40% pulses and an output setting of 7 (final temperature <12°C). The mixture obtained was centrifuged for 8 min at 1500 *g* at 0°C. Solid PEG 8000 was dissolved in the supernatant at 0°C to a final concentration of 7% (w/w) and the mixture was incubated for 10 min on ice. The precipitate was removed by centrifugation (8 min at 1500 *g* at 0°C) and the PEG 8000 content of the supernatant was adjusted to 17% (w/w) by addition of solid PEG. After incubation and centrifugation as above, the protein "pellet" was collected and dissolved in 5 ml of 50 m*M* TEA–HCl buffer (pH 7.6).

Two-phase systems

The systems were prepared by weighing out the required amounts of solutions of 40% (w/w) PEG 3400 and 25% (w/w) dextran 500, together with salt, buffer and enzyme solution, plus water to the final weight (normally 4 g). The systems were equilibrated at 25°C, centrifuged for 5 min at 700 *g* and samples of known volume were withdrawn and analysed. The partition coefficient of the enzyme, *K*, defined as the ratio of the concentrations of enzyme (obtained by activity measurements) in the upper and lower phases, was calculated. The partition coefficients of the free dye and of dye–dextran were determined by absorbance measurements at 400 nm using equally diluted phases without dye as blanks.

Centrifugal counter-current distribution

The apparatus for counter-current distribution has been described in detail elsewhere [13]. The operating unit consists of an inner plate with 60 cavities for the upper phases surrounded by an outer ring containing corresponding cavities for the

lower phases. The volume of each of the outer cavities was 0.96 ml. All operations were carried out at 3°C. The apparatus was loaded with 1.7 ml of phase system (of which 0.85 ml was the lower phase) in each chamber formed by the disk and the ring. The systems in chambers 0–2 were loaded with yeast extract in the phase system. Fifty-six partitioning steps were carried out automatically with shaking for 1.5 min followed by centrifugation for 15 min after each transfer. When the distribution was complete, 1.7 ml of 10 m*M* TEA–HCl buffer (pH 7.6) containing 1.5 m*M* 2-mercaptoethanol were added to each chamber, giving one-phase systems which were analysed for protein and enzyme activities.

Enzyme assays

The enzyme activities were determined spectrophotometrically at 340 or 240 nm (enolase) and 22°C using a Hitachi 100-60 double-beam spectrophotometer connected to an LKB 2210 potentiometric recorder. Descriptions of the assays can be found elsewhere: glucose-6-phosphate dehydrogenase [14], enolase [15], hexokinase [16] and glyceraldehydephosphate dehydrogenase [17]. Samples (phases, mixed systems or diluted systems from counter-current distribution experiments) of 25–100 μl were mixed with assay solution to a final volume of 2.9 ml. The polymers present in the sample had no detectable effect on the activity measurements.

Protein assay

Protein was assayed by using Coomassie Brilliant Blue G according to Bradford [18] at 595 nm with bovine serum albumin as standard.

RESULTS AND DISCUSSION

It is well known that the addition of salts to aqueous (two polymers) two-phase systems affects the partitioning of polyelectrolytes [8,10,12,19]. This salt effect was studied earlier by partitioning of proteins of various charges. This influence is assumed to be connected with the relative affinities of the two ions of the salt for the phases and it has been related to the presence of an interfacial potential [12,19]. In the two-phase system used below, based on dextran and PEG, anions such as chloride, bromide and iodide have an increasing affinity (in that order) for the PEG-rich upper phase. Likewise, the cations lithium, sodium and potassium show increasing affinity for the lower dextran-rich phase. The relative affinity for the anion and the cation of the salt for the two phases can be expressed by hypothetical partition coefficients, K_- and K_+, describing the partitions that the ions should have if they could partition independently of each other [19]. When the salt is a 1:1 electrolyte, the partition coefficient, K, of a polyelectrolyte with net charge Z is given by

$$K = K_0 \, (K_-/K_+)^{Z/2} \tag{1}$$

were K_0 is the partition coefficient of the polyelectrolyte in a system with no interfacial potential [19].

