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Multiple Protein Kinases from Human Lymphocytes

IDENTIFICATION OF ENZYMES PHOSPHORYLATING EXOGENOUS HISTONE AND CASEIN

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1. Cell-free lysates of human peripheral blood lymphocytes contained two casein kinase activities and two histone kinase activities, which could be separated by chromatography on DEAE-Sephadex. 2. Neither of the casein kinase activities were stimulated by cyclic AMP. The major activity was eluted from DEAE-Sephadex between 0.4 and 0.45M-KCl, had a molecular weight of approx. 130000 (sucrose density gradients) and was stimulated by KCl (maximum 150mM). It also formed higher-molecular-weight aggregates when centrifuged in sucrose gradients containing 150mM-KCl. The minor activity was not retained by DEAE-Sephadex, had a molecular weight of approx. 50000 and was not stimulated by KCl. 3. The major histone kinase activity was stimulated by cyclic AMP and was eluted from the DEAE-Sephadex column between 0.05 and 0.2M-KCl. The other activity was not stimulated by cyclic AMP and was insensitive to the rabbit muscle protein kinase inhibitor. 4. Evidence was obtained suggesting that the lymphocyte casein kinases were located primarily in the nuclei.

Phosphorylation of non-histone chromosomal proteins has been implicated in the regulation of gene expression in eukaryotes (for references see Stein *et al.*, 1974). For example, gene activation after the interaction of lymphocytes with phytohaemagglutinin is correlated with an increase in the phosphorylation of nuclear acidic proteins (Kleinsmith *et al.*, 1966). As part of a study on the role of protein phosphorylations in lymphocyte activation, we are attempting to characterize the protein kinases present in these cells. Extracts of human peripheral blood lymphocytes have previously been shown to catalyse the phosphorylation of both histone and casein (Murray *et al.*, 1972). Only the phosphorylation of histone was stimulated by cyclic AMP. A description of the fractionation and further characterization of the lymphocyte enzymes responsible for the phosphorylation of these exogenous substrates is presented in this paper.

Materials and Methods

Substrates and activators

[γ-^{32}P]ATP was prepared as described by Glynn & Chappell (1964). Dephosphorylated casein was prepared by the method of Reimann *et al.* (1971) and dialysed against 0.2M-KCl and then against 10mM-Hepes [2-(*N*-2-hydroxyethylpiperazin-*N'*-yl)-ethanesulphonic acid] buffer, pH7.5.

Unlabelled ATP, ADP, AMP, cyclic AMP, cyclic GMP, Ficoll, catalase, cytochrome *c*, dextran, whole calf thymus histone (type IIA) and phosvitin were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A.

Alcohol dehydrogenase (yeast) and Hepes were obtained from Calbiochem (Aust.) Pty. Ltd., Sydney, Australia. Casein was purchased from British Drug Houses Ltd. (Poole, Dorset, U.K.) and Hypaque (sodium diatrizoate) was obtained from Winthrop Laboratories, Sydney, Australia.

Preparation of lymphocytes

Lymphocytes were isolated from human peripheral blood (430ml) collected into plastic blood packs containing disodium citrate (2g) and dextrose (1.7g) in 70ml of water. All blood donors were Australia-antigen-negative.

The blood was defibrinated by adding iso-osmotic (0.11M) $CaCl_2$ (100ml) and stirring with a glass rod at 27°C. As the fibrin clot formed it was wound around the glass rod. The bulk of the erythrocytes were removed through sedimentation by adding 150ml of 6% (w/v) dextran in 0.154M-NaCl and incubating at 37°C for 1–2h in a plastic measuring cylinder. The upper layer was siphoned off and centrifuged at 300*g* for 10min in two 250ml polycarbonate centrifuge bottles. The sedimented cells were resuspended in 30ml of autologous serum (supernatant from the previous step) and incubated for 30min on a cottonwool column (30cm×1.5cm), surrounded by a water jacket kept at 37°C (the

cottonwool was exhaustively washed with glass-distilled water and dried before use). The non-adherent lymphocytes and contaminating erythrocytes were eluted from the column with 100ml of autologous serum at 37°C and the resulting cell suspension was centrifuged at 300*g* for 10min. The lymphocytes were separated from the erythrocytes by a modification of the method of Mendelsohn *et al.* (1971). The cells, suspended in 20ml of 0.154M-NaCl buffered with 10mM-sodium phosphate (pH7), were layered over Hypaque–Ficoll gradients (2ml of a mixture containing 20ml of 85% Hypaque, 9g of Ficoll and 130ml of water) in three 15ml conical plastic centrifuge tubes and centrifuged at 1600*g* for 15min. The lymphocytes were located at the NaCl/Hypaque–Ficoll interface and were carefully removed with a pipette, resuspended in 10ml of 0.154M-NaCl buffered with 10mM-sodium phosphate (pH7) and collected by centrifugation at 1600*g* for 15min.

