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Determination of the Polyamine Content of Rat Heart Mitochondria by the Use of Heparin–Sepharose

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ABSTRACT

Heparin–sepharose has been utilized to remove polyamines adsorbed to the cytoplasmic surface of rat heart mitochondria. The results obtained can be summarized as follows:

1. Heparin–sepharose removes 90% of the spermine, 98% of the spermidine, and 98% of the putrescine adsorbed.
2. Polyamine contents of chromatographed mitochondria amount to 2.66 and 0.36 nmol spermine and spermidine, respectively, per mg of mitochondrial protein.

Index Entries: Heparin–sepharose, polyamine determination by; mitochondria, polyamine content of; polyamines; in rat heart mitochondria; rat heart mitochondria, polyamine in; sepharose–heparin, polyamine determination by.

INTRODUCTION

The polyamines—putrescine, spermidine, and spermine—are widely distributed in prokaryotic and eukaryotic cells and reach intracel-

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lular concentrations approaching the millimolar range (*1*). Not much is known, however, of their subcellular distribution. Because they are highly charged cations at cellular pH values, it is generally accepted that they are bound to anionic cell constituents such as ribosomes, DNA, RNA, and membranes (*2–8*). This hypothesis is supported both by many polyamine-binding studies in vitro (*5,6,9,10*) and by direct measurements of the association of polyamines to cellular components (*2,4,8,11*). This association, however, may be the result of the secondary redistribution of polyamines because of their high affinity for cellular polyanions, during cell disruption and fractionation from their primary compartments to other cellular locations. In *Neurospora crassa*, according to tracer experiments, 70–78% of spermidine and putrescine are sequestered in vivo in some fashion (*12,13*). One third of the sequestered spermidine, furthermore, is in a vacuolar pool that is nonexchangeable in in-vitro experiments. (*13*). Nonexchangeable polyamines were also reported in synaptosomal membranes of rat brain by Seiler and Deckardt (*14*). Polyamines thus appear to have an organellar compartmentation whose physiological significance is unknown; the sequestered polyamines may have either a regulatory effect on the cytoplasmic pool or a local intraorganular physiological effect. A major drawback in the study of the extent and physiological importance of the subcellular compartmentation of polyamines is given by the difficulty in discriminating between the adsorbed and the truly sequestered polyamine.

Recently we have shown that the resin heparin–sepharose has a high affinity and capacity for polyamines (*15*). We also proved the suitability of this resin to remove polyamines from biological materials (*11,16*). In the present report we utilized heparin–sepharose to remove polyamines adsorbed to rat heart mitochondria and this allowed us to determine the intraorganular polyamine pool. The results obtained prove the usefulness of heparin–sepharose in the study of polyamine compartmentation and let us foresee its utilization in the study of polyamine transport. Furthermore, the determination of the polyamine content of rat heart mitochondria gives an estimate of the polyamine concentration that may have a physiological significance in the control of mitochondrial functions.

RESULTS AND DISCUSSION

Intact and coupled rat heart mitochondria isolated by the method described by Sordhal et al. (*17*) were added with labeled polyamines to label the adsorbed pools. The adsorbed polyamines were removed as previously described (*11*). After 5 min incubation, heparin–sepharose was added and the suspension was poured onto a chromatographic column. The column was washed with the buffer, and the radioactivity present in the eluate was determined. The experiments were repeated in

triplicate with several different mitochondrial preparations and the results were highly reproducible. The heparin–sepharose column is able to remove 90 ± 2% of the labeled spermine, 98 ± 1% of the labeled spermidine, and 98 ± 2% of the labeled putrescine (Table 1). Under similar experimental conditions and in the absence of mitochondria, the heparin–sepharose column is able to completely remove polyamines from the solution. Heparin–sepharose is able to completely remove from the mitochondrial suspension labeled spermidine and putrescine, and to a lesser extent, labeled spermine. Thus, either labeled spermine is strongly bound to some component of the cytoplasmic membrane of mitochondria or it has been transported into the mitochondrial compartment and is inaccessible to the resin.

To prevent possible overestimation of the spermine mitochondrial pool, because of the uncomplete removal of the tracer polyamine, separate aliquots of intact and coupled rat heart mitochondria were directly added with heparin–sepharose to remove the polyamines adsorbed during the isolation procedures.

The polyamines associated to the mitochondria before and after the heparin–sepharose chromatography were determined as described by Seiler et al. (*18*).

The results obtained (Table 1) represent the polyamines sequestered by the mitochondria. They amount to 2.66 and 0.36 nmol of spermine and spermidine, respectively, per mg of mitochondrial protein (*19*). Mitochondria have an almost undetectable putrescine content. Its quantitative determination is unreliable because of technical difficulties. Spermidine is present in the mitochondria in a low amount, most of it being removable by the resin. Despite the fact that the resin is able to sequester a good deal of the adsorbed spermine, significant quantities of this amine are present in rat heart mitochondria. Our study indicates a different content of polyamines in the mitochondria compared to the whole tissue (*20*). The result, together with the ability of rat heart

TABLE 1
Removal of Polyamines from Mitochondrial Preparations by Heparin–Sepharose Chromatography

	Labeled polyamine added, dpm		Polyamine content, nmol/mg protein	
Polyamine	Untreated mitochondria	Heparin–sepharose-treated mitochondria	Untreated mitochondria	Heparin–sepharose-treated mitochondria
Spermine	5×10^5	$5 \pm 1 \times 10^4$	5.89 ± 2.60	2.66 ± 0.52
Spermidine	5×10^5	$1 \pm 0.5 \times 10^4$	2.60 ± 1.04	0.36 ± 0.15
Putrescine	5×10^6	$1 \pm 1 \times 10^5$	0.10 ± 0.03	—

mitochondria to interact with spermine in such a way as to render it inaccessible to the resin, suggests the existence, in mitochondria, of a transport system specific for spermine. At present the physiological role of spermine in mitochondrial functions cannot yet be conclusively stated. A possible involvement of this amine in the maintenance of the correct membrane potential ($\Delta \psi$) has been proposed (*21,22*).

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