TABLE I

PARTITION COEFFICIENTS, K, OF DEAE-DEXTRAN, LABELLED WITH PROCION YELLOW HE-3G, IN THE PRESENCE OF KBr OR K_2SO_4

System composition: 8% dextran 500, 8% PEG 3400, 0.33% Procion Yellow HE-3G–DEAE-dextran 500, and various amounts of salt. The dye–DEAE-dextran was treated beforehand with the same salt used for the partitioning and dialysed against water. Temperature, 25°C; pH, 7.3.

Concentration of salt (mmol kg^{-1})	Log K	
	With KBr	With K_2SO_4
0	−0.27	−0.94
0.5	0.16	−1.45
5	1.11	−2.06
10	1.69	−2.10
25	1.67	−2.11
50	1.74	−1.49

Partition of dye-labelled DEAE-dextran

The effect of two salts, potassium bromide and potassium sulphate, on the partition of the dye–DEAE-dextran derivative is shown in Table I. The DEAE-dextran was "labelled' with covalently bound Procion Yellow HE-3G (PrY), which made it possible to determine the partitioning of the polyelectrolyte. The positive charge of the DEAE-dextran, 1.8 mmol g^{-1}, was reduced by around 25% owing to the presence of negatively charged groups on the dye, 0.4 0.5 mmol g^{-1}. Partitioning in salt-free systems was effected with DEAE-dextran that had been in contact with either potassium bromide or sulphate followed by dialysis. The polyelectrolyte can therefore be assumed to carry either bromide or sulphate, respectively, as counter ions, which affects the partition (Table I). The partition coefficient of the positively charged dextran was changed by as much as 7000-fold by exchanging the salt from 25 mM potassium sulphate (K = 0.0078) to 50 mM potassium bromide K = 55). For full effect the salt concentration had to be at least double the concentration of (net) positive charges on the dextran, which was 4.4–4.7 mmole kg^{-1} as calculated from the degree of substitution and the concentration of the dextran derivative in the system (3.3 g kg^{-1}). Therefore, salts can be used to adjust the partitioning of strongly charged polyelectrolytes.

Effect of DEAE-dextran on partitioning of an enzyme

The presence of DEAE-dextran in a system affected the partitioning of proteins which, if negatively charged, could be assumed to interact with the positive polyelectrolyte. This was studied by using a pure enzyme, glucose-6-phosphate dehydrogenase (G6PDH, from yeast), which has an isoelectric point [20] of 5.5–6.0 and therefore had a negative net charge at the pH value used, 7.6 (Table II). In systems without DEAE-dextran the G6PDH had a low partition coefficient of $\leqslant$ 0.1 (except for phosphate), which varied with the type of salt used. By addition of DEAE-dextran the K value of the enzyme could be changed as much as 1700-fold (with potassium iodide) (Table II). The two salts used in Table I, potassium sulphate and bromide,

TABLE II

PARTITION COEFFICIENTS, K_{G6PDH}, OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN SYSTEMS CONTAINING DIFFERENT SALTS AND WITH OR WITHOUT DYE–POLYMERS AND/OR CHARGED DEXTRAN

Enzyme (G6PDH, 2 U ml^{-1}) was partitioned in systems containing 8% (w/w) dextran 500, 8% (w/w) PEG 3400, 2.5 m*M* TEA–HCl buffer (pH 7.6), salts, 20 m*M* (K_2SO_4 or Li_2SO_4) or 50 m*M* (other salts), and with or without 0.33% DEAE-dextran 500 (DEAE-Dx), 0.33% Procion Yellow HE-3G–DEAE-dextran 500 (PrY–DEAE-Dx), 0.004% dextran sulphate 500 or 0.004% dextran sulphate with 0.1% Procion Yellow HE-3G–PEG 8000 (PrY–PEG). Temperature, 25°C; pH, 7.6.