The isolated lymphocyte fraction consisted of 90–95% lymphocytes contaminated with 0–5% granulocytes and/or monocytes, and less than 5% erythrocytes. The yield of purified lymphocytes from 430ml of whole blood ranged from approx. 200×10^6 to 600×10^6 cells.

Since human peripheral blood contains only 34% B lymphocytes (Wilson & Nossal, 1971) and B lymphocytes selectively adhere to cottonwool (Raff, 1973) the isolated lymphocyte fraction is expected to consist mainly of T lymphocytes.

Preparation of lymphocyte extracts

Purified lymphocytes were lysed by hypo-osmotic shock in 10mM-Hepes–7mM-2-mercaptoethanol, pH7.4 (100×10^6cells/ml of extraction buffer), and homogenized briefly by hand in a close-fitting glass Dounce homogenizer. The homogenate was dialysed at 4°C against the extraction buffer, and centrifuged at 25000*g* for 30min. The supernatant was stored at −15°C and the pellet discarded. Protein was determined by the method of Lowry *et al.* (1951) with bovine serum albumin (Cohn fraction V) as standard.

Sucrose-density-gradient centrifugation

Linear sucrose gradients (5–20%, w/v; 4.8ml) contained 20mM-Hepes buffer, pH7.4, 7mM-2-mercaptoethanol and KCl as indicated in the Figure legends. Gradients were centrifuged at 2°C for 10h at 280000$g_{av.}$ in a 6×5ml Ti rotor in an MSE Superspeed 65 centrifuge. Fractions (four drops) were collected and assayed for casein kinase and histone kinase activities by using an assay time of 30min. Molecular weights were estimated by the method of Martin & Ames (1961), with yeast alcohol dehydrogenase (0.5mg; mol.wt. 150000), horse heart cytochrome *c* (8mg; mol.wt. 12400) and catalase (0.2mg; mol.wt. 250000) as molecular-weight standards. The presence of contaminating protein kinase in the alcohol dehydrogenase (Kemp *et al.*, 1973) necessitated that these standards be run in parallel with the gradient tubes containing the lymphocyte fractions.

Assay of protein kinase activity

Reaction mixtures (final volume 100μl) contained 5μmol of Hepes buffer, pH7.4, 1μmol of $MgCl_2$, 5nmol of [γ-^{32}P]ATP (specific radioactivity greater than 37mCi/mmol) and 0.57mg of dephosphorylated casein or 0.1mg of whole calf thymus histone. The reaction was started by the addition of enzyme and incubated at 37°C for 10min unless otherwise stated. Assays were terminated by adding 50μl of 100mM-EDTA containing bovine serum albumin (50mg/ml, pH7.4). Samples (100μl) were dried on paper discs which had been pretreated with 50μl of 10mM-ATP, and the discs washed as described by Murray *et al.* (1972).

Characterization of the phosphorylated product formed with histone as a substrate has been reported (Murray *et al.*, 1972). The phosphorylated product obtained when casein was used as a substrate with crude lymphocyte extract in assays with or without added 150mM-KCl was alkali-labile (87 and 78% respectively; 0.1M-NaOH, 100°C, 20min) and stable in acid (94 and 100% respectively; 0.1M-HCl, 100°C, 20min). After partial acid hydrolysis (2M-HCl, 100°C, 4h) and electrophoresis on paper (4h, 40V/cm) in formic acid–acetate acid–water (1:4:45, by vol.), radioactivity was associated with the serine phosphate marker and the threonine phosphate marker. In the absence of added KCl, 35% of the radioactivity was unhydrolysed, 4% was associated with threonine phosphate, 16% with serine phosphate, 8% with di- and tri-serine phosphates [(Ser-*P*)$_2$, (Ser-*P*)$_3$] (Williams & Sanger, 1959) and 37% with P_i.

Protein kinase inhibitor

The heat-stable inhibitor of cyclic AMP-dependent protein kinases was prepared from rabbit muscle by the method of Walsh *et al.* (1971). The supernatant containing the inhibitor was further dialysed against 5mM-Hepes–1mM-EDTA, pH7.3. The activity of the inhibitor was characterized by using the cyclic AMP-dependent protein kinase from rabbit muscle (Walsh *et al.*, 1968). Addition of 10.2μg of inhibitor protein per assay caused between 90 and 95% inhibition of the muscle protein kinase activity.