Salt	Log K_{G6PDH}				
	No additional	DEAE-Dx	PrY–DEAE-Dx	Dx sulphate	PrY–PEG + Dx sulphate
KH_2PO_4/K_2HPO_4	−0.68	−2.25	−2.96	−1.18	−0.08
Li_2SO_4	−0.95	−2.70	−2.15	1.28	2.22
K_2SO_4	−1.25	−2.66	−1.98	−1.96	−0.13
$KHCO_3$	−1.67	−0.24	0.58	–	–
$NaClO_4$	−2.08	0.13	1.37	−1.77	–
KI	−2.10	1.13	1.87	–	–
LiCl	−2.24	−0.25	1.07	−0.21	0.46
$NaNO_3$	−2.25	0.70	1.89	−2.52	−1.53
CH_3COOK	−2.32	−0.66	1.00	−2.05	−0.97
KBr	−2.41	0.28	1.81	−1.69	−0.87
KCl	−2.39	0.08	1.86	−1.61	−0.94

changed the partitioning of the dye–DEAE-dextran by −1.1 (K_2SO_4) or +1.9 (KBr) log *K* units on changing from a system without salt to one containing 25 mmole kg^{-1}. The change in the partitioning of the G6PDH by introducing DEAE-dextran in the systems was −1.4 (for K_2SO_4) and +2.7 (for KBr) log *K* units, respectively. This shows that the partitioning of the enzyme changed in the same order as the DEAE-dextran was assumed to do, estimated from the salt effects on the dye-labelled DEAE-dextran. When Procion Yellow HE-3G (PrY) was bound to the DEAE-dextran additional effects were observed on the partitioning of G6PDH. This dye is known to be an affinity ligand for G6PDH and therefore should support the interaction between the enzyme and the polyelectrolyte. This was also found to be true in most instances with the exception of sulphates. The additional change was in the range 0.6–1.8 log *K* units. The strongest total change in the partition coefficient was 17 800-fold (with KCl, $\Delta \log K = 4.25$).

Effect of dextran sulphate on partitioning of G6PDH

The salts which gave high partition coefficients for positively charged polyelectrolytes, such as DEAE-dextran, should give low *K* values to a negatively charged polyelectrolyte such as dextran sulphate. As both the polyelectrolyte and the G6PDH in this case are negatively charged, the enzyme should be excluded from the phase containing dextran sulphate. Consequently, dextran sulphate should change the *K* value of G6PDH in the same direction as DEAE-dextran does. This effect was also obtained in most instances (Table II), and the log *K* value of G6PDH changed in the

predicted direction. However, the change was, with one exception, less than that with DEAE-dextran. Lithium sulphate and sodium nitrate gave changes in the partition opposite to the predicted directions, which may indicate strong ion-pair formation. Addition of PrY–PEG, which was concentrated in the upper phase, to the system containing dextran sulphate increased the log K value by 0.7–1.8 unit (Table II). The use of another PEG-bound ligand, Cibacron Blue F3G-A together with dextran sulphate showed a considerably smaller effect on the partition coefficient of G6PDH, with an increase of only 0–0.8 log K units.

Combination of charged dextran and affinity ligand

The method of binding the ligand to one polymer and the charged groups to another polymer may be a fruitful means of increasing the selectivity in the partitioning of proteins. By using, *e.g.*, potassium phosphate buffer as the steering salt the DEAE-dextran can be located in the lower phase while dye–PEG is confined in the upper phase. In such a system the ligand is extracting the enzyme towards one phase while the positively charged dextran attracts proteins with a negative net charge to the other phase. The partitioning of G6PDH was shown to depend on the concentrations of salt, polymer-bound dye and DEAE-dextran (Table III). A high salt concentration eliminated both the binding between the ligand and enzyme and the electrostatic action of the DEAE-groups.

Another possibility is to use DEAE-dextran and highly substituted PrY-dextran. As the PrY is strongly negatively charged, the dye–dextran becomes a negative polyelectrolyte. By using potassium chloride in the system, DEAE-dextran was concentrated in the upper phase and the dye–dextran in the lower phase. The partitioning of G6PDH in such systems is shown in Fig. 1. Considerable precipitation and formation of a small third (intense yellow) phase was observed. This was probably due to complex formation between the two polyelectrolytes. More than 50% of PrY–dextran and enzyme were removed from the bulk phases (Fig. 1).

The relative effects of dextran sulphate and PrY–PEG (in a system with potassi-

TABLE III

PARTITIONING OF G6PDH WITH PrY–PEG 8000 AT VARIOUS CONCENTRATIONS OF POTASSIUM PHOSPHATE BUFFER AND DEAE-DEXTRAN 500

G6PDH (3.0 U ml^{-1}) was partitioned in the system containing 8% dextran 500, 7.6% PEG 3400, 0.4% PrY–PEG 8000, 2.5 mM TEA–HCl buffer (pH 7.6), potassium phosphate buffer (pH 7.6) and DEAE-dextran 500. Temperature, 25°C.