Subcellular fractionation of lymphocytes

The first fractionation procedure used was based on that described by Fisher & Mueller (1971).

The lymphocyte pellet was suspended in 50mM-$MgCl_2$ (approx. 50×10^6cells/ml) for 2min (2°C). The suspension was centrifuged at 300*g* for 5min, and the cell pellet resuspended in 50mM-Tris–HCl–5mM-$MgCl_2$, pH7.4 (approx. 100×10^6cells/ml). The supernatant of the 5mM-$MgCl_2$ wash was retained. The cells were disrupted with 150 strokes of a tight-fitting Dounce-type homogenizer. The whole homogenate was centrifuged at 300*g* for 5min. The particulate fraction was resuspended in 1ml of 0.44M-sucrose–5mM-$MgCl_2$ and layered over a discontinuous sucrose gradient of 2ml of 1.6M-sucrose–5mM-$MgCl_2$ and 2ml of 0.88M-sucrose–5mM-$MgCl_2$. Nuclei were pelleted by centrifugation at 100000*g* for 60min. A 'membrane' fraction was obtained by combining the 0–0.88M-sucrose and the 0.88–1.6M-sucrose layers together with the pellet obtained by centrifuging the 300*g* supernatant at 100000*g* for 60min.

Lymphocytes were also fractionated by a method based on the procedure of Chauveau *et al.* (1956) for preparing rat liver nuclei. Freshly purified lymphocytes were suspended in 2.2M-sucrose–20mM-Hepes–6mM-2-mercaptoethanol–5mM-$MgCl_2$, pH7.3, and homogenized by 12 strokes in a power-driven glass/Teflon homogenizer. The homogenate was centrifuged at 62000*g* for 30min to give a nuclear pellet; the supernatant fraction was diluted to 1.1M-sucrose with 20mM-Hepes–6mM-2-mercaptoethanol–5mM-$MgCl_2$ and re-centrifuged at 77000*g* for 45min. The nuclear pellet fraction was resuspended in 0.44M-sucrose–20mM-Hepes–6mM-2-mercaptoethanol–5mM-$MgCl_2$, pH7.3, layered over 2.2M-sucrose in the same buffer and centrifuged at 77000*g* for 45min. The supernatant from this centrifugation is referred to as the 'nuclear wash' fraction. A 'membrane' fraction was obtained by combining the pellet obtained from centrifuging the 1.1M-sucrose supernatant (see above) and the fraction obtained from the 0.44M/2.2M-sucrose interphase. The combined fractions were diluted with 1 volume of 10mM-Hepes–0.5mM-EDTA–0.5mM-EGTA [ethanedioxybis(ethylamine)tetra-acetate], pH7.3, centrifuged at 77000*g* for 45min and the pellet was collected.

All subcellular fractions were dialysed against 10mM-Hepes–0.5mM-EDTA–0.5mM-EGTA, pH7.3, overnight before assay of protein kinase activities.

Results

Fractionation of casein kinase and histone kinase by ion-exchange chromatography

Protein kinases in the lymphocyte extract could be separated into four main classes by chromatography on DEAE-Sephadex (Fig. 1). A portion of the casein kinase activity (fraction CK_2) did not bind to DEAE-Sephadex, and was eluted as a bimodal peak. This activity was eluted as a single peak if 100mM-KCl was added to the equilibration buffer. The major casein kinase activity (fraction CK_1) was eluted from the column at a KCl concentration of approx. 0.4M (Fig. 1). The activities of neither peak CK_1 nor peak CK_2 were stimulated by the inclusion of cyclic AMP (2μM) in the assays.

Two distinct peaks of histone kinase activity were also eluted from the column (HK_1 and HK_2; see Fig. 1). The fractions containing histone kinase activity were reassayed in the presence and absence of cyclic AMP (Fig. 2). Activity in peak HK_1 was clearly stimulated by cyclic AMP, whereas that in peak HK_2 was poorly stimulated. The slight stimulation of peak HK_2 activity by cyclic AMP is attributed to overlap of the two peaks and the relatively high activity of peak HK_1 in the absence of cyclic AMP to the dissociation of catalytic and regulatory subunits. Histone kinase peak HK_1 was inhibited 90% by the inclusion of 32.5μg of the heat-stable protein inhibitor in the assays; peak HK_2 activity was inhibited only by 30%. The heat-stable protein inhibitor has been shown to inactivate the dissociated catalytic subunit of cyclic AMP-dependent protein kinases (Walsh & Ashby, 1973). On the basis of this criterion peak HK_1 may be classified as a cyclic AMP-dependent protein kinase holoenzyme. The relative insensitivity of peak HK_2 to the heat-stable inhibitor indicates that it is not the dissociated catalytic subunit of a cyclic AMP-dependent holoenzyme. Again the inhibition of peak HK_2 by the inhibitor can be attributed to the overlap of the two histone kinase peaks.

The presence of two histone kinases was also suggested by assays on the original crude lymphocyte extracts. The basal histone kinase activity was insensitive to the heat-stable protein inhibitor. However, the inhibitor abolished the increase in histone kinase activity obtained by adding 2μM-cyclic AMP.

The rates of casein phosphorylation by both fractions CK_1 and CK_2 were linear with respect to time for periods of at least 20min and enzyme concentration up to 10.8 and 18.4μg of protein respectively. Assays of fraction CK_1 were carried out in both the presence and absence of KCl. Salt stimulation of this casein kinase activity will be considered further below. Kinetic parameters for both ATP and Mg^{2+}, also determined in the presence and absence of KCl, are summarized in Table 1.

Fraction CK_2 preferentially phosphorylated phosvitin, whereas fraction CK_1 preferred casein as substrate. For this comparison the usual casein kinase assay conditions were used, except that the

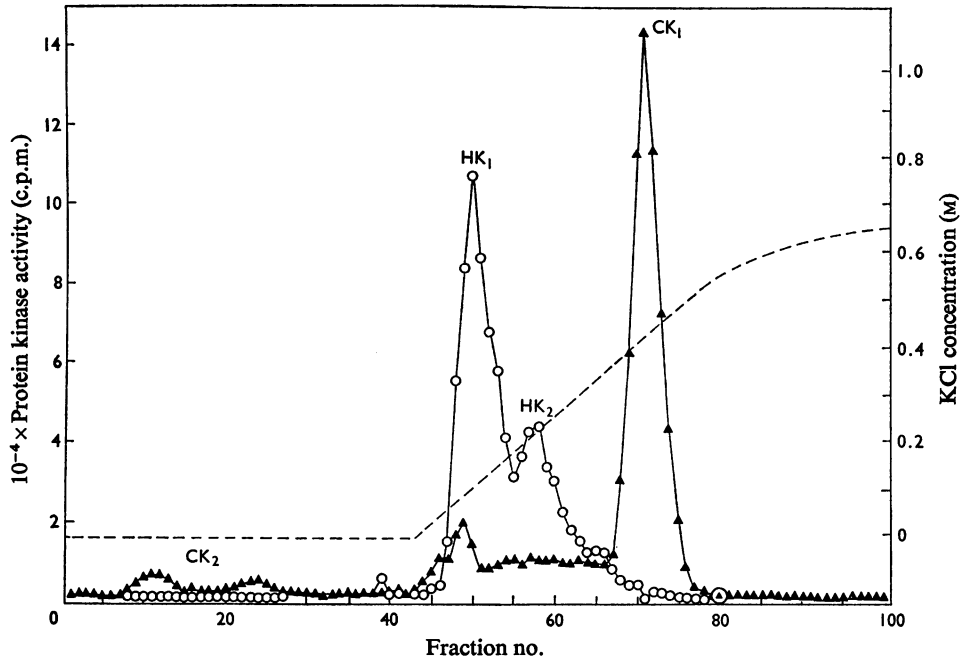

Fig. 1. *Chromatography of lymphocyte extract on DEAE-Sephadex*

The column (0.9cm×25cm) was equilibrated with 50mM-Tris–HCl buffer (pH7.9). Extract (9.12mg of protein) was loaded on the column and eluted with the equilibration buffer. At fraction 43 a gradient of KCl in the same buffer was started. The column was eluted at a constant rate of 6ml/h and 1.5ml fractions were collected. All fractions were assayed for histone kinase in the presence of 2μM-cyclic AMP (○) and casein kinase in the absence of added KCl (▲). The salt gradient was monitored by using a refractometer with refractive-index determinations on every fifth fraction as indicated by the broken line.

[γ-^{32}P]ATP concentration was 89.8μM and the phosvitin concentration was 3.5mg/ml in the assay. In the absence of added KCl, fractions CK_1 and CK_2 phosphorylated phosvitin at 88% and 414% of the rate obtained with casein. Phosphorylation of phosvitin by fraction CK_2 was inhibited by 80% by 150mM-KCl, whereas phosphorylation of phosvitin by fraction CK_1 was stimulated by 54% by 150mM-KCl. The corresponding inhibition by KCl of fraction CK_2 and the stimulation by KCl of fraction CK_1 when casein was used as substrate were 31% and 125% respectively.