Concentration of phosphate buffer (mM)	Concentration of DEAE-dextran (%)	Log K_{G6PDH}
0	0.066	0.28
2	0.013	0.48
5	0.000	1.75
5	0.041	0.39
5	0.330	0.04
10	0.066	2.61
50	0.000	−0.99
50	0.206	−0.84
50	0.330	−0.91

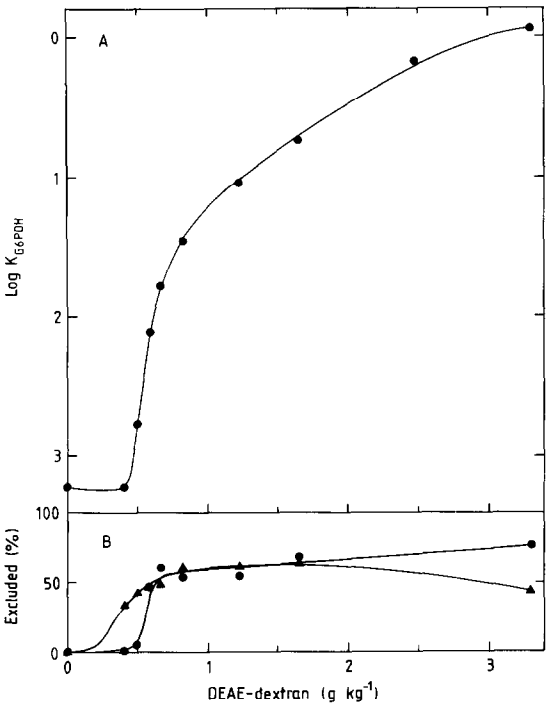

Fig. 1. Effect of DEAE-dextran concentration (given in g DEAE-dextran per kg system) on (A) the partitioning of G6PDH and (B) the amount of (●) G6PDH and (▲) PrY–dextran excluded from the phases (as a percentage of the total amount in the system) and recovered at the interface. System composition: G6PDH (2.3 U ml^{-1}), 8% (w/w) dextran 500, 8% (w/w) PEG 3400, 50 m*M* KCl, 2.5 m*M* TEA–HCl buffer (pH 7.6), 0.12% Procion Yellow HE-3G–dextran 2000 and various concentrations of DEAE-dextran 500. Temperature, 25°C.

um phosphate) were tested on a crude protein extract from baker's yeast (Table IV). In this system both ligand–PEG and dextran sulphate are in the upper phase. Very low concentrations of the dextran sulphate were sufficient to counteract the effect of the ligand. By using other ligand carriers or by increasing their concentrations, the K values of the enzyme could be increased again (Table IV). The strongest effect was found for ligand–Ficoll.

When the other combination of ligand and charged dextran was used (dye–PEG, DEAE-dextran, phosphate buffer) for partitioning of yeast extract, the enzyme G6PDH (Fig. 2) was effected by the concentration of DEAE-dextran. The partition coefficient of the protein, in general, decreased gradually from log $K = -0.1$ (no DEAE-dextran) to log $K = -1.1$ (2.5 g DEAE-dextran kg^{-1}).

Counter-current distribution

The combination of dextran sulphate and the ligand PEG was used to study the separation of enzymes, present in an extract of yeast, by counter-current distribution (CCD) (Fig. 3). The system contained sodium phosphate buffer, which caused both of the "steering" polymers to be in the upper phase. With solely dextran sulphate the

TABLE IV

PARTITIONING OF PROTEIN EXTRACT FROM BAKER'S YEAST IN SYSTEMS AT VARIOUS CONCENTRATIONS OF DEXTRAN SULPHATE WITH DIFFERENT POLYMERS CARRYING PROCION YELLOW HE-3G (PrY)

Yeast extract (containing 0.29 U ml^{-1} G6PDH) was partitioned in systems containing 8% (w/w) dextran, 7.6% (w/w) PEG 3400, various concentrations of dextran sulphate and 25 m*M* potassium phosphate (pH 7.0), with or without PrY–PEG 8000 (PrY–PEG), PrY–dextran 2000 (PrY–Dx), PrY–DEAE-dextran 500 (PrY–DEAE-Dx), or PrY–Ficoll 400 (PrY–Ficoll). Temperature, 25°C.

Dx sulphate (%)	Additional dye–polymer	Log K_{G6PDH}
0.0000	PrY–PEG, 0.40%	0.32
0.0025	PrY–PEG, 0.40%	0.07
0.0050	PrY–PEG, 0.40%	−0.25
0.0125	PrY–PEG, 0.40%	−0.77
0.0250	PrY–PEG, 0.40%	−1.14
0.0500	PrY–PEG, 0,40%	−1.45
0.1000	PrY–PEG, 0.40%	−1.55
0.0125	PrY–PEG, 0.65%	0.05
0.0125	PrY–Dx, 0.25%	−0.34
0.0125	PrY–DEAE-Dx, 0.33%	−0.98
0.0125	PrY–Ficoll, 1.75%	0.70

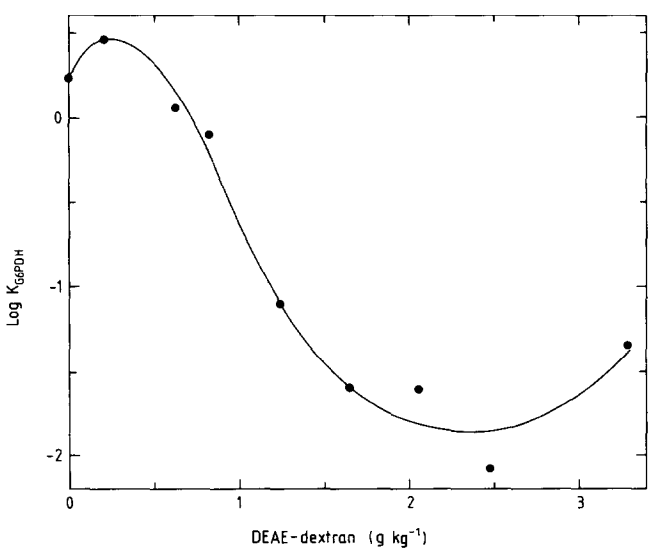

Fig. 2. Partitioning of protein extract from baker's yeast in systems at various concentrations of DEAE-dextran 500 (as a percentage of the total amount in the system). Yeast extract (containing 0.14 U ml^{-1} G6PDH) was partitioned in the system composed of 8% dextran 500, 7.6% PEG 3400, 0.4% Procion Yellow HE-3G–PEG 8000, 2.5 m*M* TEA–HCl buffer (pH 7.6) and 5 m*M* potassium phosphate buffer (pH 7.6). Temperature, 25°C.