Stimulation of casein kinases by KCl

It has previously been shown that lymphocyte histone kinase activity is inhibited by KCl (Murray *et al.*, 1972). The effect of KCl on casein kinase activity in crude lymphocyte extracts and on fractions CK_1 and CK_2 is shown in Fig. 3. The activity of the crude extract and of fraction CK_1 was clearly stimulated by KCl, with a maximum near 150mM; a slight inhibition of CK_2 activity was observed. Linear rates of casein phosphorylation were observed in the presence or absence of KCl, so the stimulation is unlikely to result from a stabilization of casein kinase activity during assay. As shown in Table 1, the stimulation by KCl is associated with a decrease in the $s_{0.5}$ (concentration required for half-maximal activity) value for $MgCl_2$. It is noteworthy that casein kinase activity in freshly prepared crude extracts of lymphocytes was only slightly (less than 10%) or not at all stimulated by KCl. The salt stimulation developed after storage of the extract for several days at −15°C. It is not known if the development of the KCl stimulation results from changes in the properties of fraction CK_1, from changes in the relative proportions of KCl-stimulated and KCl-inhibited kinases or from loss of a separate regulatory molecule during storage.

Fractionation of lymphocyte protein kinases by sucrose-density-gradient centrifugation

The sedimentation pattern of casein kinase activity in sucrose density gradients, both in crude extracts and in partially purified fractions, was markedly affected by KCl. When crude extract was sedimented in the presence of 600mM-KCl two peaks of activity were observed (see Fig. 4*a*; approx. molecular weights 130000 and 50000). After dialysis, inclusion of 150mM-KCl in assays stimulated the activity of the larger-molecular-weight species by 200% and that of the smaller species by only 18%. Figs. 4(*b*) and 4(*c*) establish that the higher-molecular-weight activity corresponds to fraction CK_1 obtained from a DEAE-Sephadex column, and the lower-molecular-weight species to fraction CK_2.

The inclusion of 150mM-KCl in gradients caused the aggregation of casein kinase activity (Fig. 5). This effect was observed both with crude extracts and with fraction CK_1 from a DEAE-Sephadex column. The position of histone kinase activity is also indicated in Fig. 5(*b*); the sedimentation of this activity was unaffected by KCl.

Subcellular distribution of lymphocyte protein kinases

The distribution of casein and histone kinase activities after fractionation by the method of Fisher & Mueller (1971) is shown in Table 2. The initial hypo-osmotic shock in 5mM-$MgCl_2$ appeared to cause the lysis of a number of cells, since about 10% of DNA and 22% of the total protein was present in the Mg^{2+} wash fraction. Also, considerable amounts of both casein kinase and histone kinase were released by the hypo-osmotic shock.

The distribution obtained by homogenization in hyperosmotic sucrose by the method of Chauveau *et al.* (1956) is also summarized in Table 2. A high proportion of both casein kinase and histone kinase activities was located in the nuclear plus nuclear wash fractions. The data suggest that histone kinase activity might be more readily leached from the nuclei than casein kinase activity. With this fractionation procedure more than 60% of the 5'-nucleotidase activity was associated with the nuclear fraction (results not shown), suggesting that plasma-membrane fragments were largely sedimented with the nuclei. This result is consistent with the results of Burgoyne (1972) who reported that Ehrlich ascites-tumour-cell nuclei prepared in the presence of high-viscosity Ficoll solutions retained the cell membrane. However, the data obtained using the method of Fisher & Mueller (1971) preclude the plasma membrane as a major (quantitative) source of casein kinase or histone kinase activity.

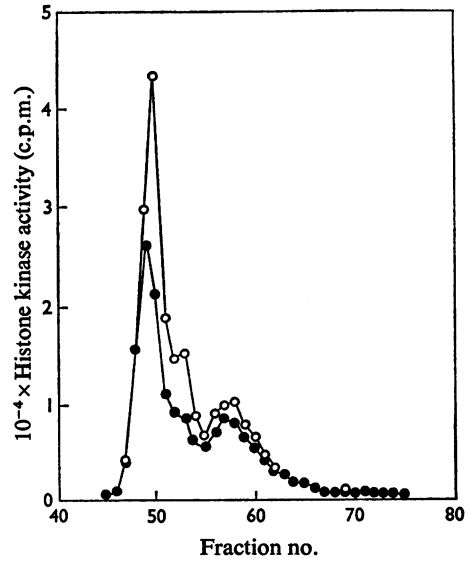

Fig. 2. *Cyclic AMP dependence of the lymphocyte histone kinase activities HK_1 and HK_2*

The fractions containing histone kinase activity from the DEAE-Sephadex column described in Fig. 1 were re-assayed in the presence (○) and absence (●) of cyclic AMP (2μM). The column fractions were stored at −15°C before assay for histone kinase activity. All other details were as described in the legend to Fig. 1.

Table 1. *Kinetic constants of partially purified lymphocyte casein kinases*

The maximum velocity (V) is expressed as nmol of phosphate transferred/min per mg of protein. Assays with ATP as the variable substrate contained 10mM-$MgCl_2$ and those with $MgCl_2$ as the variable substrate contained 90μM-ATP. Kinetic constants (±S.E.M., for seven determinations) were determined by fitting the kinetic data to the Michaelis–Menten equation by using the method of least squares.

Substrate ...	$MgCl_2$		ATP	
Fraction	$s_{0.5}$ (mM)	V	$s_{0.5}$ (μM)	V
CK_1	7.9±0.7	3.4±0.14	10.9±0.5	2.2±0.03
CK_1+150mM-KCl	1.4±0.1	4.8±0.12	11.4±0.6	4.3±0.08
CK_2	1.3±0.3	0.11±0.006	23.4±1.6	0.12±0.003

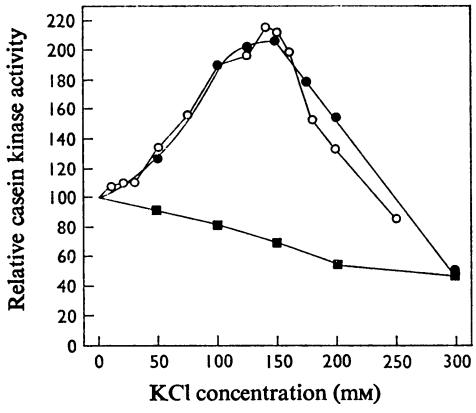

Fig. 3. *Effect of KCl on the casein kinase activity of crude extracts of partially purified fractions CK_1 and CK_2*

Assays were carried out as described in the Materials and Methods section with crude extract (○; 17μg of protein), with fraction CK_1 (●; 2.7μg of protein) and with fraction CK_2 (■; 18.4μg of protein). The casein kinase activities in the absence of KCl were 260, 1845 and 85pmol/min per mg of protein respectively.

Lack of adequate quantities of lymphocytes prevented the direct assessment of the relative contributions of all subcellular fractions. Although the membrane fraction reported in Table 2 would be expected to be contaminated with mitochondria, this fraction contains such a small proportion of the total histone kinase activity that the contribution of the mitochondria is likely to be small.

After centrifugation of a crude lymphocyte extract at 100000*g* for 60min about 80 and 90% of the casein kinase and histone kinase activity (assayed in the presence of 150mM-KCl and 2μM-cyclic AMP respectively) remained in the supernatant. Consequently it is unlikely that major amounts of these activities are associated with the ribosomes. It is not possible from the results reported here to determine the localization of the different classes of histone kinase and casein kinase.

With the normal reservations associated with cell-fractionation studies, the present data suggest that a large proportion of the casein kinase activity is located in the lymphocyte nuclei.

Discussion

The two histone kinases described in this paper (HK_1 and HK_2) appear to be similar to those obtained from hypo-osmotic extracts of human tonsillar lymphocytes (Farago *et al.*, 1973). An additional histone kinase was reported in tonsillar lymphocyte nuclei, which did not bind to DEAE-cellulose and which was not detected in the present studies. Since the tonsillar lymphocytes, unlike the purified lymphocytes used in the present study, would be

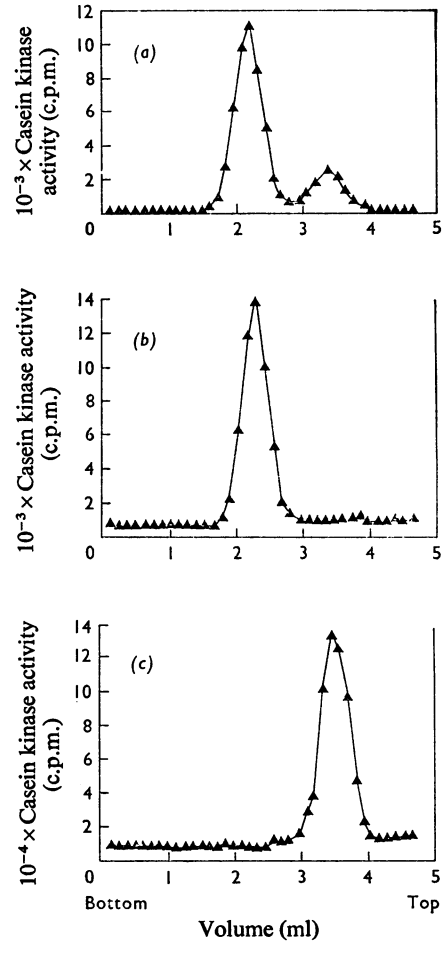

Fig. 4. *Sucrose-density-gradient centrifugation of casein kinase activity peaks CK_1 and CK_2 eluted from a DEAE-Sephadex column*