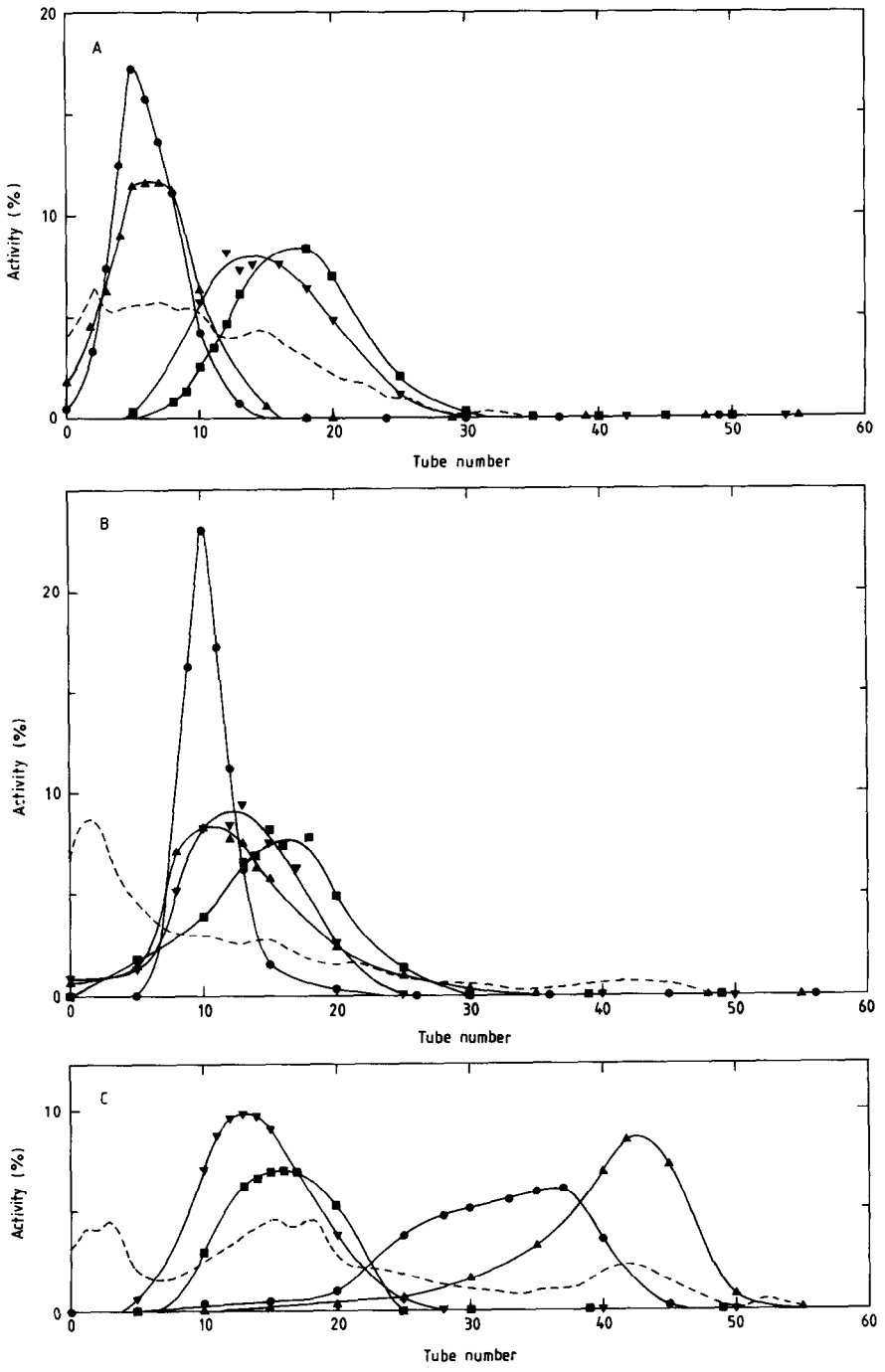

Fig. 3.

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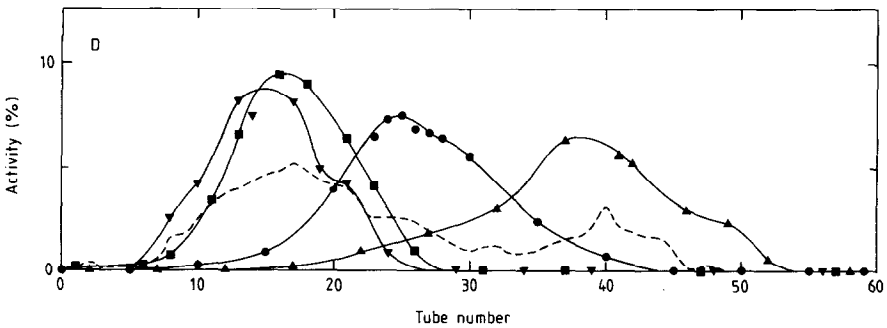

Fig. 3. Counter-current distribution of extract from baker's yeast. (A) The biphasic system contained 10% dextran 500, 5.4% PEG 3400, 0.004% dextran sulphate, 25 m*M* potassium phosphate buffer (pH 7.0), 5 m*M* glucose-6-phosphate and 9 m*M* dithiothreitol. (B) System as in A but it also contained 0.3% Cibacron Blue F3G-A–PEG 8000 and the concentration of PEG 3400 was reduced to 5.1%. (C) System as in A but with 0.1% Procion Yellow HE-3G–PEG 8000 and the concentration of PEG 3400 was 5.3%. (D) As in C but with 0.015% dextran sulphate. The diagrams show the distribution of (●) G6PDH, (▼) enolase, (■) hexokinase, (▲) glyceraldehyde phosphate dehydrogenase and (- - -) protein. Temperature, 4°C.