Sucrose gradients containing 600mM-KCl were prepared and centrifuged as described in the Materials and Methods section. All fractions were assayed for casein kinase activity (▲) in the presence of 150mM-KCl (derived from the gradient). The column fractions CK_1 and CK_2 were dialysed against 50mM-Tris–150mM-KCl buffer, pH7.7, and concentrated by high-pressure ultrafiltration. All samples were adjusted to 600mM-KCl before loading on the sucrose gradients. (*a*) Control gradient: lymphocyte crude extract (302μg of protein). (*b*) A portion of peak CK_1 obtained from a DEAE-Sephadex column (155μg of protein). (*c*) Peak CK_2 from a DEAE-Sephadex column (76μg of protein).

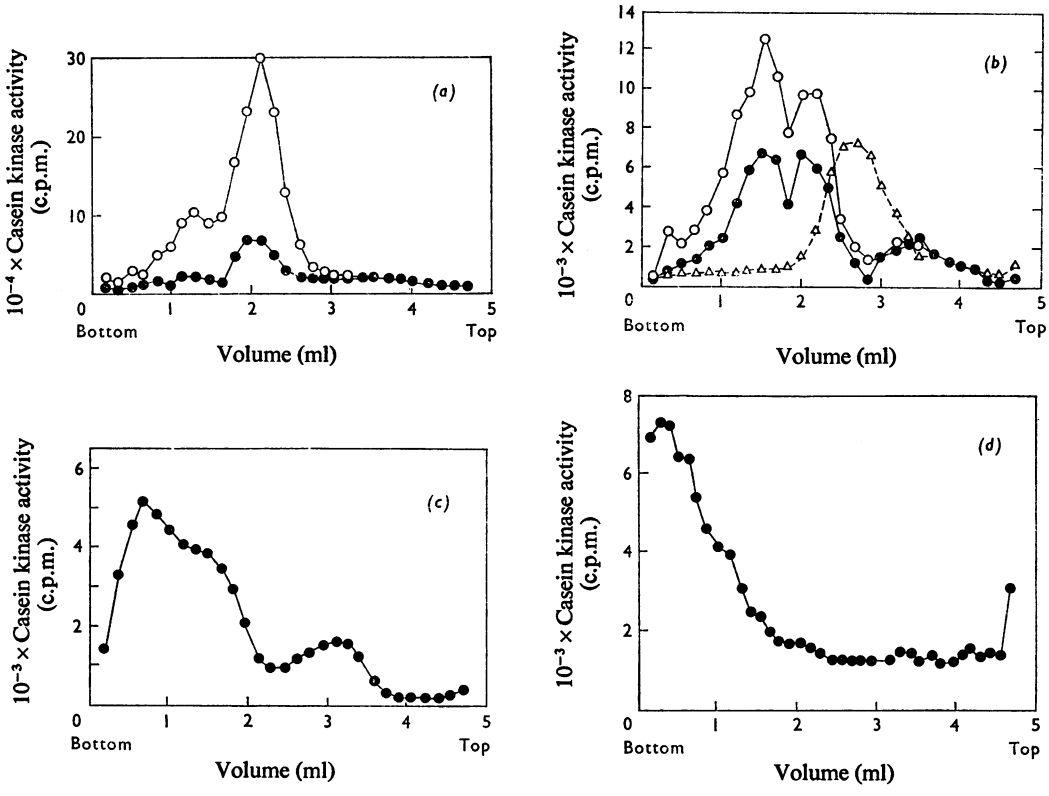

Fig. 5. *Effect of* 150 mM-KCl *on the sedimentation pattern of casein kinase in sucrose density gradients*