measured enzymes, glucose-6-phosphate dehydrogenase (G6PDH), glyceraldehyde-phosphate dehydrogenase (GAPDH), hexokinase (HK) and enolase, moved only slowly in the CCD process (Fig. 3A). Addition of Cibacron Blue–PEG to the system moved both G6PDH and GAPDH slightly towards higher tube numbers whereas the two other enzymes remained in their positions (Fig. 3B). This CCD experiment demonstrates a weak interaction between the two dehydrogenases and Cibacron Blue despite the negative results obtained in single-partition experiments above. Procion Yellow–PEG showed much stronger effect (Fig. 3C). No significant difference in the CCD patterns was found if the system contained no or 0.004% of dextran sulphate. Both G6PDH and GAPDH travelled ahead of the two other enzymes and were also partly separated. By increasing the concentration of dextran sulphate both G6PDH and GAPDH moved less to the right (Fig. 3D). The form of the CCD curves shows that these enzymes do not behave as homogeneous substances. Whether this is due to interactions between the enzymes and dextran sulphate or if the enzymes are present in multiple forms is not clear. The counter-current distribution machine gives nearly perfect distribution profiles with pure substances [13], which excludes technical errors.

The parameter G (partition ratio defined as the ratio of homogeneous enzyme in the mobile and stationary parts of the system [21]) of the most dominant region of the activity peaks was calculated from the position of the peak, $\hat{\imath}$, using the relationship [21] $G = \hat{\imath}/(n-\hat{\imath})$, where n is the number of transfers (Table V). From the G values and the volumes of the phases the K values were also calculated. To obtain a good separation of two (homogeneous) enzymes in CCD, the ratio, β, between their respective G values should be >3 or <0.33. This requirement is fulfilled for the peak components of the pair of enzymes GAPDH and enolase without dye–PEG (β = 0.32), and with the two concentrations of PrY–PEG (β = 10 and 6, respectively), as calculated from the G values in Table V. The optimum separation, which corresponds to CCD with no stationary upper phase, can be seen from the ratio between the K values (Table V) of the enzymes in the latter two systems giving β = 16 and 8. For the

TABLE V

PARTITION RATIOS (G VALUES) AND PARTITION COEFFICIENTS (K VALUES) OF THE MAIN ENZYME PEAKS IN THE COUNTER-CURRENT DISTRIBUTIONS PRESENTED IN FIG. 3A–D

The enzymes are G6PDH, enolase, hexokinase (HK) and glyceraldehyde phosphate dehydrogenase (GAPDH). The G values descibe the relative mobility of the peak along the counter-current distribution train [21] and they are related to the K values by the equation $G = 0.75K/(0.1K + 0.85)$ using the volumes of stationary and mobile phases given under Experimental. Cb–PEG = Cibacron Blue F3G-A–PEG 8000.

Figure	Dextran sulphate (%)	Dye–PEG (%)	G6PDH		Enolase		HK		GAPDH	
			G	K	G	K	G	K	G	K
3A	0.004	None	0.077	0.088	0.31	0.37	0.45	0.54	0.10	0.11
3B	0.004	Cb–PEG, 0.3	0.20	0.23	0.28	0.33	0.34	0.40	0.20	0.23
3C	0.004	PrY–PEG, 0.1	1.50	2.1	0.28	0.33	0.38	0.45	2.9	5.4
3D	0.015	PrY–PEG, 0.1	0.77	0.97	0.34	0.40	0.41	0.49	2.1	3.3

pair of (homogeneous) G6PDH and GAPDH, the combination of PrY–PEG and 0.004% dextran sulphate gave a less useful β value (1.9), whereas an increase in the dextran sulphate level to 0.015% gave a higher β value (2.7). The optimum conditions established from the β values obtained from the K values (Table V) show that good separations could be obtained with CCD avoiding the stationary part of the upper phase. In this instance the β values were 3.6 and 4.3, respectively.

CONCLUSIONS

The results show that polyelectrolytes, forced into one of the phases of the two-phase system by the choice of salt, can be used to affect strongly the partitioning of proteins. The polyelectrolytes can also be used simultaneously in an antagonistic or cooperative way with phase-localized (polymer-bound) affinity ligands.

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