(*a*) Crude lymphocyte extract (458 μg of protein) was centrifuged on a gradient in the absence of KCl as described in the Materials and Methods section and assayed for casein kinase activity in the absence (●) and presence (○) of 150 mM-KCl. (*b*) Crude lymphocyte extract (458 μg of protein) was centrifuged in the presence of 150 mM-KCl and assayed for casein kinase activity in the absence (●) and presence (○) of 150 mM-KCl. Fractions were also assayed for histone kinase activity (△). (*c*) Crude lymphocyte extract (320 μg of protein) was centrifuged in the presence of 150 mM-KCl and assayed for casein kinase activity in the absence of added salt (37.5 mM-KCl was derived from the gradient samples). (*d*) Fraction CK_1 from a DEAE-Sephadex column (155 μg of protein) was centrifuged in the presence of 150 mM-KCl and assayed for casein kinase activity in the absence of added salt (37.5 mM-KCl was derived from the gradient samples).

expected to contain both B and T cells it is possible that the extra kinase is derived from B lymphocytes. Alternatively the activity may represent the dissociated catalytic subunit of the cyclic AMP-dependent holoenzyme.

Salt stimulation of both casein kinase and phosvitin kinase has been reported in a number of cases (Desjardins *et al.*, 1972; Rabinowitz & Lipmann, 1960; Rodnight & Lavin, 1964; Traugh & Traut, 1974), but neither the molecular basis nor the physiological significance of this effect is known. The sucrose-density-gradient studies indicate that maximum activity of fraction CK_1 occurs at a KCl concentration that causes the enzyme to aggregate (150 mM). There is, however, no direct evidence to suggest that these two effects are causally related. It is interesting that the phosphorylation of endogenous proteins by rat liver nucleoli protein kinases is maximally stimulated by 150 mM-KCl (Grummt, 1974).

Although the endogenous substrates of fractions CK_1 and CK_2 are not known, the apparent preference of these enzymes for acidic protein substrates makes them possible candidates for the kinases responsible for phosphorylation of nuclear acidic proteins (Desjardins *et al.*, 1972). The lymphocyte kinase CK_1 has a number of properties in common with the rat liver nuclear casein kinase NII reported by Desjardins *et al.* (1972). Both activities are insensitive to cyclic AMP, are optimally stimulated by 150 mM-KCl and are eluted from DEAE-Sephadex at similar salt concentrations. However, the lympho-

Table 2. *Subcellular distribution of casein kinase and histone kinase activity in human lymphocytes*

Protein kinase assays were carried out as described in the Materials and Methods section. Histone kinase was assayed in the presence of 2μM-cyclic AMP, and casein kinase activity in the presence of 150mM-KCl. 5'-Nucleotidase and alkaline phosphatase were assayed as described by Fisher & Mueller (1971). The results presented were obtained in single experiments; a similar distribution pattern was obtained in two separate experiments.

	Enzyme activity (percentage distribution)					
Fraction and method	Casein kinase	Histone kinase	5'-Nucleotidase	Alkaline phosphatase	Protein (%)	DNA (%)
Fisher & Mueller (1971)						
Mg^{2+} wash	41	64	13	53	22	10
Supernatant	27	30	8	26	33	5
'Membrane'	6	1	69	16	18	7
Nuclei	26	5	10	5	27	68
Chauveau *et al.* (1956)						
Whole homogenate	100	100	—	—	100	100
Nuclei	45	33	—	—	39	74
Nuclear wash	10	34	—	—	16	0
Supernatant	19	28	—	—	22	0
Membrane; whole cells	27	3	—	—	18	23

Table 3. *Classification of human lymphocyte protein kinases*

	Protein kinase			
Criterion	CK_1	CK_2	HK_1	HK_2
Stimulation by cyclic AMP	−	−	+	−
Sensitivity to protein kinase inhibitor	−	−	+	−
Stimulation by KCl	+	−	−	−
Inhibition by KCl	−	+	+	+
Substrate preference	Casein	Phosvitin	Histone	Histone
Classification type*	III	III	I	III

* As described by Traugh & Traut (1974).

cyte enzyme has a lower $s_{0.5}$ value for ATP and a lower sedimentation coefficient in sucrose gradients carried out in the absence of added salt. Desjardins *et al.* (1972) have equated their rat nuclear casein kinases (NI and NII) with the chromatin-associated kinases described by Takeda *et al.* (1971; A_2 and A_1 respectively) and by Ruddon & Anderson (1972; IV and I respectively). The second lymphocyte casein kinase (CK_2) can be distinguished from the rat liver nuclear kinase NI because it is not stimulated by KCl. There is, however, insufficient information available to determine the degree of similarity of kinase CK_2 with the phosvitin kinase from rat liver cytosol (Baggio & Moret, 1971).

The properties of the four lymphocyte protein kinases are summarized in Table 3. They have been classified according to the scheme of Traugh & Traut (1974) in which type I, type II and type III refer to cyclic AMP-dependent enzyme (RC), free catalytic subunit (C) and other protein kinases respectively.

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